

申 报	系列：教师系列 教学为主型
	专业：畜牧
	职 称： 副 教 授 (教学型 A 类)

业绩成果材料

(申报人的业绩成果材料包括论文、科研项目、获奖以及其他成果等)

单 位 (二级单位) 动物科学学院

姓 名 柳广斌

材料核对人:

单位盖章:

核对时间:

华南农业大学制

目 录

一、教学研究业绩

1. 教学研究项目

- 1.1 基于创新创业能力培养的“赛教融合”养羊学课程改革与实践（申请书及立项通知） 1
- 1.2 入选第三批卓越青年教师百人计划（立项通知及实施方案） 31
- 1.3 “养羊学”思政示范课堂（申请书及立项通知） 38
- 1.4 《养羊学》课程内容优化及微课、翻转课堂在教学实践中的应用研究（申请书、立项通知及验收结果公示） 51

2. 教改论文

- 2.1 检索证明 72
- 2.2 基于“项目驱动”的养羊学课程教学改革与实践 73
- 2.3 华南地区高等农林院校养羊学课程改革探索 86

3. 教学成果奖证书

- 3.1 第九届广东省教育教学成果奖一等奖“以实践创新能力为导向的动物生产类课程教学模式改革与实践” 93
- 3.2 校级教学成果奖一等奖“以实践创新能力为导向的动物生产类课程教学模式改革与实践” 94

4. 教学比赛证书

- 4.1 第四届广东省高校教师教学创新大赛二等奖（排位1） 95
- 4.2 第六届广东省高校青年教师教学大赛三等奖 96
- 4.3 华南农业大学 2022 年度“本科课堂教学十佳教师” . 97
- 4.4 华南农业大学 20220-2021 学年青年教师教学优秀奖一等奖 98
- 4.5 第三届广东省高校教师教学创新大赛二等奖（排位3） 99

5. 编写教材：教学辅导材料《奶牛生产实践教程》 100

二、科研项目

1. 主持

- 1.1 清远黑山羊快速良种快速扩繁关键技术建立（合同及入账凭证） 106
- 1.2 miR-128 及 miR-450 对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究（合同及验收书） 103
- 1.3 广东湖羊高产耐热新品系多基因聚合育种技术开发与示范（合同及验收书） 155
- 1.4 广东肉用黑山羊经济杂交配套方案开发（合同及验收书） 186
- 1.5 黑山羊养殖技术示范推广及科技小院平台建设（协议） 217
- 1.6 山羊繁殖及配套技术示范推广（合同） 223
- 1.7 黑山羊杂交配套技术开发及新品系培育（合同） ... 236
- 1.8 韶关市仁化县牛羊产业园山羊养殖技术服务（合同） 255
- 1.9 温氏优良肉用山羊及绵羊品种选育及配套饲养技术研发 266
- 1.10 肉牛标准化饲养管理技术开发（合同） 275
- 1.11 以牧草为主要原料的饲料配方设计及加工技术服务（合同） 285

2. 主要参与

- 2.1 雷州山羊新种质资源引进与优质基因快速传递技术开发（合同） 295
- 2.2 广东丘陵山地“林-草-羊”生态循环种养模式构建与示范（合同） 316
- 2.3 雷州山羊繁育体系建立及快大高繁新品系培育（合同） 337
- 2.4 广东雷州黑山羊快大高繁新品系培育（合同） 352
- 2.5 南方荷斯坦奶牛重要经济性状全基因组选择（合同） 366
- 2.6 辣木对荷斯坦奶牛生产性能和牛奶品质的影响研究（合

同)	378
2.7 黄梁木对乐至黑山羊饲用效果和肉品质的影响研究与应用示范(合同)	391

三、论文、著作等

1. 检索证明	405
2. 以第一作者发表本专业论文情况	
2.1 Effects of chronic heat stress on mRNA and miRNA expressions in dairy cows	410
3. 以通讯作者发表本专业论文情况	
3.1 Effects of moringa polysaccharides on growth performance, immune function, rumen morphology, and microbial community structure in early-weaned goat kids	421
3.2 Transcriptome analysis reveals mRNAs and long non-coding RNAs associated with fecundity in the hypothalamus of high-and low-fecundity goat.....	434
3.3 Integrating Analysis to Identify Differential circRNAs Involved in Goat Endometrial Receptivity	445
3.4 雄激素受体抑制剂恩杂鲁胺对山羊卵泡颗粒细胞增殖凋亡的影响	463
3.5 Screening of SNP Loci Related to Leg Length Trait in Leizhou Goats Based on Whole-Genome Resequencing	477
3.6 Selection and Regulatory Network Analysis of Differential CircRNAs in the Hypothalamus of Goats with High and Low Reproductive Capacity	499
3.7 miR-128-3p Regulates Follicular Granulosa Cell Proliferation and Apoptosis by Targeting the Growth	

Hormone Secretagogue Receptor	514
3.8 Expression profile and bioinformatics analysis of circRNA and its associated ceRNA networks in longissimus dorsi from Lufeng cattle and Leiqiong cattle	530
3.9 Transcriptome analysis reveals pituitary lncRNA, circRNA and mRNA affecting fertility in high- and low-yielding goats	542
3.10 Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats	555
3.11 Identification and Comparative Analysis of Long Non-coding RNAs in High- and Low-Fecundity Goat Ovaries During Estrus	572
3.12 湖羊和川中黑山羊在华南地区适应性研究	583
3.13 华南地区舍饲川中黑山羊与雷州山羊的生产繁殖性能及死亡状况研究	591
3.14 牛血清蛋白及大豆卵磷脂对山羊精子保存效果的影响	599
3.15 湖羊和川中黑山羊 GDF9、BMPR-IB、GnRHR 基因多态性及其与产羔数的关联分析	509
3.16 华南地区不同耐热性荷斯坦奶牛血液生化指标比较	619

四、科研成果

1. 科技奖励证书

1.1 2023 年度广东省农业技术推广奖二等奖“华南地区奶牛健康养殖关键技术与示范推广”	629
1.2 2021 年度广东省农业技术推广奖一等奖“畜禽资源保护与利用技术示范推广”	630

1.3 2020 年度广东省农业技术推广奖一等奖“华南地区羊的繁育及配套技术研发与示范推广”	631
1.4 2019 年度广东省农业技术推广奖二等奖“优质肉牛生态高效养殖技术研发与示范”	632
2. 知识产权	
2.1 专利授权证书：一种提高湖羊产羔数的多位点聚合效应分析的育种方法	633
2.2 专利授权证书：BMP6 基因作为黑山羊产羔数性状的分子标记	634
2.3 专利授权证书：chi-miR-128-3p 作为山羊卵泡成熟 miRNA 标志物的应用	635
2.4 专利授权证书：一种影响湖羊产羔性状的 SNP 标记及其应用	636
2.5 专利授权证书：chi-miR-450-5p 作为山羊卵泡成熟 miRNA 标志物的应用	637
2.6 专利授权证书：一种提高山羊产羔数的多基因聚合效应分析的育种方法	638
2.7 专利授权证书：一种影响湖羊产羔性状的 SNP 标记及其应用	639
2.8 专利授权证书：一个与中国南方荷斯坦奶牛 305 天产奶量显著相关的 SNP 分子标记及应用和选育方法.....	640
2.9 专利授权证书：一个与南方荷斯坦奶牛乳脂率相关的 SNP 分子标记、试剂盒及应用和选育方法	641
2.10 专利授权证书：一种保定、称重和体尺测量一体式装置	642

五、其他业绩

1. 指导学生学科竞赛

1.1 第九届中国国际“互联网+”大学生创新创业大赛广东省分赛银奖	643
1.2 第四届牛精英挑战赛特等奖	644
1.3 中国国际大学生创新大赛（2024）广东省分赛银奖 .	645
1.4 第十四届“挑战杯”广东大学生创业计划竞赛铜奖 .	646
1.5 第三届全国农林高校“牛精英”挑战赛二等奖	647
2. 个人荣誉	
2.1 校级“教书育人”先进个人证书	648
2.2 动物科学学院 2022 年度“教学十佳”	649
2.3 动物科学学院 2021 年度“教学十佳”	650
2.4 动物科学学院 2019 年度青年教师教学观摩比赛一等奖	651
3. 社会服务	
3.1 生产实践证明	652
3.2 担任科技推广专家证明	653
3.3 华南农技通社会服务工作量统计	660

5

广东省高等教育教学研究和改革项目

申 请 书

学 校 名 称 : 华南农业大学 (盖章)

项 目 名 称 : 基于创新创业能力培养的
“赛教融合”《养羊学》课程
改革与实践

项 目 负 责 人 : 柳广斌

负 责 人 职 称 : 讲师

联 系 电 话 : 18802085530

填 表 日 期 : 2023. 12. 12

广东省教育厅 制

申请者的承诺与成果使用授权

本人自愿申报广东省本科院校教育教学改革项目，认可所填写的《广东省本科院校教育教学改革项目》（以下简称为《申请书》）为有约束力的协议，并承诺对所填写的《申请书》所涉及各项内容的真实性负责，保证没有知识产权争议。课题申请如获准立项，在研究工作中，接受广东省教育厅或其授权（委托）单位、以及本人所在单位的管理，并对以下约定信守承诺：

1. 遵守相关法律法规。遵守我国著作权法和专利法等相关法律法规；遵守我国政府签署加入的相关国际知识产权规定。

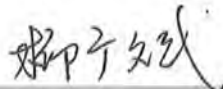
2. 遵循学术研究的基本规范，恪守学术道德，维护学术尊严。研究过程真实，不得以任何方式抄袭、剽窃或侵吞他人学术成果，杜绝伪注、伪造、篡改文献和数据等学术不端行为；成果真实，不重复发表研究成果；维护社会公共利益，维护广东省高等教育教学改革项目的声誉和公信力，不以项目名义牟取不当利益。

3. 遵守广东省本科院校教育教学改革项目有关管理规定以及广东省财务规章制度。

4. 凡因项目内容、成果或研究过程引起的法律、学术、产权或经费使用问题引起的纠纷，责任由相应的项目研究人员承担。

5. 项目立项未获得资助项目或获得批准的资助经费低于申请的资助经费时，同意承担项目并按申报预期完成研究任务。

6. 同意广东省教育厅或其授权（委托）单位有权基于公益需要公布、使用、宣传《项目申请·评审书》内容及相关成果。

项目负责人（签章）： 

2023年12月13日

一、基本情况

项目 简 况	项目名称	基于创新创业能力培养的“赛教融合”《养羊学》课程改革与实践						
	是否申报 省委托教改	否						
	项目性质	1.研究性教改 2.实践性教改√						
	起止年月	2023年12月13日 - 2026年12月13日						
项目 负 责 人	姓名	柳广斌	性别	男	出生年月	1982.4		
	专业技术职务/ 行政职务	讲师/无		最终学位/授予国家		博士/中国		
	所在 学校	学校名称	华南农业大学		手机号码	18802085530		
		通讯地址	广东省					
	主要教学 工作简历	时间	课程名称	授课对象	学时	所在单位		
		2016-2023	养羊学	动物科学	32	动物科学学院		
		2015-2023	畜牧学	动物医学	56	动物科学学院		
		2021-2023	动物科学概论	农经	32	动物科学学院		
		2014-2021	养牛学	动物科学	32	动物科学学院		
		2022-2023	动物生产类综合 实习	动物科学	120	动物科学学院		
	曾主持项目情况 (限填近 3年主持 项目情况)	项目级别	项目名称				获批时间	
		校级	“养羊学”思政示范课堂				2022	
		校级	华南农业大学第三批卓越青年教师百人计划				2022	
		校级	《养羊学》课程内容优化及微课、反转课堂在教学实践中的应用研究				2019	
项目 组	总人数	职称			学位			
		高级	中级	初级	博士后	博士	硕士	参加单位数
	6	4	2	0	0	6	0	1
	主要成员 (不含主 持人)	姓名	性别	出生 年月	职称	工作 单位	分工	签名
		孙宝丽	女	1981.9	教授	动科	思政元素融入	孙宝丽

		刘德武	男	1966.7	教授	动科	考核方案设计	
		李耀坤	男	1986.7	副教授	动科	课堂教学设计	
		郭勇庆	男	1981.6	讲师	动科	实践案例分析	
		邓铭	男	1986.1	高级实验师	动科	教学素材收集	

二、立项依据¹

2-1 项目实施的意义和现状分析（要求必须立足合本专业、本学院、本校或全省教学改革实际，从问题出发有针对性地提出）

（1）项目实施意义

畜牧业是我国农业的重要组成部分，畜牧业高质量发展关系到民生大计。我国是一个养羊大国，养羊业在促进畜牧产业结构调整、保障粮食安全和保护生态环境等方面起到重要作用，是助推畜牧业增产、农民增收，助力乡村振兴的主要途径之一，同时也符合广东省大力发展草食动物的方针与政策。《养羊学》是我国高等农林院校动物科学专业一门具有较强实践性的重要基础专业课，内容密切联系生产，紧扣实际需求，具有较强的理论性、实践性、可操作性和前瞻性，课程的主要任务是为养羊业健康、高效和可持续发展培养高素质专业人才。因此，积极开展《养羊学》课程教学模式改革，对加强养羊业高素质专业人才培养，推动畜牧业高质量快速发展意义重大。

在当前的《养羊学》教学中，存在课堂理论与生产实践脱节、跨学科科技趋势介绍不足、创新创业能力培养缺乏等问题，这阻碍了高素质人才的培养。在这种情况下，将课程教学与“互联网+”“挑战杯”等赛事进行融合对培养学生创新创业能力提高综合素质具有巨大意义。该项目组在前期教学中以《养羊学》课程为基础，组织学生参加了2023年第九届中国国际“互联网+”大学生创新创业大赛广东分赛并获得银奖，在竞赛过程中学生的创新创业能力得到显著提升，这让项目团队进一步明确：借助学科比赛和专业竞赛等途径高效培养高素质养羊业精英的教学融合模式值得进一步探索。

¹ 表格不够，可自行拓展加页；但不得附其他无关材料。下同。

基于以上背景和实践探索，本项目以立德树人为核心，以学科比赛或创新创业竞赛为重要载体，创新《养羊学》课程知识体系、创新课程建设模式、改革教学方法、强化实践环节、优化考核方式，构建基于创新创业能力培养的“赛教融合”教学模式，真正实现教学相长，培养品德高尚，热爱畜牧业，具有主动思考和创新能力的，具备养羊生产的基本理论、知识和技能，可在相关领域和部门从事技术研发、应用推广和服务管理等工作的创新型和实践型复合高素质优秀人才。

（2）现状分析

《养羊学》作为动物科学专业的生产类专业课，课程教学是培养具有养羊科学技术和创新创业能力人才的重要途径，在长期的教学过程中，课程团队在教学方法、教学内容、生产实践等方面逐步改革，取得了一定的教学成效。但是随着时代的快速发展，畜牧业逐步进入“智慧养殖”时代，呈现出多学科多产业交叉融合的发展趋势，对人才的创新创业能力及综合素质等方面提出了更高更新的要求，因此，《养羊学》课程的知识体系及德育环节亟待进一步创新和改革。当前，《养羊学》教学的主要薄弱环节表现在：

① 思想政治素质方面的教育有待进一步加强

十八大以来，党中央高度重视高校思想政治教育工作，全面构建高校思政工作体系，紧紧抓住高校立德树人的根本任务。习近平总书记明确指出，要用好课堂教学这个主渠道，提升思想政治教育亲和力，满足学生成长发展需求和期待，使各类课程与思想政治理论课同向同行，形成协同效应。但过去很长一段时间，由于主客观原因的共同作用，思政教学在许多高校处于“边缘化”的地位，其工作内容与学科专业之间也呈现“疏离化”的状态，专业课程中涉及思政方面的内容更是少之又少，在一定程度上影响了学生专业综合素质的培养与提升，因此，专业课程应补充和加强思政内容建设，将立德树人贯穿于整个课程。

② 教学方式单一，知识体系较为封闭，缺乏跨学科知识拓展

当前，高等院校本科课堂教学普遍仍以教师为中心，多采用灌输和填鸭式的教学方法。教师自顾自讲课，全程多通过PPT授课，偶尔附带少量教学视频，少量板书或无板书，教学模式过于单一，极度缺乏与学生的互动、沟通和交流。加之过于依附单一教材，教学内容更新不及时而显得较为陈旧，极度缺乏前沿性和先进性，与生产实践脱节严重，很难吸引学生同步参与教学活动。这种一元单向式的传统陈旧教学形式容易引发学生无聊、厌烦等不良学习情绪，导致学生在课堂上玩手机、睡觉、私下聊天及思想漫游等，严

重影响课堂教学氛围及学生学习效率。

③ 偏理论、轻实践，学生知识综合运用及创新创业能力较弱

目前，我国养羊业发展愈发迅速，对从事相关行业的人才要求越来越高，特别是实践及创新创业能力。而当今养羊学教学仍以传统的课堂教学模式为主，实践机会基本为零，教师侧重理论知识的讲授，不能与实践充分结合，未能让学生将课堂所学的理论知识付诸于实践，缺乏在实践中解决问题的机会；另外，学生较为关注的课程考核内容也基于局限于“教学大纲”“教材”和“课堂讲授”，为获取高分学生往往考前突击复习、死记硬背，考后迅速遗忘知识，从而导致学生实践动手能力及知识综合运用能力较弱，不能达到使学生真正掌握知识和技能的教學目的。

④ 考核制度缺乏创新，评价方式单调

《养羊学》考核方式仍是传统的以期末考核为主，忽视了对学生综合能力的考察，多以学生试卷考分的高低来评价学生学习成绩和对所学知识的掌握程度，导致学生死记硬背，缺少学习活力，“上课记笔记、考前背笔记、考后忘笔记”，这种单一的考核制度及评价方式不利于培养学生创新思维、知识综合运用和实践动手能力，应加强对“全过程考核”的重视程度与实践。

主要参考文献

- [1]张建斌, 杨华, 杨升, 蒲蕾, 洪亮, 李留安, 秦顺义. 新农科背景下动物科学一流专业建设研究. 畜牧与兽医, 2023, 55(08): 138-144.
- [2]仲庆振, 赵元, 杜东菊, 杨春馥, 孙泽威. 新农科背景下动物科学专业大学生创新能力的培养策略. 教育教学论坛, 2022(28): 181-184.
- [3]郝小燕, 杨春合, 李碧波, 牟春堂, 张春香. 《羊生产学》教学的改革探索——以山西农业大学为例. 畜禽业, 2022, 33(10): 47-49.
- [4]卢琦, 田兴舟, 陈祥. 《羊生产学》课程思政元素挖掘与探讨. 中国畜禽种业, 2022, 18(07): 61-62.
- [5]马海玉, 刘玲玲, 刘武军. “羊生产学”课程智慧教学实践创新探索. 大学, 2021(31): 158-160.
- [6]段春辉, 高立杰, 张英杰, 刘月琴. 《羊生产学》课程思政案例库建设的探索与实践. 畜禽业, 2021, 32(07): 86-87.
- [7]柳广斌, 孙宝丽, 刘德武. 华南地区高等农林院校养羊学课程改革探索. 学园, 2020, 13(29): 18-19.

2-2 项目实施基础（包括与本项目有关的工作积累和已取得的工作成绩；学校对项目的支持情况，含有关政策、经费及其使用管理机制、保障条件等，可附文件材料）

（1）与本项目有关的工作积累和已取得的工作成绩

① 项目主持人教学经验丰富，《养羊学》教学改革基础扎实。

2014年至今，项目负责人一直承担动物科学专业本科生《养羊学》课程教学，不断更新完善教学材料，探索创新教学模式，教学效果良好，本科课堂教学评价在学院内一直名列前茅。

目前，项目负责人主持并顺利结题校级教改项目“《养羊学》课程内容优化及微课、翻转课堂在教学实践中的应用研究”1项，通过项目实施初步优化了《养羊学》课程的教学大纲，制作了“微课”视频，并采用了QQ群、雨课堂等现代教学工具进行线上线下相结合的教学实践（图1）。结合课程授课情况，在总结上课经验的基础上，公开发表教改论文“华南地区高等农林院校养羊学课程改革探索”1篇。通过多年教学活动，已积累了丰富的教学经验，并于2022年入选华南农业大学第三批卓越青年教师百人计划，为本项目的顺利开展奠定了坚实基础。

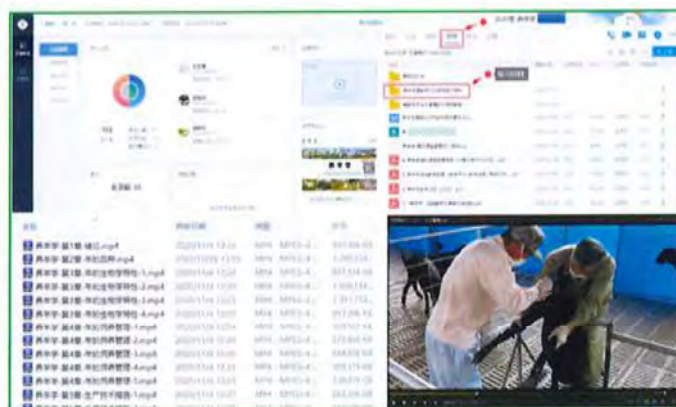


图1 雨课堂、QQ、微课视频在《养羊学》教学中的应用实践

② 项目负责人及团队教学技能娴熟，网络教学平台的使用较为熟练，指导学生参加学科竞赛经验丰富。

项目负责人及团队成员线上课程建设技能娴熟，具有丰富的课程信息化建设经验，可熟练应用华南农业大学在线教育平台、中国大学mooc平台、腾讯课堂、雨课堂等多个教学平台，熟悉多样化教学模式的建设与实践，并且了解学生的学习兴趣和喜爱的教学方式。

项目负责人多次参加教学竞赛，获得广东省第六届高校（本科）青年教师教学大赛三等奖、华南农业大学2022年度“本科课堂教学十佳教师”、华南农业大学2020-2021学年青年教师教学优秀奖一等奖等奖励（图2），具有优秀的教学能力。



图2 项目负责人获得的教学竞赛奖励

项目负责人及团队成员经常深入生产一线提供养殖技术指导，解决实际问题，具有丰富的生产实践经验。项目负责人指导学生参加创新创业竞赛，并获得第九届中国国际“互联网+”大赛广东省分赛银奖（第一指导教师）、2023年华南农业大学“创客杯”大学生创新创业大赛金奖（第二指导教师）等奖励（图3），具有丰富的创新创业竞赛指导经验。



图3 项目负责人指导学生参加创新创业竞赛获奖

③ 项目负责人已有的教学成果

2023.9 第九届中国国际“互联网+”大赛广东省分赛，银奖，第一指导教师。

2023.6 华南农业大学2022年度“本科课堂教学十佳教师”。

2023.5 2023年华南农业大学“创客杯”大学生创新创业大赛，金奖，指导教师，排位二。

2023.4 第三届全国高校教师教学创新大赛广东分赛，二等奖，排位三。

2022.9 广东省第六届高校（本科）青年教师教学大赛，三等奖。

2022.7 华南农业大学2022年“三育人”评选-“教书育人”先进个人。

2022.1 华南农业大学2020-2021学年青年教师教学优秀奖，一等奖。

2022.1 华南农业大学动物科学学院2021年度“教学十佳”。

2021.5 第四届全国农林高校“牛精英挑战赛”，特等奖，指导教师。

2020.3 第九届广东省教育教学成果奖，一等奖，排位八。

2020.1 华南农业大学动物科学学院2019年度青年教师教学观摩比赛，一等奖。

2019.5 华南农业大学教学成果奖，一等奖，排位七。

2018.7 第三届全国农林高校“牛精英挑战赛”，二等奖，指导教师。

④ 项目负责人及团队成员发表的教改论文

李耀坤,刘德武,孙宝丽,柳广斌,郭勇庆,邓铭.“互联网+赛教融合”养牛学课程改革与实践[J].草业科学,2022,39(10):2237-2244.

柳广斌,孙宝丽,刘德武.华南地区高等农林院校养羊学课程改革探索[J].学园,2020,13(29):18-19.

李耀坤,刘德武,孙宝丽,邓铭.“畜牧产业经济学”课程教学设计与实践[J].教育教学论坛,2020,(39):205-207.

张舒,赵敏杏,苏民强,孔令旋,韩印如,黄超群,邓铭,孙宝丽,刘德武,李耀坤*.“牛精英挑战赛”助力培育牛业精英[J].中国乳业,2020(S1):98-102.

郭勇庆,邓铭,李耀坤,柳广斌,刘德武,孙宝丽.借鉴“牛精英计划”模式促进创新型畜牧兽医人才培养[J].中国乳业,2020(S1):64-68.

孙宝丽,柳广斌,郭勇庆,李耀坤,邓铭,刘德武.动物生产类课程教学模式的创新与实践.安徽农学通报,2020,26(15):166-168.

李耀坤,郭勇庆,柳广斌,孙宝丽.《养牛学》课程课堂教学改革与实践[J].科教文

汇. 2019, 6 (461): 74-76.

郭勇庆, 邓铭, 孙宝丽, 李耀坤, 柳广斌, 刘德武. 草食动物生产学课程教学改革的探索. 畜牧与饲料科学, 2019, 40(12): 69-72.

邓铭, 李佳颖, 王涵沁. 高校学生社区党员志愿服务工作探析[J]. 山西青年. 2017(19): 189-190.

⑤ 项目负责人和项目组成员所承担的教学改革和科研项目情况

A. 项目负责人承担的教学改革和科研项目情况

[1]柳广斌, 2022年校级课程思政示范项目, “养羊学”思政示范课堂, 华南农业大学教改项目, 主持;

[2]柳广斌, 华南农业大学第三批卓越青年教师百人计划, 华南农业大学教改项目;

[3]柳广斌, 校级教改项目, 《养羊学》课程内容优化及微课、反转课堂在教学实践中的应用研究, 主持;

[4]柳广斌, 指导校级大学生创新创业项目1项;

[5]柳广斌, miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究, 广东省自然科学基金项目, 主持;

[6]柳广斌, 广东东肉用黑山羊经济杂交配套方案开发, 广东省科技厅公益研究与能力建设项目, 主持;

[7]柳广斌, 广东湖羊高产耐热新品系多基因聚合育种技术开发与示范, 广东省科技厅公益研究与能力建设项目, 主持;

[8]柳广斌, 肉用黑山羊优势杂交方案及高效养殖技术示范推广, 华南农业大学项目, 主持。

B. 项目组成员承担的主要教学改革和科研项目情况

[1]孙宝丽, 动物生产教学团队建设的研究与实践, 广东省教学研究与改革项目;

[2]孙宝丽, 华南农业大学广州风行牛奶有限公司实践教学基地, 广东省教学质量工程项目;

[3]孙宝丽, 基于创新能力培养的《养兔学》教学模式改革研究与实践, 华南农业大学教改重点项目;

[4]孙宝丽, 动物生产教学团队建设的研究与实践, 华南农业大学教改重点项目;

[5]孙宝丽, 动物生产学, 华南农业大学线下课程建设项目;

[6]孙宝丽,《养兔学》教学过程和教学环节优化的研究和实践,华南农业大学校级教改项目;

[7]孙宝丽,以《养牛学》为例探索实践类专业课教学模式改革,华南农业大学校级教改项目;

[8]孙宝丽,动物科学专业实践教学体系创新与实践,广东省教改项目;

[9]孙宝丽,辣木对荷斯坦奶牛饲用效果和牛奶品质的影响研究,广东省科技厅公益研究与能力建设项目;

[10]孙宝丽,基于物联网技术的集约化养兔管理信息平台,广东省科技厅农业攻关项目;

[11]孙宝丽,基于物联网的集约化养兔质量安全溯源信息平台,广东省农村信息化建设专项资金项目;

[12]孙宝丽,基于物联网的集约化兔场管理信息平台,省部产学研结合项目;

[13]刘德武,华南农业大学教改项目,《普通畜牧学》教学过程和教学环节优化的研究和实践(2005),华南农业大学教改项目;

[14]李耀坤,基于微课的翻转课堂在《养牛学》中的开发与应用,华南农业大学教改项目;

[15]李耀坤,指导广东省大学生创新创业项目1项,校级大学生创新创业项目2项;

[16]李耀坤,广东现代农业产业技术体系南方现代草牧业创新团队岗位专家项目;

[17]李耀坤,优质肉牛高效安全养殖技术应用与示范“南方湿热条件下优质肉牛高效安全养殖技术应用与示范”,国家重点研发计划子课题;

[18]李耀坤,南方荷斯坦奶牛重要经济性状全基因组选择,2019广东省基础与应用基础研究基金委员会,企业(温氏)联合基金重点项目;

[19]李耀坤,陆丰黄牛保育种技术集成与指导,广东省现代种业提升项目;

[20]李耀坤,肉牛优质高效生产关键技术示范推广,广东省公益研究与能力建设项目;

[21]李耀坤,雷州山羊不同生长发育阶段肌肉组织 miRNA 的鉴定及其调控机制研究,广东省青年创新人才项目;

[22]李耀坤,南方高温高湿气候条件下肉牛生产关键技术集成研究与示范。

[23]郭勇庆,《草食动物生产学》教学过程和教学环节优化的研究与实践,华南农业

大学教改项目

[24]郭勇庆, 基于多组学技术研究典型日粮诱导奶牛乳脂合成抑制的分子机理, 国家自然科学基金;

[25]邓铭, 畜牧类专业校外实践教学基地管理和学生实践学习模式创新研究, 华南农业大学教改项目;

[26]邓铭, 温氏班“校企协同实训”, 省级创新课程;

[27]邓铭, 大学生党员培养教育管理机制研究, 华南农业大学党建项目;

[28]邓铭, 三共建探索研究生党支部组织生活新途径, 华南农业大学党建项目;

[29]邓铭, 种养一体化家庭生态农场建设, 广东省公益研究与能力建设项目;

[30]邓铭, 基于TPC模式的温氏班《养牛学实训》课程改革与实践, 华南农业大学教改重点项目。

(2) 单位对项目的支持情况

① 华南农业大学出台了《华南农业大学本科教学质量与教学改革工程项目建设管理办法》等文件, 对全面提高本科教学质量起到了保证, 为项目工作的顺利开展提供了良好的制度保障。

② 华南农业大学动科学院拥有国家级实验教学示范中心、教育部人才培养创新实验区、国家级农科教人才培养基地等平台, 电脑、网络、图书等基础设施设施雄厚, 学校、学院有各种规格会议室、多媒体课室可用, 这为本项目的研究和建设提供了平台支持。

③ 华南农业大学动物科学学院与省内养羊企业共建中国农村专业技术协会广东连山山羊“科技小院”、华南农业大学“永根科技站”、“华南农业大学连山壮族瑶族自治县数字农业研究院产学研合作基地”、“广东省南方现代草牧业(羊)创新团队黑山羊智能养殖示范基地”、“广东农技服务乡村行轻骑兵”、“华南农业大学动物科学学院实践教学基地”等产学研合作平台, 可为学生的生产实践提供良好的实习条件。

④ 华南农业大学具有健全的财务和项目管理制度, 并在经费使用和管理等方面为本项目提供了经费保障。

三、项目实施方案及实施计划

3-1 具体改革内容、改革目标和拟解决的关键问题

(1) 具体改革内容

① 以立德树人为核心，优化课程知识体系，完善课程教学内容，实现专业和思政教育的有机融合，拓展科技前沿及跨学科知识，促进现代化“四有新人”专业人才的培养

习近平总书记在全国教育大会上指出：“要把立德树人融入思想道德教育、文化知识教育、社会实践教育各环节，学科体系、教学体系、教材体系、管理体系要围绕这个目标来设计，教师要围绕这个目标来教，学生要围绕这个目标来学”，指明了立德树人为教育之本。因此，本项目以立德树人为核心，将正确的世界观、人生观和价值观塑造，尊重科学、尊重规律，创新思维、辩证思维和合作共享精神培养，尊法、懂法、守法，家国情怀、责任担当、生态文明等“思政”元素全面系统融入课程教学。同时结合网络、不同教材、专业文献等养羊相关资料和素材，针对每个知识点就某些新理论、新现象、新热点进行有效的图文及视频补充，完善课程知识体系建设，拓展科技前沿及跨学科知识，促进学生专业与思想政治素质全面均衡发展。

② 以学生为中心，构建“创新型”教学团队

打破《养羊学》课程长期以来固定教师负责制模式，构建养羊学课程“创新型”教学团队。将接受过《养羊学》等动物生产类专业课程培训，且具有海外留学经历，理论基础扎实，创新思维和科研能力突出的老师联结起来，建立以年轻教师为主的养羊学教学团队，实现教师队伍的有机整合。建立教师协同授课与指导模式，开展协同制订教学方案、协同实施教学、协同指导实习和协同评价教学效果。同时，在教学过程中邀请养羊行业企业家、公司高管或技术人员等为学生就某个专题授课，从新的视角为学生输送新颖知识，激发学生探索与求知欲，帮助学生了解产业和行业的发展，更加清晰地认识学习的重点方向与内容，促进教学效果以及人才培养质量的提升。

③ 构建“赛教融合”的创新创业能力培养教学模式

教学模式对提升教学效果具有至关重要的作用，项目拟创新构建“赛教融合”教学模式。授课过程融合“互联网+”“挑战杯”等创新创业大赛理念，充分利用互联网工具和平台，主要包括多媒体、网络教学平台以及网上教学资源等，结合动画和动态模拟等手段，系统融入计算机科学和人工智能等基础知识，培养适应现代化、集约化、自动化和信息化发展的智慧畜牧业高素质专业人才。“赛教融合”是对人才培养模式的新要

求、新尝试和新实践，不仅能提升教师教学水平，还能更加高效地锻炼学生专业技能，学生通过参加专业比赛，可将课堂上获取的知识和掌握的技能融合并应用于生产实际问题的解决，可有效激发学生学习兴趣，提升学生学习效果和专业技能，这对高质量优秀人才培养至关重要。

④ 注重过程考核，创新课程成绩评定方式

课程考核是提高教学质量、检验教学效果和培养合格人才的重要环节，项目针对《养羊学》课程性质与任务，创新开展“线上+线下”注重过程评价的课程成绩评定方式，以适应新形势下高职专业人才培养的需要。课程不再采用传统单一的期末考试进行考核，新的考核方式注重过程评价，强调全面考核，成绩评定贯穿整个授课过程，考核内容主要包括预习任务（5%），课堂考勤（5%）、课堂互动（10%），课堂测试（10%）、课后作业（10%）、实践报告（10%）及课程论文（50%）等方面，评价综合采取学生互评、教师评价等多种形式进行，全方位系统评价学生的学习效果，促进学生理论与实践等方面的全面提升。

（2）改革目标

① 完善教学内容，拓展科技前沿及跨学科知识，并促进学生专业与思政教育同向同行，使学生在掌握全面的专业知识的同时，引导其形成正确的理想信念、政治认同、家国情怀、专业素质及道德修养。

② 加强《养羊学》课程教学团队建设，优化教学平台组成，丰富教学手段，创新“赛教融合”教学模式，充分激发学生学习兴趣，提升学习效率，增强学生专业技术及自主学习能力。

③ 采用线上线下结合型“理论+实践”综合考核方式替代传统考核方式，将考核贯穿于学习全过程，构建强化过程考核的综合评定体系。

通过项目建设，将立德树人贯穿于课程始终，突出《养羊学》培养高素质综合应用型人才、服务现代畜牧业的特色，同时为其他高校相关课程教学模式的改革和建设提供示范，起到带动作用。

（3）拟解决的关键问题

① 解决课程教学内容知识覆盖不够全面、缺乏科技前沿及交叉学科拓展的问题。将思想政治理论教育全面融入课程教学，同时结合网络、不同教材、专业文献等养羊相关资料和素材，完善课程知识体系建设，促进学生专业与思政素质全面均衡发展。

② 解决教学手段单一，课堂知识与生产实践脱钩，缺乏创新创业能力培养的问题。充分利用中国大学mooc平台、雨课堂、腾讯课堂等教学平台资源，结合“互联网

“+”及“创新杯”等创新创业竞赛，构建“赛教融合”型教学模式，强化过程管理，创新课程考核方式，培养学生创新创业能力，提升学生专业综合素养。

3-2 实施方案、实施方法、具体实施计划（含年度进展情况）及可行性分析(项目负责人和项目组成员如何协作保证项目顺利完成。)

（1）实施方案

当前，多数高等院校专业课程与思想政治方面的教育结合不够紧密，在一定程度上影响了学生专业综合素质的培养与提升。课堂教学仍多以“灌输”为主，忽视学生情感激发；教学课件经常依附单一教材，授课内容更新不及时显得较为陈旧，极度缺乏前沿性和先进性，与生产实际脱节严重，学生实践动手能力及知识综合运用能力较弱；课程成绩评定仍采用传统单一的闭卷笔试形式进行，考核不够全面系统，影响了对学生知识掌握及运用程度的检阅，不利于学生学习效果的提升。因此，本项目拟创新《养羊学》课程教学模式，以期为新时期背景下现代畜牧业发展所需的高素质专业人才培养提供助力。

习近平总书记指明立德树人为教育之本。本项目严格落实以立德树人为核心，①将“思政”元素全面系统融入课程教学，全力提升学生思想道德素养；②优化课程知识体系，完善课程教学内容，增强学生专业综合知识；③组建“创新型”教学团队，激发学生创新思维；④结合“赛教融合”和创新课程考核机制，增强学生实践动手能力；⑤引入“线上+线下”教育理念，强化学生自主学习能力；项目旨在探究富有活力、促进学生全面而有个性发展的课堂教学新模式，切实提高教育教学的质量和水平，为培养和造就大批高素质创新型专业人才奠定坚实基础（图4）。



图4 《养羊学》课程教学改革核心思路

(2) 实施方法

① 以立德树人为核心，优化课程知识体系，完善课程教学内容

A. 将思想政治理论教育全面融入《养羊学》课程教学

以立德树人为核心，结合课程知识点，将与科学养殖、食品安全、环境保护、脱贫攻坚和乡村振兴相关的学生世界观、人生观和价值观塑造，尊重科学、尊重规律，创新思维、辩证思维和合作共享精神培养，尊法、懂法、守法，家国情怀、责任担当、生态文明等“思政”元素全面系统融入课程教学（图5、表1）。



图5 《养羊学》课程“课程思政”元素融入实施流程

表1 《养羊学》课程知识体系及思政元素

章节设置	教学内容	★思政元素
第一章 绪论	讲解羊的分类、起源及驯化等知识，介绍中国养羊业的发展状况。	★结合国际形势及国家发展，说明脱贫攻坚、乡村振兴、绿色农业等国家政策及行业发展趋势，激发学生家国情怀。
第二章 羊的主要产品与品种	按在不同生产用途讲解绒毛用羊、乳用羊及肉用羊的产品特性及主要品种。	★结合现代种业发展，介绍行业杰出科学家的事迹，培养学生科学家精神。
第三章 羊的生物学特性及行为特点	讲解羊的行为及生理特征，介绍现代生物学技术在羊上的研究进展。	★结合羊的生物学特点，介绍羊在绿色农业、可持续发展中的作用，进一步带入脱贫攻坚、乡村振兴等理念。培养学生知农爱农情怀。
第四章 羊的饲养管理	结合养羊业发展，讲解羊饲养方式及各类型羊的现代化饲养管理方法	★通过生产实践案例，介绍养羊生产在脱贫攻坚、乡村振兴中的重要作用，培养学生的社会担当和责任感。
第五章 羊的生产性能提高技术措施	讲解羊的现代育种、繁殖及育肥技术研究进展及应用，介绍信息化技术在羊养殖中	★介绍学术前沿，结合授课老师个人科研经验，邀请学生参与科研项目，激发学生科研兴趣。

的应用。

第六章 羊场建设与经营管理

介绍现代化智慧牧场建设及企业化羊场经营管理。

★通过企业实际案例，结合物联网+、挑战杯等创新创业竞赛，培养学生创新创业精神。

B. 结合网络、不同教材、专业文献等相关资料和素材，更新和拓展授课内容

当前《养羊学》课程教材主要包括张英杰老师主编的《羊生产学》（第四版）、赵有璋老师主编的《中国养羊学》。除此之外，关于养羊生产的辅助资料还包括国家畜禽遗传资源委员会组编的《中国畜禽遗传资源志-羊志》、赵兴绪主编的《羊的繁殖调控》等。这些教材各有特色，侧重点也不尽相同，在制作授课课件时，应综合汲取以上教材的精华，优化教学大纲，提升授课内容的质量；另外，针对每个知识点就某些新理论、新现象、新热点进行有效的图文及视频补充，充实教学内容，帮助学生在有限时间内获取较多有用知识，促进学生专业与思想政治素质全面均衡发展（图6）。



图6 《养羊学》课程知识内容补充和完善思路

C. 结合当代生产经验和实际，引入案例教学，加强理论与实践的联系

紧跟形势，充分借助网络资源以及教师本人的生产实践经验和认知，将当前养羊业发展中先进的养殖技术、模式、理念、产业发展政策，以及前沿基础理论研究等融入课程（图7）。



图7 《养羊学》课程引入案例教学

②以学生为中心，构建“创新型”教学团队

A. 构建教授引领，以年轻教师为主的创新型教学团队，提升学生创新能力

打破《养羊学》课程长期以来固定教师负责制模式，构建养羊学课程“创新型”教学团队：2名教授、1名副教授、2名讲师、1名高级实验师（兼事务管理）。其中4名教师具有海外留学经历，理论基础扎实，创新思维和科研能力突出，接收新知识新理论速度快、效率高。建立以年轻教师为主的养羊学教学团队，实现教师队伍的有机整合，建立教师协同授课与指导模式，开展协同制订教学方案、协同实施教学、协同指导实习和协同评价教学效果，为提高学生的创新能力奠定基础（图8）。



图8 《养羊学》“创新型”教学团队

B. 邀请一线专家参与课程教学，增强学生实践认知。

在教学过程中，还可邀请养羊行业企业家、公司高管或技术人员等为学生就某个专题授课，不仅能从新的视角为学生输送新颖知识，还能激发学生探索与求知欲，帮助学生了解产业和行业的发展，更加清晰地认识学习的重点方向与内容，这对教学效果以及人才培养质量的提升具有重要作用（图9）。



图9 邀请生产一线专家开展课程授课

③ 构建“赛教融合”的创新型教学模式。

A. 引入“线上+线下”教育方法，创新教学模式

养羊学授课过程中要避免仅采用传统的单向传递式的以教师为中心的教学模式，摒弃“灌输”和“填鸭式”的教学方法，充分利用“线上+线下”教学渠道，主要包括多媒体、网络教学平台以及网上教学资源等，可通过慕课、雨课堂、腾讯课堂、钉钉、智慧树等网络教学平台，开展线上教学活动；同时，利用“中国大学MOOC”及“微信公众号”等向学生推送优质的最新知识内容；除了传统的文字、图片和视频外，也可多运用动画和动态模拟等手段，来进行抽象知识的讲解。另外，授课过程还可融入计算机科学、通信和人工智能等基础知识，将现代能化投料机器人，畜舍温度、湿度、通风等信息智能收集、分析和调控融入课程教学，培养适应现代化、集约化、自动化和信息化发展的畜牧业高素质专业人才（图10）。在授课过程中，也可就某个知识点安排学生查阅相关资料，由其代替老师进行讲解，并展开讨论和辩论，一定程度的反转课堂，实现师生角色的互换，激发学生学习兴趣和热情，加深其对知识的理解与掌握。



图10 创新“互联网+”教学模式

B. “赛教融合”，培养创新创业能力，提升学生专业综合素养。

竞赛是检验教学成果的一种有效方法，也是检验和提升人才培养质量的重要途径。“以赛促学，赛教融合”通过使学生参加比赛，将课堂上获取的知识和掌握的技能融合并应用于比赛和生产实际问题的诊断与解决，可有效激发学生学习兴趣，提升学生学习效果。《养羊学》相关的创新创业及专业技能比赛主要包括中国国际“互联网+”大学生创新创业大赛、“挑战杯”全国大学生创业设计大赛、全国大学生动物科学专业技能大赛等。学生作为比赛的主体，在专业教师的指导下，深入生产一线，深入开展生产实践训练，指导学生在实际生产过程中应用所学专业知

提升意义重大（图11）。



图11 “赛教融合” 教学理念

④ 创新课程成绩评定方式。

课程考核作为教学过程中一个重要环节，应予以充分重视，要着眼于科学全面地评价学生的综合素质，强化创新能力、应用能力和实践能力的培养，不再采用传统单一的笔试闭卷形式进行考核，创新构建“线上+线下”注重过程评价的课程成绩评定模式；新的课程考核方式注重过程评价，强调全面考核，成绩评定要贯穿在整个授课过程中，考核内容主要包括思政学习讨论与反馈（共计10%，其中思政问答3%，思政讨论2%，思政反思5%），线上的在线签到（2%）、线上测试及在线讨论（10%），线下的随课测试（8%）、课堂问答（3%）、课后作业（7%）、实践报告（10%）及期末笔试闭卷考试（50%）等方面，平时成绩占课程总成绩的50%，最终根据各部分所占权重计算课程总分；评价采取学生互评、教师评价等多种形式进行，全方位综合评价学生的学习效果，促进学生理论与实践等方面的全面提升（图12）。

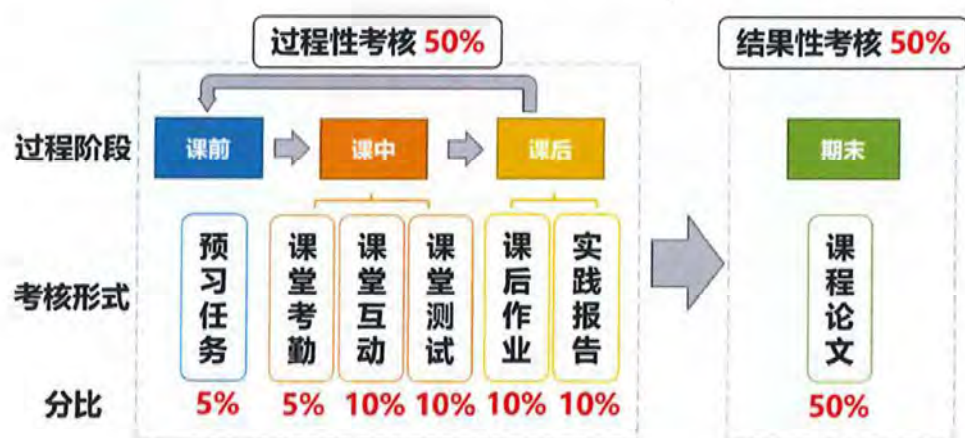


图12 注重过程考核，创新成绩评定新形式

④ 具体实施计划

2024.01-2024.06, 构建“创新型”养羊学教学团队;授课内容中补充强化思政知识点,优化完善课程教学内容;

2024.07-2025.06, 将“线上+线下”相关技术和平台应用于养羊学教学过程,并将“互联网+”“挑战杯”及“动科专业技能大赛”等专业比赛融入课堂活动,创新构建“赛教融合”的养羊学教学新模式;

2025.07-2026.06, 构建创新型课程成绩评定方式;养羊学教学新模式和手段的应用;

2026.07-2026.12, 项目总结与补充完善,发表教改论文,并完成结题报告的撰写。

⑤ 可行性分析

A. 本项目负责人具有9年本科教学经验,主讲《养羊学》《养牛学》《畜牧学》《动物科学概论》,指导学生参加创新创业竞赛并获得第九届中国国际“互联网+”大赛广东省分赛银奖(第一指导教师)、华南农业大学“创客杯”大学生创新创业大赛金奖(第二指导教师),同时参加过多次教学竞赛,获得过广东省第六届高校(本科)青年教师教学大赛三等奖,华南农业大学2022年度“本科课堂教学十佳教师”、华南农业大学2020-2021学年青年教师教学优秀奖一等奖、华南农业大学2022年“教书育人”先进个人称号等奖项。本项目团队成员也具有丰富的教学研究经验,曾主持过多项省级教改项目,并获的过第三届全国高校教师教学创新大赛广东分赛暨广东省高校教师教学创新大赛二等奖、第九届广东省教育教学成果奖一等奖、华南农业大学教学成果奖一等奖等奖励,因此本项目主持及团队成员有足够能力承担本项目。

B. 本项目团队为南方现代草牧业(羊)产业技术体系创新团队,项目组成员均有多年从事草食动物科学与教育研究的工作经验,主持过多项国家级及省级科研项目,与多家省内羊企业开展产学研合作,进行养殖技术的开发与推广,可为本项目的研究提供宝贵的实践经验与案例素材。

C. 项目团队所在动物科学学院,重视教学改革与课堂思政研究,学院定期组织集体听课、教学研讨及教学观摩比赛,开展“雨课堂”等现代化教学工具的应用培训,帮助教师提升教学能力改善教学效果。同时,动科学院拥有国家级实验教学示范中心、教育

部人才培养创新实验区、国家级农科教人才培养基地等平台，电脑、网络、图书等基础设备设施雄厚，可为本项目实施提供有利的平台支撑。

同时动物科学学院与省内养羊企业共建中国农村专业技术协会广东连山山羊“科技小院”、华南农业大学“永根科技站”、“华南农业大学连山壮族瑶族自治县数字农业研究院产学研合作基地”、“广东省南方现代草牧业（羊）创新团队黑山羊智能养殖示范基地”、“广东农技服务乡村行轻骑兵”、“华南农业大学动物科学学院实践教学基地”等产学研合作平台，可为学生的生产实践提供良好的实习条件。

综上所述，本项目在人员、经验、资源及平台等方面均有良好的条件，可保证本项目的顺利实施。

3-3 项目预期成果及其实践运用预期（包括成果形式、预期推广、应用范围、受益面等）

（1）预期成果

- 1）融入课程思政，拓展科技前沿，补充完善《养羊学》教学课件1份；
- 2）建设“赛教融合”《养羊学》教学模式1种；
- 3）发表1-2篇教学研究论文。

（2）预期推广、应用和受益面

项目充分利用华南农业大学动科学院“产学研”合作办学的典型资源优势，结合学院优质师资力量，借助学院雄厚软/硬件教学基础，根据行业对高素质应用创新型人才培养的需求和学生自身目标定位，着力创制新型、实用型《养羊学》教学模式，全力培育高素质、高知识、高技能的复合型专业人才，从而为我国养羊业发展，为乡村产业振兴和畜牧业腾飞提供助力。

项目成果主要在高等农林院校选修了《养羊学》课程的学生中推广应用，并供其他相关教学团队参考，着重在实践性较强的动物生产类课程中推广，如《养猪学》《养牛学》《养禽学》《动物生产学》《畜牧学》《动物科学概论》等课程。同时为同类型高校相关课程的建设 and 改革提供示范，起到带动作用。另外，项目取得的成效，也可推广至全省乃至全国范围内的畜牧及相关行业，用于开展养殖及相关技能培训，提高员工职业素养和专业技能，从而为我畜牧业发展和社会进步提供优秀人才。

3-4 本项目的特色与创新点

(1) 加强思政教学引领，重构课程知识体系，教学理念创新。

全面贯彻以立德树人为核心，将思想政治理论教育充分融入《养羊学》课堂教学，同时结合不同网络平台的专业材料、不同教材、不同文献和著作等相关课程素材，重构《养羊学》课程知识体系，将思想素质教育、专业知识教育、创新意识培育、生产技能实训等融入课堂教学，提高学生自我认知能力及主动获取知识能力，增强发现问题、分析问题和解决问题的能力，促进学生世界观、人生观、价值观的塑造与完善，增强学生自身责任感与使命感，树立起为国家发展和社会进步而努力奋斗的理念与目标。

(2) 创新性以学科竞赛为着力点，注重实践能力培养，以赛促教，增强学生学习动力和行业认同感。

中国国际“互联网+”大学生创新创业大赛、“挑战杯”全国大学生创业设计大赛及全国大学生动物专业技能大赛与《养羊学》课程目标及教学内容高度相关，本项目首次将其引入《养羊学》课堂教学，建立在读学生与养羊行业相关单位的纽带，将专业学习与行业实践充分结合，强化实践能力的培养，增进学生对行业现状和未来的认识，形成与社会需求对接的培养计划，给学生创新思维的释放提供平台，同时也为企业引进优秀人才搭建平台，培养畜牧业未来之星。另外，在“竞赛奖励”的驱动下，更能明确学生学习目标，以及对本专业的认可度，激发学生学习动力，提高课堂凝聚力，增强人才培养效果。

(3) 增强课程过程考核指标，创新课程成绩评定方式。

课程成绩评定贯穿整个授课过程，整合“线下+线下”考核方式，增强过程化管理，创新课程成绩评定模式。课程考核内容主要包括预习任务、课堂签到、课堂互动、课堂测试、课后作业、实践报告及课程论文等方面，评价采取学生互评、教师评价等多种形式进行。同时在授课过程中可选择表现优异的学生，协同畜牧企业开展专业实践综合培训，择优参加大学生科技创新创业项目、中国国际“互联网+”大学生创新创业大赛、“挑战杯”全国大学生创业设计大赛及全国大学生动物专业技能大赛等学术项目和竞赛。

四、建设经费预算（根据项目获得省级立项后实际建设需要制定预算，预算要经学校财务部门审核批准）

预算经费总额	3（万元）（需财务部门加盖公章）	
科目名称	预算额	项目支出用途
出版/文献/信息传播/知识产权事务费等	1.5	主要用于图书及文献购买、发表教改论文的版面费、资料打印等
材料费	0.3	教学相关材料购置等
交通费/差旅费/会议费等	0.9	前往实践基地参观调研、收集教学素材等
专家咨询费等	0.3	项目结题时专家评审劳务费等

五. 项目佐证材料清单

（可以证明项目立项必要性、可行性、创新性的相关佐证材料，请分条列明，限 20 条以内，内容另附）

1. 国务院办公厅印发《关于进一步支持大学生创新创业的指导意见》（项目紧跟国家号召，提高学生创新创业能力，为农业培养高素质人才，项目实施较为必要）；
2. 《华南农业大学本科教学质量与教学改革工程项目建设管理办法》（学校政策支持，为项目顺利实施提供保证，条件可行）；
3. 项目组成员发表教改论文（团队成员已发表多篇教改论文，能力可行）；
4. 项目组成员获国家级及省级教学成果奖（具有一定的前期积累，综合实力可行）；
5. 项目组成员获省级及校级教学比赛奖励及荣誉称号（积极开展教学能力提升，实力可行）；
6. 项目组成员指导学生参加国家级及省级比赛获奖（具备指导学生参加创新创业竞赛经验，能力可行）。

六、学校意见

学校保证给予该项目建设（研究）所需的各项条件，包括足额的经费支持，并严格遵守省教育厅项目管理规定，督促和保障项目顺利实施。

主管校长签字：

薛红



2021年12月14日

广东省教育厅

粤教高函〔2024〕9号

广东省教育厅关于公布 2023 年度广东省 本科高校教学质量与教学改革工程建设 项目立项名单的通知

各本科高校：

按照《广东省教育厅关于开展 2023 年度广东省本科高校教学质量与教学改革工程项目申报推荐工作的通知》等文件安排，经学校遴选推荐、省教育厅审核、公示等环节，现将 2023 年省本科高校质量工程建设项目立项名单予以公布，并就有关事项通知如下：

一、立项情况

确定立项建设省级实验教学示范中心 28 个、校企联合实验室 29 个、科产教融合实践教学基地 71 个、大学生社会实践教学基地 35 个、教师教学发展中心 5 个、课程教研室 167 个、现代产业学院 32 个、专项人才培养计划 62 项、高等教育教学改革项目 802 项。具体立项名单见附件。

二、项目管理

（一）本次公布项目均为省质量工程建设项目，建设项目经学校组织建设、校内结题并通过省教育厅统一组织项目验收后，正式认定为省级项目。

（二）项目正式实施前，请确保已对项目建设目标、建设举措、预期成果、建设进度安排等进行科学论证，论证专家应不少于5人，且至少有三分之一来自外校。论证后的目标、任务等将作为项目结题验收时的重要依据。

（三）项目日常管理由学校主管部门负责，学校应统筹做好项目中期检查、校内结题验收等工作。校内结题时，邀请校外评审专家人数不得少于专家总人数的三分之二。满足以下条件的项目，经学校正式申请，可以参与省教育厅统一组织的项目验收：

- 1.项目已完成立项时设定的主要建设任务和目标；
- 2.项目已取得标志性建设成果，且该成果已在教学实践中得到检验和有效应用；
- 3.已按照要求完成项目校内结题；
- 4.符合当年度省统一验收规定的其他条件。

各校质量工程建设项目管理情况，将作为学校下一年度项目立项限额的参考依据。

（四）项目实施过程中，其名称、建设内容（任务）、建设目标、建设周期、主要负责人、预期成果等发生重大变更的，需由时任项目负责人在发生变更后及时提出，经学校项目主管部门审核后由学校正式来函说明原因；擅自变更上述内容的，验收评定

时列为不通过。

三、其他事项

（一）2023 年度各校向省教育厅推荐并获得立项的项目，学校须将项目校内评审、推荐及论证相关材料妥善保存，留底备查。

（二）各校要统筹本校“冲补强”提升计划资金及自有资金对立项项目予以资助，项目获得学校资助情况将作为项目结题验收时重要考察因素之一。如项目建设中取得具有推广价值的优秀成果，请及时形成书面材料报省教育厅高教处。

联系人：李成军、窦月月，联系电话：020-37626882。

附件：2023 年度广东省本科高校教学质量与教学改革工程建设项目立项名单



公开方式：依申请公开

校对入：李成军

509	高等教育教学改革项目	暨南大学	“一体五驱”工程认证专业课程“多维融合”教学创新体系的探索与实践——以“包装振动与冲击”课程为例	杨松平
510	高等教育教学改革项目	暨南大学	融入国产操作系统和OBE理念的操作系统原理课程教学改革与实践	张继连
511	高等教育教学改革项目	暨南大学	“思政教育”融入大学生专业实习教学的体系构建与路径探索	赵建刚
512	高等教育教学改革项目	华南农业大学	一流农科高校基础实验课程思政教学一体化改革与实践 ——以大学物理实验课程为例	劳媚媚
513	高等教育教学改革项目	华南农业大学	人工智能背景下的新文科艺术专业人才培养研究与创新：以动画专业为例	王柯
514	高等教育教学改革项目	华南农业大学	人工智能赋能生态化大学英语混合教学改革研究	苏君
515	高等教育教学改革项目	华南农业大学	新农科背景下基于“数字标本”的智慧植保实验体系建设与实践	李云锋
516	高等教育教学改革项目	华南农业大学	基于创新能力培养的统计学专业数据分析类实验课程改革探索与实践	周燕
517	高等教育教学改革项目	华南农业大学	“激发内在动力，提升工程能力” ——基于项目驱动教学创新方法的研究与实践	孔莲芳
518	高等教育教学改革项目	华南农业大学	工程结构设计软件课程的“融合+分层”教学	李文雄
519	高等教育教学改革项目	华南农业大学	“三台协同，以美育人” ——以课程改革为核心的高校舞蹈美育建设研究与实践	郑琳喆
520	高等教育教学改革项目	华南农业大学	融合数据分析思维和学科交叉的线性代数课程教学创新与实践	张伟峰
521	高等教育教学改革项目	华南农业大学	双一流高校“科一教一思”融合培养学生高阶思维的实验教学改革创新——以遗传学实验为例	李楠
522	高等教育教学改革项目	华南农业大学	“新农科”背景下植物学课程教学改革创新研究与实践	白玫
523	高等教育教学改革项目	华南农业大学	劳动教育融入设计类专业课程路径探索 ——以纤维艺术设计课程为例	林汉聪
524	高等教育教学改革项目	华南农业大学	高阶思维视域下种子生物学“四维融合”混合式教学模式改革与实践	周玉亮
525	高等教育教学改革项目	华南农业大学	工程认证背景下基于知识图谱的软件工程一流专业建设和提升的研究与实践	王金凤
526	高等教育教学改革项目	华南农业大学	“双一流”建设视野下安全教育模式嵌入环境化学实验室课程体系 的实践与优化	高婷
527	高等教育教学改革项目	华南农业大学	基于创新创业能力培养的“赛教融合”养羊学课程改革与实践	柳广斌
528	高等教育教学改革项目	华南农业大学	基于知识图谱的农业院校课程思政建设探索——以公共数学基础课为例	张娜

华南农业大学文件

华南农人〔2022〕181号

关于公布华南农业大学第三批卓越青年教师百人计划人选的通知

各学院、部处、各单位：

为贯彻落实《华南农业大学高水平本科教育实施意见》（华南农办〔2019〕62号），进一步加强我校青年教师队伍建设，重点培育一批卓越青年教师，整体提升我校青年教师教育教学能力，根据《华南农业大学卓越青年教师百人计划实施方案》，学校组织开展了第三批卓越青年教师百人计划遴选工作，经个人申报、单位推荐、学校资格审查及遴选等程序，确定何永奇等25位教师入选第三批卓越青年教师百人计划（详见附件），现予以公布。

附件：华南农业大学第三批卓越青年教师百人计划入选名单

华南农业大学
2022年12月10日

- 1 -

附件

华南农业大学第三批卓越青年教师 百人计划入选名单

序号	单位名称	姓名
1	农学院	何永奇
2	林学与风景园林学院	邱 权
3	林学与风景园林学院	周 玮
4	园艺学院	刘朝阳
5	兽医学院	梁晓欢
6	兽医学院	刘 翠
7	动物科学学院	张世海
8	动物科学学院	柳广斌
9	资源环境学院	徐会娟
10	资源环境学院	舒迎花
11	海洋学院	陈世俊
12	生命科学学院	邓凤如
13	工程学院	王 昱
14	食品学院	郑倩望
15	水利与土木工程学院	张 敏
16	材料与能源学院	孙理超
17	数学与信息学院、软件学院	刘木伙

18	数学与信息学院、软件学院	殷建军
19	经济管理学院	张奕婧
20	公共管理学院	韩 丽
21	人文与法学学院	孙宗美
22	外国语学院	张秀娟
23	艺术学院	李雷鸣
24	马克思主义学院	王 超
25	马克思主义学院	林晓燕

公开方式：主动公开

华南农业大学党政办公室

2022 年 12 月 30 日印发



信息名称:华南农业大学卓越青年教师百人计划实施方案 华南农办〔2020〕4号

生成日期:

信息生成机构:信息公开专栏

发文字号:

公开方式:

访问次数:107

华南农业大学卓越青年教师百人计划实施方案

华南农办〔2020〕4号

为深入贯彻习近平新时代中国特色社会主义思想 and 全国教育大会精神,落实全国高等学校本科教育工作会议要求,认真实施《华南农业大学高水平本科教育实施意见》,紧密围绕全面提高人才培养能力,着力提升我校教师教学能力和水平,学校组织实施卓越青年教师百人计划(以下简称“百人计划”),特制定方案如下。

一、指导思想

坚持以习近平新时代中国特色社会主义思想为指导,全面贯彻党的教育方针,落实立德树人根本任务,遵循教育规律和教师成长发展规律,整体提升青年教师的师德修养、教育教学能力和水平,建设一支高素质、专业化、创新型的青年教师教学队伍,为学校高水平大学建设和争创“双一流”目标提供师资保障。

二、主要目标

遴选100名42岁以下优秀青年教师,并进行年度动态分流和补充。通过组织百人计划教师参加校院两级教师教学发展中心和各学院基层教学组织举办的校内外多元化教学培训、研讨、交流、比赛等活动,并在教改项目、质量工程、活动经费方面予以政策支持,培育一大批青年教师教学竞赛种子选手和各级各类教学名师后备人选,整体提升我校青年教师教育教学能力。

三、基本要求

(一)年龄要求

入选青年教师当年1月1日未满42周岁。

(二)思想素质

忠诚党的教育事业,爱国爱校,教书育人,模范遵守职业道德,具有强烈的责任感和事业心,热爱本科教学。

(三)教学要求

服从学校本科教学安排,积极承担本科课堂教学任务,每年承担本科生课程2门次以上;入选前一年度两个学期的课堂教学学生评教得分(含理论课和实验课)均在本学院排名前50%,出国、休产假、因

病等客观原因未承担本科教学任务的可不考虑学生评教得分。

（四）其他条件

未受过党纪政纪处分者；未出现过教学差错或事故；未受到《华南农业大学教职工师德师风失范行为处理实施办法》处理者。

四、组织实施

百人计划的组织实施由学校教师教学发展中心具体负责，并由校中心指导学院教师教学发展分中心同步开展相关工作。

（一）动态遴选

每年12月份开展下一年度遴选工作，符合条件的青年教师自愿报名，学院教师教学发展分中心进行资格审查后推荐至学校教师教学发展中心，入选后享有百人计划的相应权利，并遵守百人计划退出机制。

（二）入选后的培养安排

百人计划组建三个固定的自然班，每年遴选并开设一个班，每班30~35人，培养阶段为三年，出国访学、生育等客观原因造成的培养期异动时间可顺延，同一人不得连续超过2个培养阶段。按照教师教学发展中心制定的计划定期开展教学能力提升活动，内容包括培训、听课、研讨、咨询、调研、考察、交流等。入选者需完成前两个年度70%以上的教学能力提升活动才能享有入选后的权利。

（三）入选后的权利

1. 入选百人计划视同主持1项校级质量工程或本科教改项目。

2. 每人每个培养阶段配置5000元专项经费，纳入教师教学发展专项经费预算，用于教改论文版面费、教学培训、教学研讨差旅、教学竞赛等教学相关支出。

3. 优先享有校院两级教师教学发展中心举办的个性化定制教学能力提升活动，包括校内外课堂教学技能强化培训、现代教育技术应用、课程建设、教学比赛等。

4. 同等条件下优先推荐国家、省级高校青年教师教学比赛候选资格。

5. 同等条件下优先获得教学类项目建设经费支持。

6. 优先获得学校开展的其他教师教学发展类项目。

五、退出机制

（一）自动退出

百人计划教师符合以下条件之一，视为自动退出。

1. 当年1月1日满45周岁的。

2. 进入百人计划后获评学校本科课堂教学十佳教师、青年教师教学优秀奖一等奖、国家和广东省高校青年教师教学竞赛奖一、二等奖以及各级各类教学名师的。

3. 连续入选超过两个培养阶段以上的。

4. 因其他客观原因如调离我校的。

（二）强制退出

百人计划教师符合以下条件之一的，强制退出并不可享有入选后的权利。

1. 没有参加活动记录的。

2. 一个培养阶段内有3个以上学期（含3个）学生评教结果在本单位排在后20%的。

3. 受党纪政纪处分的。

4. 出现教学差错或教学事故的。

5. 受到《华南农业大学教职工师德师风失范行为处理实施办法》处理的。

六、其他

本方案自公布之日起实施，由教务处负责解释。



扫描关注学校微信



华农企业号 您的办事大厅

SCAU Copyright © 2014-2017 华南农业大学 .All rights reserved. 地址：广州市天河区五山华南农业大学

华南农业大学

课程思政示范课堂申报书

学院名称（盖章）动物科学学院

所属课程名称养羊学

课堂所在章节第二章 羊的主要品种

第 1 节 肉用羊的主要品种

课堂主讲人柳广斌

申报日期2022.9.15

本科生院制

二〇二二年

填 写 要 求

- 1.以 word 文档格式如实填写各项。
- 2.表格文本中外文名词第一次出现时，要写清全称和缩写，再次出现时可以使用缩写。
- 3.所填内容必须真实、可靠。
- 4.如表格篇幅不够，自行调整，但页码须清楚。
- 5.著作、教材、论文须已刊登在正式期刊上或为正式出版物，截止时间为 2022 年 6 月 30 日。

3. 教材版权页:

图书在版编目 (CIP) 数据

羊生产学 / 张英杰主编. —4 版. —北京: 中国农业出版社, 2019. 12
普通高等教育农业农村部“十三五”规划教材 全国
高等农林院校“十三五”规划教材
ISBN 978-7-109-26273-7

I. ①羊… II. ①张… III. ①羊—饲养管理—高等学校—教材 IV. ①S826

中国版本图书馆 CIP 数据核字 (2019) 第 283416 号

中国农业出版社出版

地址: 北京市朝阳区麦子店街 18 号楼

邮编: 100125

责任编辑: 何 微

版式设计: 杜 然 责任校对: 刘丽香

印刷: 北京万友印刷有限公司

版次: 1995 年 5 月第 1 版 2019 年 12 月第 4 版

印次: 2019 年 12 月第 4 版北京第 1 次印刷

发行: 新华书店北京发行所

开本: 889mm×1194mm 1/16

印张: 19.75

字数: 560 千字

定价: 54.00 元

版权所有·侵权必究

凡购买本社图书, 如有印装质量问题, 我社负责调换。

服务电话: 010-59195115 010-59194918

二、课堂设计

1. 课堂教学目标和思政育人目标

(1) 知识与技能目标

掌握国内外肉用羊的主要品种及特征。理解现代育种技术研究的基本策略。了解我国畜牧业发展的政策方向与育种技术前沿信息。具备养羊生产实践及科研工作中发现、分析和解决问题的能力。

(2) 过程与方法目标

1) 课堂上, 引导学生观察和比较不同品种之间羊的外貌特征和生产性能的差异, 启发学生发觉动物外貌特征与生产性能之间的内在联系, 培养学生发现问题的能力。通过“如何选育高生产性能种羊”的问题引发学生思考, 介绍现代育种技术前沿, 增强学生对分子生物学、计算机科学等多学科交叉领域的了解。

2) 课堂下, 针对本节课教学内容通过“雨课堂”布置关于品种资源利用与乡村振兴相融合的作业, 进一步促进学生对教学难点的思考与探索。通过“雨课堂”布置预习任务, 为下堂课做好铺垫。利用 QQ 及微信群与学生保持沟通交流, 及时答疑解惑。

(3) 情感态度与价值观目标

了解国家、养羊业及育种技术的发展状况。理解种质资源保护及开发利用对我国农业发展的重要意义, 通过“打好种业翻身仗”“种业科技就要自立自强”“乡村振兴”等重要思想的融入, 提高学生爱国主义情怀与时代使命感。

2. 专业教学与思政教育的融合设计

本节课有 3 个思政点:

(1) 品种资源介绍与国家“振兴种业”方针政策的融合

通过课前预习任务, 引入种质资源是畜牧业的“芯片”的概念, 举例美国制裁华为事件, 进一步说明芯片是我国的“卡脖子”技术。进而引出中央 2021 年发布的“中央一号”文件, 强调“加快推进农业现代化, 打好种业翻身仗”我们当代畜牧从业人员的历史使命, 帮助学生建立正确价值观, 强化学生勇于“使命担当”的责任心。

(2) 育种前沿技术与弘扬“科学家精神”的融合

通过育种技术发展，引出习近平主席于 2022 年 4 月 10 日考察海南省三亚市崖州湾种子实验室时的讲话，强调“种业科技就要自立自强。要弘扬袁隆平老一辈科技工作者的精神，十年磨一剑，久久为功，把这件大事抓好。”，培养学生“科学家精神”。

(3) 地方品种利用与“扶贫攻坚”及“乡村振兴”的融合

通过本地品种特点介绍，强调地方品种资源在我国“扶贫攻坚”及“乡村振兴”中发挥的重要作用。指出我国现代农业种养循环及可持续发展的需要，帮助学生理解我国羊的地方品种资源在开发利用中的潜力。并通过作业的形式引导学生思考以养羊作为切入点在家乡创业为乡村振兴做贡献的可能性，培养学生“家国情怀”。

3.教学方法、手段和载体途径（课堂讲授主要采用的方法，辅助信息技术手段，所使用的课件、案例、教材、素材等。）

(1) 教学方法

采用引导与启发的教学策略，以学生为本，从国家种业发展、科技自强、乡村振兴等需求为导向，通过类比、设问、举例、应用等方法，引导学生思考，激发主观能动性，培养学生应用知识的能力。通过思政元素融入，加强学生思想政治教育。

(2) 教学手段

1) 课堂上：利用多媒体课件（图片及视频），配合板书对重要知识点进行讲解。通过提问与学生进行课堂互动，并对教学效果进行评价。

2) 课堂下：利用“雨课堂”工具布置课后作业及预习任务，老师在线批改，及时获取反馈意见。利用“雨课堂”、QQ 及微信群设置讨论区，与学生保持沟通交流，及时答疑解惑，了解学生知识掌握情况并进行教学评价。

(3) 教学资源应用

1) 学生可通过“雨课堂”及“QQ 群”在线查看课件、视频及微课等。



图 1：雨课堂及 QQ 群共享资源

2) 教材及参考书

- [1] 《羊生产学》（第四版），张英杰主编，中国农业大学出版社，2019；
- [2] 《羊生产学》（第2版），张英杰主编，中国农业大学出版社，2015；
- [3] 《中国养羊学》，赵有璋主编，中国农业出版社，2013；
- [4] 《中国畜禽遗传资源志-羊志》，国家畜禽遗传资源委员会组编，中国农业出版社，2011。

3) 本节建议参考文献

- [1] 王志武, 李俊, 赵鹏, 郭宏宇, 田晖, 王婷, 孙锐锋. 优种肉用羊特克塞尔羊、萨福克羊、陶赛特羊选育试验[J]. 中国草食动物科学, 2021, 41 (06): 72-74.
- [2] 杜子文, 高福光, 周利军, 乌云塔娜, 贾福平, 刘心宽. 草原短尾羊肉用性能[J]. 兽医导刊, 2021(13): 138-139.
- [3] Belhaj K, Mansouri F, Tikent A, et al. Effect of Age and Breed on Carcass and Meat Quality Characteristics of Beni-Guil and Ouled-Djellal Sheep Breeds[J]. Scientific World Journal, 2021, 2021: 5536793.

4) 课外教学基地

- ① “畜禽育种国家地方联合工程研究中心”实验室；
- ② “广东省畜禽种质资源库”实验室；
- ③ “广东省南方现代草牧业（羊）创新团队”试验羊场。



图2: 广东省畜禽种质资源库及实验室

三、教学内容（须列出课堂教学中课程思政点的融入方式和教学方法，以及预期达成效果等。）

授课内容	课程思政融入点	融入方式与教学方法	预期成效
种质资源重要性及我国种业发展	类比种质资源是畜牧业的“芯片”，举例美国制裁华为事件，进一步说明芯片是我国的“卡脖子”技术。引出中央 2021 年发布的“中央一号”文件，强调“加快推进农业现代化，打好种业翻身仗”是当代畜牧从业人员的历史使命。	通过课前预习任务的分析，引入种质资源是畜牧业的“芯片”的概念。通过类比手机芯片解释种质资源重要性。通过国家政策分析解释我国种业发展方向。	帮助学生建立正确人生观价值观，强化学生勇于“使命担当”的社会责任心。
最新育种技术成果及育种工作重要性	通过育种技术发展，引出习近平主席于 2022 年 4 月 10 日考察海南省三亚市崖州湾种子实验室时的讲话。弘扬袁隆平老一辈科学家的科学精神，强调育种工作及技术研究对我国种业发展的重要性。	通过对最新科研成果的举例介绍，引出习主席讲话内容，弘扬科研工作者的奉献与科学精神。	培养学生“科学家精神”。
地方品种资源利用与脱贫攻坚及乡村振兴	通过本地品种特点介绍，强调地方品种资源在我国“脱贫攻坚”及“乡村振兴”中发挥的重要作用。指出我国现代农业种养循环及可持续发展的需要，帮助学生理解我国羊的地方品种资源在开发利用中的潜力。后续通过作业的形式引导学生思考以养羊作为切入点在家乡创业为乡村振兴做贡献的可能性。	通过课后作业的形式引导学生拓展思维，进一步思考与理解地方品种资源利用与脱贫攻坚及乡村振兴的关系。	培养学生“家国情怀”。

*教学活动计划不适用于本表格式表述的内容部分，可另加附页加以描述。

四、课堂应用及评价情况

4.1 课堂的主要特色及亮点自评（500 字以内）

（1）教学方法灵活

课堂采用“雨课堂”教学工具进行线上线下相结合的教学方式。线上在课前及课后通过教学软件布置预习及作业任务，学生通过网络对相关资料进行查阅与总

结，培养其信息收集及分析总结的能力，提高其主动思考的能力。同时通过 QQ 及微信群等通信软件及时对学生的疑问进行解答促进师生交流。线下课堂灵活运用多媒体教学手段，使用丰富的图片与视频资源，使教学内容形象生动，印象深刻。通过类比、设问、举例、应用等方法，引导学生思考，激发主观能动性。通过提问等课堂互动方法培养学生应变及语言表达能力。

（2）课程思政巧妙融入

针对课堂的知识点，精心设计，巧妙融入我国种业振兴、科技发展、扶贫攻坚、乡村振兴等国家重要发展政策方向，培养学生的“家国情怀”与“责任担当”精神。通过重要我国重要育种成果的介绍，激发学生的“科学家精神”，引导学生形成正确的人生观、世界观和价值观，达到思政育人润物无声的效果。

（3）开展创新创业教育

通过课题组最新研究成果的介绍，激发学生对科研与实践的兴趣。充分利用教师所在科研平台及养殖合作基地，为学生提供开展科研及生产实践的机会。帮助学生了解科研工作及企业经营的运作方式，增强学生创业就业的能力。

4.2 校内评价、同行专家或社会评价情况（请据实列明学校、院系和学生评价情况；同行专家的基本信息、评价时间、评价内容或媒体报道版面等。800 字以内）

（1）学生评价：本课程收到学生好评，在最近一次学生评教中（2021-2022-2 期末评教问卷）分数为 95.8 分，101 人参评，参评率为 99.02%。评语中学生给予了积极正面的反馈。

序号	回答
1	柳老师上课非常有趣生动，非常不错。
2	宝藏老师!!! 太好啦!!!
3	无，老师很搞笑
4	老师辛苦了
5	好，有趣
6	很好!
7	不错
8	暂无
9	无
10	w

图 3：学生评价

(2) 学校同行评价：最近一次学校同行评价中（时间：2022-03-01，听课地点：3503，听课内容“羊的品种”）分数为 97 分。听课同行对课程教学效果给予了肯定，具体评价为“教学目标全面，理论紧密联系实际，教学方法较为灵活”。详细报告见附件。

(3) 教学比赛成绩：本课程为“华南农业大学 2020-2021 学年青年教师教学优秀奖”参赛课程，其中本节课为现场比赛展示内容，获一等奖。同时本课程参加了“广东省第六届高校（本科）青年教师教学大赛”，获三等奖。

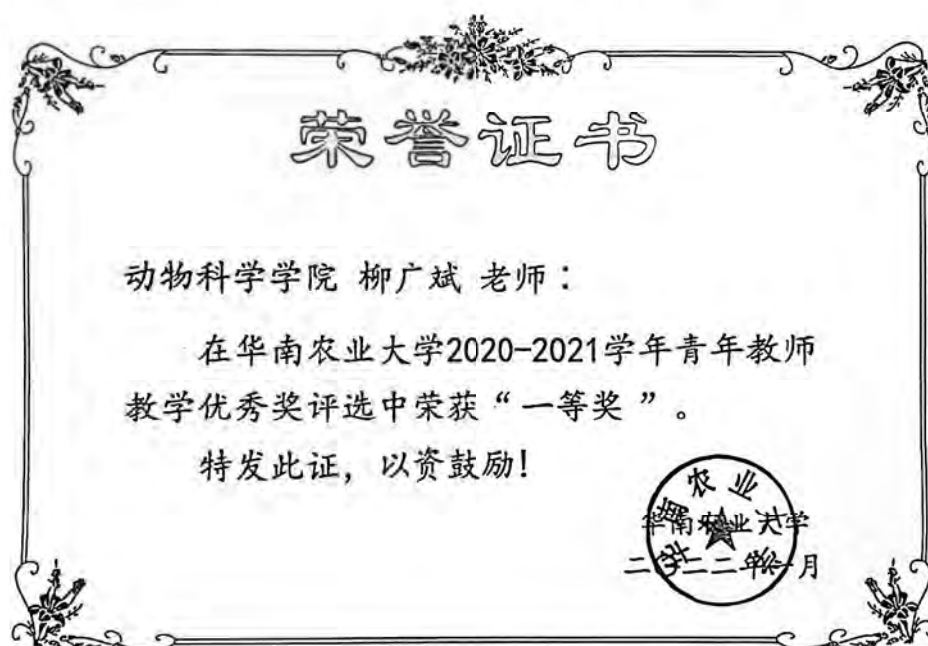


图 4：华南农业大学 2020-2021 学年青年教师教学优秀奖一等奖

*有关课堂评价的佐证材料可据实另附。

五、附件材料清单

1.课堂教案（必须提供）

（主讲人签字）

2.课堂授课课件（必须提供）

（一份完整的课堂授课课件，PDF 文档。）

3.教学（课堂或实践）实录视频或其他能够反映教学氛围的材料（必须提供）

（完整的一节课课堂实录，至少 30 分钟，技术要求：分辨率 720P 及以上，MP4 格式，图像清晰稳定，声音清楚。教师必须出境，视频中需标注教师姓名、单位；要有学生的镜头，并须告知学生可能出现在视频中，此视频会公开。）

4.其他能够佐证课堂教学效果的材料，不超过 2 份（选择性提供）

以上材料均可能在网上公开，请严格审查，确保不违反有关法律及保密规定。

六、课程承诺与保证

- 1.主讲人保证所提供各项材料不存在政治性、思想性、科学性和规范性问题；
- 2.主讲人保证所使用的教学资源知识产权清晰，无侵权使用的情况；
- 3.主讲人保证所提供各项材料不涉及国家安全和保密的相关规定，可以在网络上公开传播与使用。
- 4.如若获评为校级课程思政示范课堂，将继续提供优质教学服务。

主讲人签字：柳竹斌

2022年9月19日

七、所在学院审查意见与承诺

我院已按有关规定对主讲人进行了资格审查，对所有申报材料进行了严格审核，并保证：

1. 主讲人不存在师德师风问题；
2. 课堂所使用的资源内容不存在政治性、思想性、科学性和规范性问题；
3. 保证对课堂正常开展教学活动提供便利支持；
4. 严格遵守省教育厅课堂教学有关规定，积极开展课堂应用和推广等工作。

主管院领导签字
(学院公章)



2022年9月19日



关于公示2022年省级课程思政改革示范拟推荐项目和校级课程思政示范项目遴选认定结果的通知

审核发布：本科生院（招生办公室） 来源单位及审核人： 发布时间：2022-11-22 浏览次数：1028

各学院（部）、各相关单位：

根据《广东省教育厅关于开展2022年课程思政改革示范项目遴选认定工作的通知》精神和学校《关于开展2022年课程思政示范项目遴选认定工作的通知》有关要求，学校组织开展了本年度课程思政示范项目遴选工作。经项目负责单位推荐和学校组织专家评审等环节，拟认定校级课程思政示范项目177项，其中试点学院3个，示范团队19个，示范门，示范课堂54个，典型案例58个。在校级认定的基础上，拟推荐11项（其中示范团队3个、示范课程3门、示范课堂报2022年省级课程思政改革示范项目。另外，经遴选，拟推荐“社会工作助力乡村振兴的从化实践”等7个项目申报2022年省级课程思政改革示范项目中的“粤教阅心”——课程思政大家谈典型案例。现将名单予以公示（见附件）。

公示期自2022年11月22日至2022年11月28日。公示期间，若对公示项目有异议者，请以书面方式向本科生院实名以及不提供具体事实材料者，不予受理。

联系人：冯安伟
联系电话：85280052

华南农业大学本
2022年11月

附件：2022年省级课程思政改革示范拟推荐项目和校级课程思政示范项目认定名单.pdf

24	课程思政示范课堂	《海洋底栖生物学》课程示范课堂	海洋学院	付京花	拟认定校级
25	课程思政示范课堂	习近平新时代中国特色社会主义思想概论	马克思主义学院	何艳玲	拟认定校级
26	课程思政示范课堂	电影音乐欣赏	艺术学院	胡远慧	拟认定校级
27	课程思政示范课堂	园艺植物生物技术	园艺学院	刘朝阳	拟认定校级
28	课程思政示范课堂	抽样调查	数学与信息学院、软件学院	杨志程	拟认定校级
29	课程思政示范课堂	华南农业大学课程思政示范课堂--《新能源材料》第四章 超级电容器	材料与能源学院	胡航	拟认定校级
30	课程思政示范课堂	“养羊学”思政示范课堂	动物科学学院	柳广斌	拟认定校级
31	课程思政示范课堂	财务管理	经济管理学院	龙思颖	拟认定校级
32	课程思政示范课堂	体育舞蹈	体育教学研究部	周文英	拟认定校级
33	课程思政示范课堂	新视野大学英语 III	外国语学院	王莹	拟认定校级
34	课程思政示范课堂	国际贸易实务（英文）	外国语学院	谢治萍	拟认定校级
35	课程思政示范课堂	人力资源管理	经济管理学院	李桦	拟认定校级
36	课程思政示范课堂	土木工程材料思政课堂	水利与土木工程学院	譙雯	拟认定校级
37	课程思政示范课堂	线虫的形态与生活史	兽医学院	元冬娟	拟认定校级
38	课程思政示范课堂	土壤生物学	资源环境学院	张池	拟认定校级
39	课程思政示范课堂	跨文化交际	外国语学院	赵勇	拟认定校级

068

项目编号: JG19 069

华南农业大学教育教学研究和改革项目

申报书

项目名称 《养羊学》课程内容优化及微课、
翻转课堂在教学实践中的应用研究

项目负责人 柳广斌

职 称 讲师

工作单位 动物科学学院 (盖章)

移动电话 18802085530

电子邮箱 gbliu@scau.edu.cn

申报日期 2019.06.20

华南农业大学 教务处 制

2019 年 6 月

78

申请者的承诺与成果使用授权

本人自愿申报华南农业大学教育教学改革项目，承诺对所填写的《申报书》所涉及各项内容的真实性负责，保证没有知识产权争议。课题申请如获准立项，在研究工作中，接受华南农业大学教务处及本人所在单位的管理，并对以下约定信守承诺：

1. 遵守相关法律法规。遵守我国著作权法和专利法等相关法律法规；遵守我国政府签署加入的相关国际知识产权规定。

2. 遵循学术研究的基本规范，恪守学术道德，维护学术尊严。研究过程真实，不得以任何方式抄袭、剽窃或侵吞他人学术成果，杜绝伪注、伪造、篡改文献和数据等学术不端行为；成果真实，不重复发表研究成果；维护社会公共利益，不以项目名义牟取不当利益。

3. 遵守华南农业大学教育教学改革项目有关管理规定以及华南农业大学财务规章制度。

4. 凡因项目内容、成果或研究过程引起的法律、学术、产权或经费使用问题引起的纠纷，责任由相应的项目研究人员承担。

5. 项目获批后务必按项目计划要求及时开展研究工作，确保研究工作如期完成。

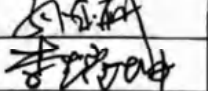
6. 同意华南农业大学或其授权（委托）单位有权基于公益需要公布、使用、宣传《项目申请·评审书》内容及相关成果。

项目负责人（签章）：

柳子斌

2019 年 6 月 20 日

一、项目及项目负责人、项目组简况

项目 简 况	项目名称	《养羊学》课程内容优化及微课、翻转课堂在教学实践中的应用研究						
	项目类别	<input type="checkbox"/> 1. 重点项目 <input checked="" type="checkbox"/> 2. 一般项目 <input type="checkbox"/> 3. 青年项目						
	起止年月	2019年8月-2021年7月						
项目 申 请 人	姓名	柳广斌		性别	男	出生年月	1982年4月	
	专业技术职务/ 行政职务		讲师/无		最终学位/授予国家		博士/中国	
	所在单位 及联系方 式	单位名称	动物科学学院			手机号码	18802085530	
		电子邮箱	gbliu@scau.edu.cn					
	主要教学 工作简历	时间	课程名称	授课对象	学时	所在单位		
		2014-2018	养牛学	本科生	32	动物科学学院		
		2015-2018	畜牧学	本科生	56	动物科学学院		
		2016-2017	养羊学	本科生	32	动物科学学院		
	主要教学 改革和科 学研究工 作简历	时间	项目名称					获奖情况
		2019	以实践创新能力为导向的动物生产类课程 教学模式改革与实践					一等奖
2018		第三届全国农林高校牛精英挑战赛					二等奖	
2017-2019		广东肉用黑山羊经济杂交配套方案开发						
2015-2018		广东湖羊高产耐热新品系多基因聚合育种 技术开发与示范						
项目 组	总人数	职称			学位			参加单位数
		高级	中级	初级	博士后	博士	硕士	
	5	3	2			5		1
	主要成员 (不含申 请者)	姓名	性别	出生 年月	职称	工作 单位	分工	签名
		刘德武	男	1966.7	教授	动科	难点剖析	
		孙宝丽	女	1981.9	副教授	动科	微课制作	
		李耀坤	男	1986.7	副教授	动科	素材收集	
郭勇庆		男	1981.6	讲师	动科	微课制作		

二、立项依据（项目研究的意义、现状分析）¹

1. 项目的意义

随着我国畜牧产业结构不断的优化与调整，近几年养羊业的发展得到了各地政府的大力支持，产业正从农户原始的放养模式向公司科学的规模化集约化养殖逐步转变，在这个过程中行业对高质量技术及管理人才的需求也在不断增加，因此如何培养符合养羊业发展需求的高素质人才成为了高校教育需要解决的重要课题。

《养羊学》是研究养羊生产技术以提高养羊业经济效益为目的的一门科学，它是高等农林院校中动物科学等专业的专业基础课，在相关专业的人才培养课程体系设置中占有重要地位。它既产生于养羊生产实践，又服务于养羊生产实践，其主要任务是通过理论和实践教学，使学生全面了解和掌握肉羊、奶山羊及绒毛用羊生产的基础理论和基本生产技能。课程教学质量的高低及教学效果的优劣将直接关系到今后能否为我国养羊产业发展培养合格人才。

目前在我省大力发展草食动物养殖的背景下，养羊业对专业人才的需求急剧增加，而我校《养羊学》课程教学内容较陈旧，教学形式单一，无法满足现代养羊业快速发展的需求，因此对现有《养羊学》课程内容及授课形式进行改革优化，提高教学效果，培养更多符合现代养羊业发展需求的专业人才是十分有必要的。本项目拟通过“微课”、翻转课堂等现代教学手段，优化课程教学内容，建设新的教学模式，促进学生自主化、个性化、多样化学习，提升《养羊学》教学质量，为草食畜牧业专业人才培养奠定基础。

2. 现状分析

《养羊学》是我校动物科学专业开设的传统课程之一，但随着时代的发展以及教学技术的进步，该课程逐步暴露出以下问题：

（1）教学内容陈旧：目前我校《养羊学》使用的教学大纲为数年前编制，教学内容主

¹ 表格不够，可自行拓展加页；但不得附其他无关材料。下同。

要参考北方养羊的经验与特点，并不符合目前养羊业发展的实际情况。例如课程中绵羊品种介绍较多，山羊较少，但实际上山羊养殖在我国华南地区更为普遍；再如课程中绒毛用羊介绍篇幅较多，肉羊较少，而实际上肉羊养殖已经发展为养羊产业的主要生产类型。此外随着养羊业的快速发展，一些新的生产技术及模式已被开发出来并在生产中得到应用，而这些新知识在目前《养羊学》课程中没有涉及，因此当前的课程内容已无法满足行业的发展需求，对《养羊学》教学内容进行改革优化势在必行。

(2) 教学方法单一：当前《养羊学》主要以课堂讲授为主，教师按照课程大纲安排对各知识点进行逐一讲解，但《养羊学》属于与实践联系较为紧密的课程，由于图片及视频等教学素材的缺乏，使得课堂教学并不生动，学生对知识的理解程度并不理想。而且由于该课程的专业性较强，单一的填鸭式教学方式往往使学生感到学习内容枯燥乏味，参与性不强，造成学习兴趣不高，影响教学效果，因此对《养羊学》的教学方式进行创新，提高教学效果是十分有必要的。

随着教学技术的进步，“微课”、翻转课堂等教学新手段可有效提高课堂教学效果。翻转课堂是利用现有的信息技术手段重新规划课前、课上、课后的教学安排，通过知识传递、知识内化、知识巩固的颠倒安排实现传统教学中的师生角色的翻转。教师不仅仅是课程内容的传授者，更多的变为学习过程的指导者与促进者，学生则由原来被动接受的听众转变为教学活动中积极主动的参与者。翻转课堂作为一种教学设计方法，它被认为是对于传统课堂教学的重大变革，现已成为全球教育界关注的焦点。“微课”作为翻转课堂的重要组成部分，具有主题突出、短小精悍、交互性宽、应用面广等突出特点，“微课”的任课教师能通过在线授课、网上作业、学生作业互评等多种方式将学生的积极性充分调动起来，明显提高了教师教学效果。除此之外，“微课”的应用不但能帮助学生查缺补漏、强化巩固知识，还为学生自主学习提供了一个知识挖掘的平台，通过“微课”设计，学生能根据自身所需搜索相应的学习资源，自主地挖掘所需的知识点、有针对性地开展学习；通过“微课”的互联网应用播放方式，允许学生使用自己的手机、平板、电脑等在各种场所随时随地开展学习，使学生对自己的学习有更多的主动权，能够有效地利用各种闲暇时间，充分提高学习效率。

虽然国内关于“微课”及翻转课堂的研究不在少数，但在《养羊学》教学中的应用案例还比较匮乏，因此对《养羊学》课程教学模式进行创新，研究“微课”及翻转课堂在《养羊学》教学实践中的应用方式与效果，激发学生的学习兴趣 and 热情，提高教学质量，是十分有必要的。

三、项目实施方案及实施计划

1. 具体改革内容、改革目标和拟解决的关键问题

1.1 具体改革内容

(1) 《养羊学》教学内容改革优化

对现有养羊学教学内容进行更新与优化，大量增加养羊新技术及新方法的讲解，满足现代养羊业对技术人才培养的需求。同时根据华南地区养羊业发展的地方特点，设置专题对南方肉羊品种及养殖模式与技术进行学习讨论，使学生了解南北养羊的差异，为南方养羊业发展培养人才。

(2) “微课”制作

通过网络收集及羊场现场拍摄养羊技术相关视频，根据教学重点与难点制作微课视频，以更形象生动的方式对知识点进行讲解，帮助学生理解并加强记忆。同时以广泛的视频素材，拓展学生视野，提高学生对养羊学的兴趣，并加深学生对行业的理解。

(3) “翻转课堂”在《养羊学》课程中实践应用

通过课前微课学习，促使学生发现并提出问题。引导学生通过对问题的进一步挖掘与分析，以自己的力量来寻找答案。并通过课堂讨论的方式，以学生为主体，对相关问题进行讨论，让所有学生参与其中，提高学生对问题的兴趣并加强理解与印象。老师在

课堂上对重点问题进行引导，在保证关键知识点传递的基础上，提高课堂师生的互动性，调动学生主观能动性，激发学生学习欲望。

（4）发展养羊实践及技能培训“第二课堂”

以广东省草食动物产业联盟为基础，建立广东地区养羊产学研实践基地，增加学生到羊场进行生产实践的机会，加强学生对养羊生产实践的理解。同时利用高校养羊专业联盟“领头羊计划”平台，加强与其他高校在养羊专业课教学上的资源共享与交流，建立为学生提供专业技能培训的渠道。

1.2 改革目标

对现有《养羊学》课程内容进行改革优化，通过“微课”及“翻转课堂”教学手段提高学生对教学内容的兴趣，加强对关键知识的认识与理解，增强课堂互动性，提高教学质量。同时充分利用“广东省草食动物产业联盟”及高校养羊专业联盟“领头羊计划”，建立生产实践基地，发展养羊学“第二课堂”，加强与其他高校及行业内专家的资源共享与交流，增加学生获得专业知识及技能培训的渠道，培养素质全面的养羊专业人才。

1.3 拟解决的关键问题

现有《养羊学》教学内容陈旧，已无法适应现代养羊业发展需求，且教学形式单一，无法引起学生兴趣，教学质量差。针对这一问题，本研究拟优化现有《养羊学》课程内容，利用“微课”教学方法突出知识重点及开拓学生视野，提高学生对课程内容的兴趣与认识，再利用“翻转课堂”增强课堂互动，充分调动学生积极性，解决学生提出的问题，最终提高教学效果。

同时利用“广东省草食动物产业联盟”成立的契机，建立生产实践基地，为学生提供参与实践了解养羊生产流程的场所，解决学生对生产实践缺乏感性认识的问题，同时也使学生对养羊行业及相关企业有更多的了解。此外，通过加入高校养羊专业联盟“领头羊计划”，解决养羊学教学缺乏共享资源及经验交流的问题，同时也可增加学生获得专

业技能培训的机会。

2. 实施方案、实施方法、具体实施计划（含年度进展情况）及可行性分析

2.1 实施方案

首先现有《养羊学》的课程内容进行优化，重新制定符合现代养羊业发展要求的教学内容，对课堂教学的大纲及课件进行更新。根据新的教学大纲内容，收集及拍摄“微课”教学材料并制作教学视频，之后采用“翻转课堂”的教学方式进行课堂实践。最后收集学生反馈意见，评估及分析教学效果，并对教学内容及形式进行进一步的改进。具体教学流程如图 1 所示。同时通过养羊联盟与养羊企业建立产学研合作关系，为设立养羊学实践基地及学生暑期生产实践课程打下基础。

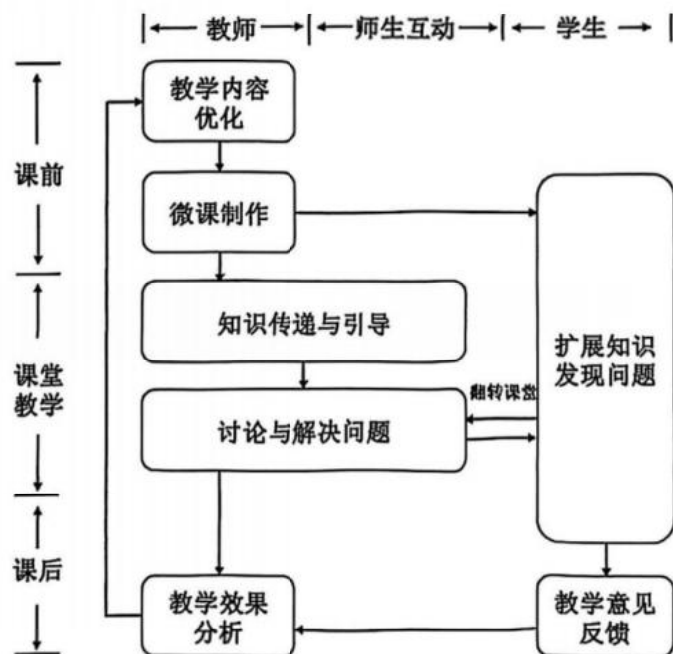


图 1：教学流程图

2.2 实施方法

(1) 优化现有教学内容及课件

根据现代养羊业发展的现状，对养羊新技术新模式新方法的资料进行收集与整理，提炼精华与知识要点，重新设计教学大纲与教学内容。以翻转课堂为主要教学方式，重新制作教学课件与教学素材。

(2) “微课”内容制作

根据新制定的教学大纲与教学内容，通过网络及羊场现场采集等方式，制作图像、音频及视频等教学材料。以知识点为基础，通过音视频处理软件制作“微课”视频。每个知识点覆盖一个多媒体“微课”，每个“微课”约10分钟。此外，针对重点知识，制作用于知识补充与拓展的“微课”资料。资料形式包括文字、图片及音视频。

(3) 翻转课堂教学实践

学生在上课前完成“微课”资料的学习，对知识点完成初步学习并提出问题，通过学生自主围绕问题进行资料收集并进行学习与分析。课堂上以学生为主体，针对学生提出的问题进行讨论，激发学生主动性，鼓励学生大胆发言，老师在重点问题上进行引导。课后通过课后“微课”拓展知识，实现对知识和技能的复习与巩固。

(4) 学生意见反馈及教学效果评估与改进

设计调查问卷，收集学生反馈意见，评价教学效果，总结分析教学内容、课件、“微课”及翻转课堂教学模式的不足，撰写教改论文，完善养羊学课程内容与模式。

2.3 具体实施计划

(1) 2019.8-2020.7: 收集资料, 优化现有养羊学教学大纲及教学内容; 制作“微课”资料; 设计翻转课堂模式, 制作教学课件。

(2) 2020.8-2021.7: 完成课件及相关教学资料的制作; 实践翻转课堂教学; 收集学生反馈意见并评估教学效果; 对养羊学课程内容及教学模式进行完善; 撰写教改论文。

2.4 可行性分析

(1) 本项目团队为南方现代草牧业(羊)产业技术体系创新团队成员, 均有多年从事草食动物科学与教育研究的工作经验, 主持过多项教改项目并获得过相关奖项。其中参与人刘德武教授为南方现代草牧业(羊)产业技术体系创新团队首席专家, 可为本项目实施提供宝贵的实践经验。

(2) 团队所在学院为“广东省草食动物产业联盟”牵头单位, 联盟内有多家养羊企业, 可为项目实施提供教学资料采集及实践提供保证。同时学院对教学研究的重视以及经费保障也有助于课题实施。

(3) 学校“教学在线”平台, 可作为“微课”应用和教学互动实施的平台; 同时目前信息技术迅速发展, 移动终端设备普及, 学生可随时随地访问“微课”教学平台进行自主学习。

(4) 该教学模式从学生角度出发, 容易在参与过程中体验到学习乐趣, 逐步提升学生的学习自觉性及独立学习能力, 为微课及翻转课堂的有效实施创造可能。

3. 项目预期成果及其实践运用预期（包括成果形式，预期推广、应用范围、受益面等）

- （1）编制新的《养羊学》课程教学大纲、讲义及教学课件；
- （2）制作一套《养羊学》“微课”教学资料；
- （3）形成一套《养羊学》翻转课堂教学模式；
- （4）撰写教改论文 1 篇。

4. 本项目的特色与创新点

（1）本项目结合现代养羊产业发展形势与人才需求，优化现有教学内容，通过“微课”及翻转课堂等先进教学手段，调动学生对养羊学学习的积极性，提高教学效果。

（2）利用“广东省草食动物产业联盟”及高校养羊专业联盟“领头羊计划”，增加学生参加生产实践及技能培训的机会，加深学生对养羊生产流程的认识，同时拓展教师的教学思路与资源。

四、项目建设基础

1. 与本项目有关的工作积累和已取得的工作成绩

本项目组成员均为我校动物科学学院动物健康养殖与安全生产专业教师，同时也是南方现代草牧业（羊）产业技术体系创新团队。团队现有教授 1 人，副教授 2 人，讲师 2 人，实验师 1 人，均有多年从事草食动物研究与教学的工作经验。其中参与人刘德武教授为南方现代草牧业（羊）产业技术体系创新团队首席专家，可为本项目实施提供宝贵的实践经验。本团队日前承担的本科生教学课程主要包括《养羊学》、《养牛学》、《畜牧学》及《动物生产学》。项目组成员已获得的教学成果及发表的教改论文如下：

- (1) 2019 华南农业大学教学成果一等奖，以实践创新能力为导向的动物生产类课程教学模式改革与实践，完成人：孙宝丽，刘德武，罗庆斌，李耀坤，邓铭，郭勇庆，柳广斌，付晓兰
- (2) 郭勇庆，邓铭，孙宝丽，李耀坤，柳广斌，刘德武. 草食动物生产学课程教学改革的探索[J]. 畜牧与饲料科学. 2019（已接收）
- (3) 李耀坤，郭勇庆，柳广斌，孙宝丽. 《养牛学》课程课堂教学改革与实践[J]. 科教文汇. 2019（已接收）
- (4) 邓铭，李佳颖，王涵沁. 高校学生社区党员志愿服务工作探析[J]. 山西青年. 2017(19): 189-190.
- (5) 孙宝丽，刘德武，谢青梅，罗庆斌，柳广斌. 动物科学专业动物生产教学团队建设的研究与实践. 黑龙江畜牧兽医, 2015（8）:261-262.
- (6) 孙宝丽，谢青梅，付晓兰，姚兵华. 以《养牛学》为例探索动物科学专业实践类课程教学方式改革. 黑龙江畜牧兽医, 2014（8）:234-235.
- (7) 孙宝丽，谢青梅，付晓兰，吴银宝，张永亮. 动物科学类专业实践教学体系的改革与实践. 家畜生态学报, 2014, 35（6）:91-93.
- (8) 孙宝丽. 《养兔学》课程教学改革的实践与探讨. 安徽农学通报, 2011, 17（10）:217-219.
- (9) 孙宝丽. 浅谈如何提高高校青年教师课堂教学质量. 教育教学论坛, 2012

(11) :31-32.

2. 单位对项目的支持情况 (含有关政策、经费及其使用管理机制、保障条件等, 可附有关文件), 尚缺少的条件和拟解决的途径

(1) 团队所在学院为“广东省草食动物产业联盟”牵头单位, 联盟内有多家养羊企业, 可为项目实施提供教学资料采集及实践提供保证。同时学院对教学研究的重视以及经费保障也有助于课题实施。

(2) 学校对教师在“微课”的建设方面给与重点支持, 多次提供校内外培训, 组织教师参加全国“微课”竞赛, 为教师进行“微课”相关的教学研究打下了坚实的基础。

(3) 校园硬件设施齐全, 如有多媒体教室、校园网络覆盖、学生拥有手提电脑、ipad、手机等现代化移动终端设备。此外, 学校“教学在线”的网络平台为“微课”的共享提供了高校、安全和便利平台, 能最大限度的实现教学资源共享。

3. 项目负责人和项目组成员所承担的教学改革和科研项目情况

(1) 教改项目:

1) 华南农业大学教改项目, 《草食动物生产学》教学过程和教学环节优化的研究与实践 (2018), 郭勇庆主持;

2) 华南农业大学教改项目, “微课”的翻转课堂在《养牛学》中的开发与应用 (2018), 李耀坤主持;

3) 广东省教学研究与改革项目, 动物生产教学团队建设的研究与实践 (2014), 孙宝丽主持;

4) 华南农业大学教改重点项目, 动物生产教学团队建设的研究与实践 (2014), 孙宝丽主持;

5) 华南农业大学教改项目, 以《养牛学》为例探索实践类专业课教学模式改革 (2012), 孙宝丽主持;

6) 华南农业大学教改项目,《养兔学》教学过程和教学环节优化的研究和实践(2010),孙宝丽主持;

7) 广东省教育厅教改项目,广州风行牛奶有限公司华南农业大学产学研教学实习基地(2014),孙宝丽主持;

8) 广东省教育厅教改项目,动物生产教学团队建设的研究与实践(2014),孙宝丽主持;

9) 华南农业大学教改项目,《普通畜牧学》教学过程和教学环节优化的研究和实践(2005),刘德武主持。

(2) 科研项目:

1) 国家自然科学基金,基于多组学技术研究典型日粮诱导奶牛乳脂合成抑制的分子机理(31872382),2018.08.16-2022.12.31,经费71.94万元,郭勇庆主持,柳广斌排第四;

2) 广东省自然科学基金,辣木多糖的分离纯化、结构鉴定及其免疫功能的调控机理研究(2017A030313158),2017.05.01-2020.05.01,经费10万元,孙宝丽主持;

3) 广东省省级科技计划项目(公益研究与能力建设),广东肉用黑山羊经济杂交配套方案开发(2017A020208050),2017.01.01-2019-12-31,经费15万元,柳广斌主持;

4) 广东省省级科技计划项目(公益研究与能力建设),广东雷州黑山羊快大高繁新品系培育(2017B020201014),2017.01.01-2019-12-31,经费50万元,刘德武主持,柳广斌排第二;

5) 广东省省级科技计划项目(公益研究与能力建设),广东湖羊高产耐热新品系多基因聚合育种技术开发与示范(2015A020209122),经费15万元,2015.07.01-2018.06.30,柳广斌主持;

6) 广东省农业厅,华南地区肉牛精粗饲料研发及推广应用,2016.09.30-2018.12.31,经费65万元,刘德武主持;

7) 广东省省级科技计划项目(公益研究与能力建设),广东湖羊高产耐热新品系

多基因聚合育种技术开发与示范（2015A020209122），经费15万元，2015.07.01-2018.06.30，柳广斌主持；

8) 广东省省级科技计划项目，辣木对荷斯坦奶牛生产性能和牛奶品质的影响研究（2016A020210083），2016.04.22-2017.12.31，经费15万，孙宝丽主持；

9) 广东省省级科技计划项目，黄梁木对乐至黑山羊饲用效果和肉品质的影响研究与应用示范（2014A020208104），2015.05.20-2016.12.31，经费20万，孙宝丽主持，柳广斌排第二。

五、经费预算

预算经费总额		4000 (元)	
序号	支出科目	预算	支出用途
1	设备费		
2	材料费（含打印费、耗材费）	1000	资料打印费用
3	图书资料费	1400	资料收集及购买费用
4	软件服务费（含课堂录像及网站制作）		
5	交通费	100	公交、地铁等交通费
6	差旅费	300	视频制作采集素材
7	会议费		
8	出版/文献/信息传播/知识产权事务费	1200	论文版面费
9	专家咨询费		
10	其他费用		

六、单位、评审小组及学校意见

所在单位意见:

同意

(公章)

单位负责人签字

2019年6月24日



评审小组意见:

同意立项

评审小组长签字:

2019年 月 日

陈平亮

学校主管部门意见:

同意立项



2019年6月24日

华南农业大学文件

华南农教〔2019〕61号

关于华南农业大学 2019 年度教育教学改革 与研究项目立项的通知

各学院、部处、各单位：

为进一步推进我校教育教学改革与研究，提高人才培养质量，根据《教育部关于加快建设高水平本科教育全面提高人才培养能力的意见》（教高〔2018〕2号）精神，学校组织开展了2019年度教育教学改革与研究项目申报工作。经组织申报、资格审查、专家评审、公示等环节，确定“马克思主义情景剧与原理课实践教学改革研究”等154项校级教育教学改革与研究项目予以立项。其中，针对学校本科人才培养改革重点领域，本年度特设思想政治理论课专项10项，课程思政专项11项，青年专项41项，金课改革专项33项。具体立项和资助情况详见附件。

请各项目负责人务必按项目计划要求及时开展研究工作，确

保研究工作如期完成，并力争取得高水平的研究成果。

附件：华南农业大学 2019 年度教育教学改革与研究项目一览表



（联系人：曹广祥、伍鹏，电话：85280052）

公开方式：主动公开

华南农业大学校长办公室

2019 年 9 月 29 日印发

	—以《旅游市场营销》课程为例			
JG19067	“线上+线下”的遗传学实验混合教学模式探索研究	郭海滨	金课改革	一般资助
JG19068	基于慕课的《林木育种学》混合式教学方法探索	骈瑞琪	金课改革	一般资助
JG19069	《养羊学》课程内容优化及微课、翻转课堂在教学实践中的应用研究	柳广斌	金课改革	一般资助
JG19070	基于“线上”+“线下”的《生物信息学》课程混合式教学模式研究与实践	王波	金课改革	一般资助
JG19071	“金课”视域下城乡规划专业中外建筑史混合式教学模式探索与实践	吴宝娜	金课改革	一般资助
JG19072	基于交互式在线教育平台应用的混合教学模式探究--以文献检索课为例	刘洋	金课改革	一般资助
JG19073	“互联网+”翻转课堂的智慧微学习空间建设研究——以华南农业大学为例	江晓庆	金课改革	一般资助
JG19074	新工科背景下高等数学混合式金课的研究与实践	刘丹	金课改革	一般资助
JG19075	“翻转+对分”混合教学模式研究--以《跨文化交际》课程为例	钟建玲	金课改革	一般资助
JG19076	《动物解剖学-感觉器官》章节基于雨课堂翻转教学模式设计与实践	许丹	金课改革	一般资助
JG19077	以打造“金课”为导向促进教师教学能力提升的探索和实践	钟建英	金课改革	自筹
JG19078	项目驱动下统计学专业实践混合式教学的创新研究	周燕	金课改革	自筹
JG19079	翻译技术辅助大学英语翻译混合式教学模式有效性研究	李志英	金课改革	自筹
JG19080	基于 SPOC “农业植物病理学实习”线上线下混合式教学的创新实践	纪春艳	金课改革	自筹
JG19081	基于翻转课堂的高校图书馆信息素养教育模式研究	关磊	金课改革	自筹
JG19082	线上线下混合式教学模式在法学专业课程中的应用研究	李福芹	金课改革	自筹
JG19083	基于翻转课堂的细胞生物学综合性实验教学改革	彭海峰	金课改革	自筹
JG19084	“雨课堂”混合式教学工具在兽医外科手术学教学中的应用研究	周沛	金课改革	自筹
JG19085	基于能力培养的多层级探究式教学改革研究及实践--以《土木工程材料》为例	李庚英		重点资助
JG19086	新工科背景下多维度提升工科高数课程教学质量的研究与实践	李娇娇		重点资助
JG19087	以科研成果促进遗传学实验教学创新改革的研究与实践	刘自强		重点资助

关于公布华南农业大学 2021 年度 校级质量工程暨教改项目验收结果的通知

各相关单位、各项目负责人：

根据学校《关于开展 2021 年校级质量工程暨教改项目结题验收工作的通知》要求，本科生院组织开展了校级质量工程暨教改项目校内结题验收工作。经项目负责人及所在单位组织专家论证、学校组织专家审议、校园网公示等程序，现将我校 2021 年度结题验收结果予以公布（详见附件）。

学校按照《华南农业大学本科教学质量与教学改革工程项目建设管理办法》（华南农办〔2011〕46 号）等相关文件要求，加强项目指导与过程管理。请各单位高度重视教学研究与教学改革工作，指导、督促立项项目按期、高质量地完成建设任务。通过验收的项目，请进一步总结项目经验和成果，做好成果的推广和应用，不断推动本科教学改革和建设。延期结题的项目，应加快项目建设进度，确保实现预期成果并在下一年度完成验收。

附件：2021 年度校级质量工程暨教改项目验收结果



序号	项目类别	项目名称	负责人	所在单位	验收结果	备注
54	教学研究与改革项目	基于雨课堂及精品网课资源的农业院校公共有机化学混合式教学改革	丁唯嘉	材料与能源学院	验收通过	
55	教学研究与改革项目	基于国际标准培养木材科学与工程新工科专业特色人才的研究与实践	古今	材料与能源学院	验收通过	
56	教学研究与改革项目	新工科背景下化工原理虚拟仿真实验教学改革与实践	袁腾	材料与能源学院	验收通过	
57	教学研究与改革项目	课程育人与科研育人相结合——《药物分析》研究性教学探索	林雅铃	材料与能源学院	验收通过	
58	教学研究与改革项目	基于“互联网+”技术在基础化学实验教学的改革与实践	肖勇	材料与能源学院	验收通过	
59	教学研究与改革项目	基于速课微课堂教学平台的物理化学教学难点突破研究	陈明洁	材料与能源学院	验收通过	
60	教学研究与改革项目	校企合作背景下材料化学专业人才培养模式探讨	张超群	材料与能源学院	验收通过	
61	教学研究与改革项目	多媒体网络技术在基础化学实验教学中的应用探索	唐小兰	材料与能源学院	验收通过	
62	教学研究与改革项目	结合从业上岗证培训的“实验动物学”课程的改革与实践	余文兰	测试中心（实验动物中心）	验收通过	
63	教学研究与改革项目	基于能力结构的卓越农林教师教学发展模式研究	朱蕾	党委教师工作部、人力资源处	验收通过	
64	教学研究与改革项目	高校创新创业教育的慕课体系建设研究	曾璇	党委学生工作部（党委研究生工作部）	验收通过	
65	教学研究与改革项目	《舞蹈鉴赏》课程中“艺术+思政”的改革探索与实践研究	殷舒	党委学生工作部（党委研究生工作部）	验收通过	
66	教学研究与改革项目	艺术类专业人才培养的创新创业教育改革研究	陈志远	党委学生工作部（党委研究生工作部）	验收通过	
67	教学研究与改革项目	基于人工智能背景下的教学教务管理信息化探索与研究	王宣琳	党政办公室（研究室）	暂缓通过	延迟结题
68	教学研究与改革项目	知识传授、文化传承、价值引领三位一体的课程思政教学改革与创新——以大学物理基础课程为例	徐初东	电子工程学院（人工智能学院）	验收通过	
69	教学研究与改革项目	《数字电子技术》课程思政建设研究	赵文锋	电子工程学院（人工智能学院）	验收通过	
70	教学研究与改革项目	可编程器件原理与应用实验在线课程建设与应用	徐海涛	电子工程学院（人工智能学院）	验收通过	
71	教学研究与改革项目	《物联网技术导论》课程校企协同育人机制创新实践研究	贾维卿	电子工程学院（人工智能学院）	验收通过	
72	教学研究与改革项目	结合企业实训的实践教学体系的创新与研究	徐梅宣	电子工程学院（人工智能学院）	验收通过	
73	教学研究与改革项目	新工科背景下通信工程专业多方协同育人模式改革与实践	俞龙	电子工程学院（人工智能学院）	验收通过	
74	教学研究与改革项目	《养羊学》课程内容优化及微课、翻转课堂在教学实践中的应用研究	柳广斌	动物科学学院	验收通过	
75	教学研究与改革项目	基于蚕病学课程的全英教学和管理模式研究	孙京臣	动物科学学院	验收通过	
76	教学研究与改革项目	在生物化学课程中构建低年级大学生研究性学习引导机制研究	孙加节	动物科学学院	验收通过	
77	教学研究与改革项目	学分制模式下动物科学专业人才培养方案的修订	吴银宝	动物科学学院	验收通过	
78	教学研究与改革项目	新时代背景下产业学院本科生实践能力提升机制研究——以华南农大一温氏集团产业学院为例	何小敏	动物科学学院	验收通过	
79	教学研究与改革项目	通过丝绸文化课程提升大学生对中华文化自信	陈芳艳	动物科学学院	验收通过	

检索证明

根据委托人提供的论文材料，委托人华南农业大学动物科学学院 柳广斌 2 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者工作单位	收录情况	影响因子	中科院大类分区
1	基于“项目驱动”的养羊课程教学改革与实践	草业科学 出版年：2024 卷期：41 9 页码：2197-2205 文献号： 文献类型：期刊论文	第一作者	C 类	华南农业大学 动物科学学院	北大核心	无	无
2	华南地区高等农林院校养羊学课程改革探索	出版年：2020 卷期：13 29 页码：18-19 文献号： 文献类型：期刊论文	第一作者	普刊类	华南农业大学 动物科学学院	CNKI	无	无

说明：论文等级和中科院大类分区按《华南农业大学学术学位论文评价方案（试行）》划分。

报告免责声明：如未盖章，报告无效



中文核心期刊
中国科学引文数据库(CSCD)核心期刊
英国CABI文摘数据库来源期刊
中国科技核心期刊
中国精品科技期刊

ISSN 1001-0629
CN 62-1069/S



草业科学

PRATACULTURAL SCIENCE

第41卷第9期 2024年9月



中国草学会 主办
兰州大学草地农业科技学院

草业科学

PRATACULTURAL SCIENCE

Sep. 2024 Vol.41 No.9 1979-2206

草业科学

CAOYE KEXUE

第 41 卷第 9 期 (总第 374 期)

2024 年 9 月

目 次

前植物生产层

- 1979 科尔沁沙地土壤碳储量插值计算对比及其影响因素分析 张钰清 陈银萍 程红元 王旭洋 陈 云 李玉强
- 1991 不同改良方式协同黑麦草对煤矸石堆场生态修复效果 卢雨霞 朱 健 曾 维 毛文件 郑汝意 王维维 范菲菲
- 2003 窝圈距离影响高寒草甸植物群落结构和土壤养分空间分布 边金霞 牛小霞 陈 娟 徐 祎 才让吉 马继红 吴昌顺 王银花
- 2012 优势物种对内蒙古典型草原营养物质生产的决定性影响 王若冰 王永慧 赵宏宇 张 斌
- 2025 黄土高原粮草复合生态系统蒸散发变化动态及其与干旱的关系 李佳璇 李辉鹏 白 璐 张金贵 王若冰 向 前 吕 陇 马景永

植物生产层

- 2035 黍子 *SRS* 基因家族的全基因组鉴定与表达分析 李佳颖 杨梦珂 宋晓涵 王 月 王禹茜 王世纪 张美春 邓 欣 王红艳
- 2046 贮藏年限对不同居群白羊草种胚细胞抗氧化特性的影响 赵奥然 曾 佳 夏方山 李晓波 朱梦馨 王 勃 李尹琳
- 2054 $^{60}\text{Co-}\gamma$ 射线辐照对紫云英生长及利用的影响 庾振宇 张江林 高雅洁 廖育林 鲁艳红 方畅宇 刘芳禧 谢 雪 肖 健 袁浩亮 屠乃美 聂 军
- 2063 荒漠草原 4 种优势植物叶片化学计量内稳性特征 霍新茹 沈 艳 马红彬 王国会 陆 颖 洪乐乐 张小菊 胡 艳 马登宝 李 东
- 2072 青海海北高寒草地 12 种植物花期物候对氮磷养分添加的响应 李永慧 袁学风 程 蓉 李宏林 武学霞 任 飞 李希来 李兰平
- 2084 灵武白芨滩柠条固沙林演替过程中林下草本植物多样性特征 于 双 李小伟 杨学霞 王瑞霞 杨君琬 马 龙
- 2094 土壤水分和种植密度对紫花苜蓿生物量分配的影响 蒋 朝 吴征江 李春阳 张振洲 武文莉 刘慧霞

2104 机理模型与遥感数据同化的草地生物量估算相关研究 王彩玲 王一鸣

动物生产层

2118 发酵苹果渣全混合日粮对肉牛育肥性能和胃肠道细菌组成的影响 朱晋佳 陈 梁 杨国杰 马志远 李 飞 郭旭生 李福厚

后生物生产层

2133 农牧交错带施氮条件下大豆和玉米间作产量及其混合青贮品质 丁 婉 邢宝龙 冯 钰

2143 饲用小黑麦与豆科作物混作生产性能及饲用品质分析 尚俊秀 姚晓华 谢德庆 姚有华

2155 添加蒲公英对苜蓿青贮品质及抗氧化活性的影响 马成艳 周小栋 马小婧 黄 帅

2164 吐鲁番市土地利用转型及生态服务功能交叉敏感性 冶建明 谢斯琦

2181 乌兰察布市草原景区旅游气候舒适度评价 陈海英 安莉娟 代海燕 李瑞青 李 多 李忆平 皇 彦

2190 奶牛生产国家在线精品课程建设与实践 牛华锋 谢晓刚 白 军 郭亚洲 赵永攀 李骁勇

2197 基于“项目驱动”的养羊学课程教学改革与实践 柳广斌 刘德武 李耀坤 郭勇庆 邓 铭 孙宝丽

封面照片：封面照片为典型草原，由内蒙古大学生态与环境学院王若冰拍摄于内蒙古锡林郭勒盟锡林浩特。

期刊基本参数: CN 62-1069/S * 1984 * m * A4 * 228 * zh * P * ¥ 21.00 * 4000 * 21 * 2024-09 * n

DOI: 10.11829/j.issn.1001-0629.2024-0274

柳广斌, 刘德武, 李耀坤, 郭勇庆, 邓铭, 孙宝丽. 基于“项目驱动”的养羊学课程教学改革与实践. 草业科学, 2024, 41(9): 2197-2205.

LIU G B, LIU D W, LI Y K, GUO Y Q, DENG M, SUN B L. “Project-driven” pedagogical reform and implementation in sheep and goat production course. Pratacultural Science, 2024, 41(9): 2197-2205.

基于“项目驱动”的养羊学课程教学改革与实践

柳广斌, 刘德武, 李耀坤, 郭勇庆, 邓 铭, 孙宝丽

(华南农业大学动物科学学院, 广东 广州 510642)

摘要: 养羊学是一门面向动物科学专业学生的专业课, 旨在培养符合现代养羊业发展需要的“强农兴农”人才。针对课程存在的教学模式陈旧、教学内容与产业脱节与社会建设融入不足等痛点问题, 华南农业大学动物科学学院围绕“立德树人”及新农科人才培养理念, 以学生发展为中心建立“项目驱动”教学模式。该模式通过实际项目驱动学生主动探索与学习, 使学生能够在项目中将理论知识应用于实践, 提升综合能力和创新思维。项目驱动教学模式强调产教融合, 并深化乡村振兴及生态文明理念融入, 提供全面的知识、能力及素养培养支撑。通过改革, 养羊学课程对学生综合素质培养的效果显著提升, 成效包括近 5 年指导的本科生发表论文 6 篇, 参加创新创业训练项目 5 项及创新创业竞赛 5 项, 并有毕业生在养羊业创业。建设产学研平台及实践教学基地 5 个, 促进了校企合作, 进一步完善了课程建设。养羊学课程的“项目驱动”教学模式可为动物生产类及与产业衔接较紧密的专业课程的教学提供参考。

关键词: 教学模式; 全过程考核; 产教融合; 教师发展; 高阶性; 创新性; 挑战度

文献标识码: A 文章编号: 1001-0629(2024)09-2197-09

“Project-driven” pedagogical reform and implementation in sheep and goat production course

LIU Guangbin, LIU Dewu, LI Yaokun, GUO Yongqing, DENG Ming, SUN Baoli

(College of Animal Science, South China Agricultural University, Guangzhou 510642, Guangdong, China)

Abstract: Sheep and Goat Production course is a specialized course for students majoring in animal science, and it aimed at cultivating talents to meet the needs of the modern sheep farming industry. To address issues such as outdated teaching models, a disconnect between teaching content and industry needs, and insufficient integration with social development, the College of Animal Sciences at South China Agricultural University has established a project-driven teaching model centered on student development, which aligned with the philosophy of fostering moral integrity and training new agricultural talents. This model drives students to actively explore and learn through real projects, enabling them to apply theoretical knowledge in practice and enhance their comprehensive abilities and innovative thinking. The project-driven teaching model emphasizes the integration of education and industry and deepens the incorporation of rural revitalization and ecological civilization concepts, providing comprehensive support for the development of knowledge, skills, and qualities. Through this reform, the effectiveness of the Sheep and Goat Production course in cultivating the comprehensive qualities of students has been significantly enhanced. In the past five years, the outcomes comprise guiding of undergraduate students to publish six papers, participate in five innovation and entrepreneurship training projects, and compete in five innovation and entrepreneurship

收稿日期: 2024-05-05 接受日期: 2024-07-09

基金项目: 广东省本科高校教学质量与教学改革工程项目 (粤教高函 (2024) 9 号); 广东省本科高校教学质量与教学改革工程项目 (粤教高函 (2023) 4 号); 华南农业大学课程思政示范项目 (“养羊学”思政示范课堂)

第一作者: 柳广斌 (1982-), 男, 山东莱西人, 讲师, 博士, 研究方向为草食动物生产与管理. E-mail: gbliu@scau.edu.cn

通信作者: 孙宝丽 (1981-), 女, 河南许昌人, 教授, 博士, 研究方向为动物营养与免疫. E-mail: baolisun@scau.edu.cn

<http://cykx.lzu.edu.cn>

competitions. Additionally, some graduates have started their own businesses in the sheep and goat farming industry. The establishment of five industry-university-research platforms and practical teaching bases has promoted school-enterprise cooperation, further improving course development. The project-driven teaching model for the Sheep and Goat Production course can serve as a reference for the teaching of other animal production-related courses that are closely connected to the industry.

Keywords: teaching model; comprehensive assessment; industry-education integration; teacher development; advances; innovation; challenge

Corresponding author: SUN Baoli E-mail: baolisun@scau.edu.cn

养羊学是华南农业大学面向动物科学专业本科三年级学生开设的专业课,对培养适应现代畜牧业需求的专业人才、推动农业绿色可持续发展以及实施国家乡村振兴战略具有重要作用^[1]。课程在前课基础上,进一步针对畜牧业中的养羊生产进行系统讲解,内容包括养羊业的发展,羊的产品、品种、生物学及行为特征、营养与饲料技术、遗传育种及繁殖技术,各类型羊饲养管理技术,羊场建设与规划、疫病防控及环境保护、经营管理等相关知识。养羊学课程旨在帮助学生掌握养羊生产的专业理论知识和实践技能,同时培养其创新思维、问题解决能力及对乡村振兴、生态文明和智慧养殖等理念的深刻理解,为养羊业可持续发展培养高素质专业人才^[2-3]。随着现代农业生产技术的快速发展,对人才综合素质有了更高需求,然而,传统教学方式存在过于依赖理论灌输、缺乏实践应用和创新思维的培养等问题。因此及时对养羊学课程进行教学改革,使其教学模式和内容符合现代养羊产业发展的需要,是十分有必要的^[4]。

1 养羊学课程存在的问题

经调研和学情分析发现,大学三年级学生已具备良好的知识基础和学习能力,并且思维活跃喜欢挑战,展现出创造性思维的潜力。然而,他们在主动学习和知识运用方面仍显不足。为充分挖掘学生潜力,需要调整课程模式,通过引导学生主动探索和自主学习,培养跨学科整合能力和创新思维,并增强合作意识和解决实际问题的能力,提升学生的综合素质,使他们更好地适应现代养羊业的发展需求,并为未来的职业发展和学术研究奠定坚实基础。结合课程学情分析,养羊学课程有以下三方面问题需要进行改进。

1.1 教学模式无法满足学生综合素质培养的需要

传统教学方式主要依赖讲授和理论灌输,学生在课程中的参与度不高,缺乏主动探索和深入学习的动力,缺少将所学知识应用于解决实际问题的思考,难以达到创新思维培养的目的。例如,课程中关于羊的营养与饲料技术的内容仅停留在书本知识上,缺乏为羊场因地制宜地选择饲料原料并进行配方设计的体验。再如,在讨论羊疫病防控策略时,学生更多是被动接受信息,而不是主动参与讨论和提出解决方案。此外,课程的考核方式仍是期末考试,无法全面评估学生的综合能力,也缺少激励学生持续投入学习的机制,比如在平时成绩中难以体现学生在团队合作和创新能力方面的表现。

1.2 教学内容与养羊产业发展存在脱节现象

课程内容更新不够及时,未能很好地体现科技前沿与学科交叉等内容,包括生物技术和智慧养殖与养羊业结合等前沿领域,学生难以接触到最新的科研成果和行业动态。例如,课程在讲解羊的遗传育种技术时,未能深入介绍基因编辑技术和基因组选择技术在羊上研究与应用的最新进展等。其次课程没有很好地利用产学研平台资源,学生实践只限于少数技术环节,没有深入参与到羊场的整体运作流程,未能充分发挥合作企业协同育人的优势,影响学生对产业认知的提升及创新创业能力的培养。此外青年教师缺乏养羊生产实践经验,可能导致教学内容的实用性和针对性降低,影响人才培养质量,使得毕业生难以满足养羊产业的发展需求。

1.3 课程对乡村振兴和生态文明建设融入不足

乡村振兴和生态文明建设是推动社会经济的全面发展,同时保持生态环境可持续性的必要条件,也是新农科建设人才培养的重要内容。养羊产业在

乡村振兴和生态文明方面发挥着重要作用,然而养羊学课程在这些内容的融入上有所不足,可能导致学生对实际应用理解不深。例如,在养羊产业与乡村振兴关系方面,缺乏对如何通过养羊业促进当地经济发展的深入思考与讨论,未能让学生真正理解养羊业对农村发展的影响和推动作用。这种不足限制了学生在未来工作中将乡村振兴和生态文明理念融入实际养羊生产中的能力。

2 养羊学课程改革目标

针对养羊学课程存在的问题,华南农业大学动物科学学院围绕“立德树人”及新农科人才培养理念,依托校企合作产学研平台,以学生发展为中心,开展养羊学课程的教学改革,以期达到以下改革目标^[5-7]:

1) 调整养羊学课程教学模式,提升学生知识综合运用和主动学习能力。通过建立项目驱动教学的教学模式改革,减少对讲授和理论灌输的依赖,引导学生主动探索和解决实际问题,培养独立学习和知识综合运用能力。鼓励学生积极参与课堂讨论和项目实践,利用多样化的教学手段如翻转课堂和案例分析,增强学习的主动性和积极性。

2) 更新教学内容,强化实践教学,培养学生创新思维和创新创业能力。将生物技术、智慧养殖等最新科研成果和行业动态纳入课程内容,确保学生掌握现代养羊业所需的最新知识和技术,培养创新思维。增加实践教学环节,利用产学研平台资源,提供更多的实践案例和实践机会,增强学生的产业

认知和创新创业能力,提升综合素质。

3) 融入乡村振兴和生态文明教育,增强学生社会责任感。在课程中全面融入乡村振兴和生态文明等重要思想理念,通过实际案例和项目学习,使学生增强社会责任感,在未来工作中能够将乡村振兴和生态文明理念融入实际养羊生产,推动养羊业的绿色发展。

3 养羊学课程改革具体举措

通过改革的实施,课程将从知识、能力及素养3个维度提升学生的综合素质,为养羊业培养具备专业基础知识、创新创业能力及社会责任感的“强农兴农”高素质人才(图1)。

3.1 建立以学生发展为中心的“项目驱动”教学模式,完善多元化评价体系

“项目驱动”是一种以实际项目为导向的教学方法,其核心在于通过实际任务和项目,引导学生在主动参与、合作探究、实际操作和反思评估中全面发展知识、能力和素养。它不仅关注学习的结果,更重视学习的过程,通过多样化的教学策略和评估方式,培养学生的综合素质和终身学习能力。通过这种方法,学生可以在真实情境中应用所学知识,解决实际问题,提升创新思维和问题解决能力,并增强跨学科整合和团队合作能力,使他们能够更好地适应和应对现实世界的挑战。养羊学课程主要以项目为导向,通过重构教学内容、优化课堂教学设计以及多元化全过程考核,建立项目驱动的教学模式。

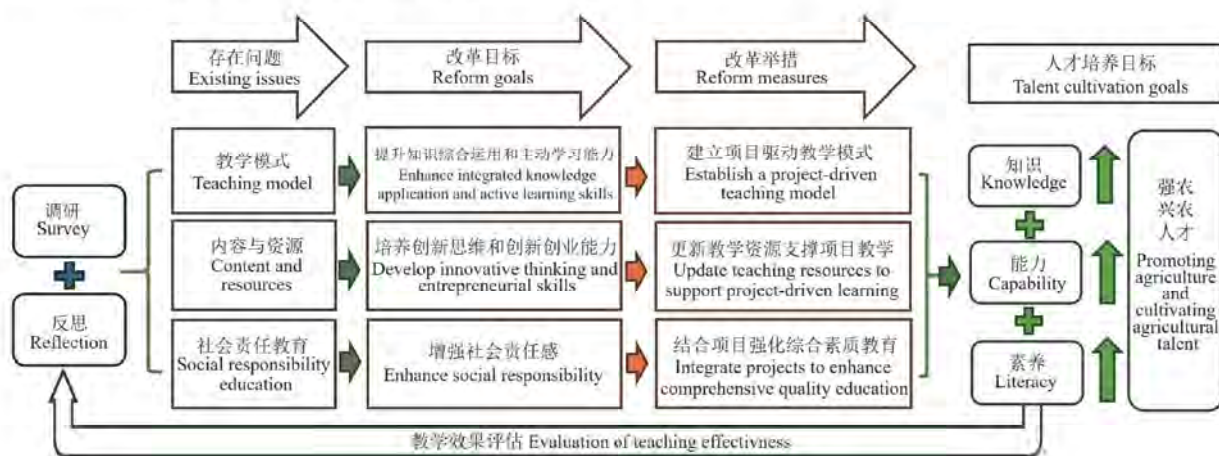


图1 养羊学课程改革思路

Figure 1 Reform ideas for sheep and goat production course

<http://cykx.lzu.edu.cn>

3.1.1 以项目为导向重构教学内容，优化教学设计，增强课程高阶性

以规划教材为基础(以姜勋平、刘桂琼主编的《羊生产学》教材为例)，根据养羊产业链环节，将课程内容划分7个项目内容(图2)。每个项目分4个阶段，从实际产业问题出发设置项目目标与内容，学生通过理论知识学习及探索思考形成解决方案，再利用翻转课堂、小组讨论提问、教师点评指导等方法进行成果展示并对方案进行进一步完善，最终完成相对完善的项目报告(图3)^[8]。通过项目的实施，提高学生的课程参与度和主动性，加强学生对

养羊产业的系统性认知，培养学生发现及解决问题的能力，更好地实现课程的高阶性。

此外，课程利用在线 MOOC 平台、雨课堂、微信及 QQ 群、养羊产业信息网、AI 智能体、牧场模拟软件及公司大数据平台等信息化软件，辅助线上+线下及课内+课外的教师教学及学生学习活动，从而提高教学效率，促进持续性学习。

3.1.2 多元化评价体系，实现全过程考核，提升课程挑战度

建立覆盖知识、能力及素质3个维度的评价考核体系，包含课上课下、课内课外的全部教学活动，

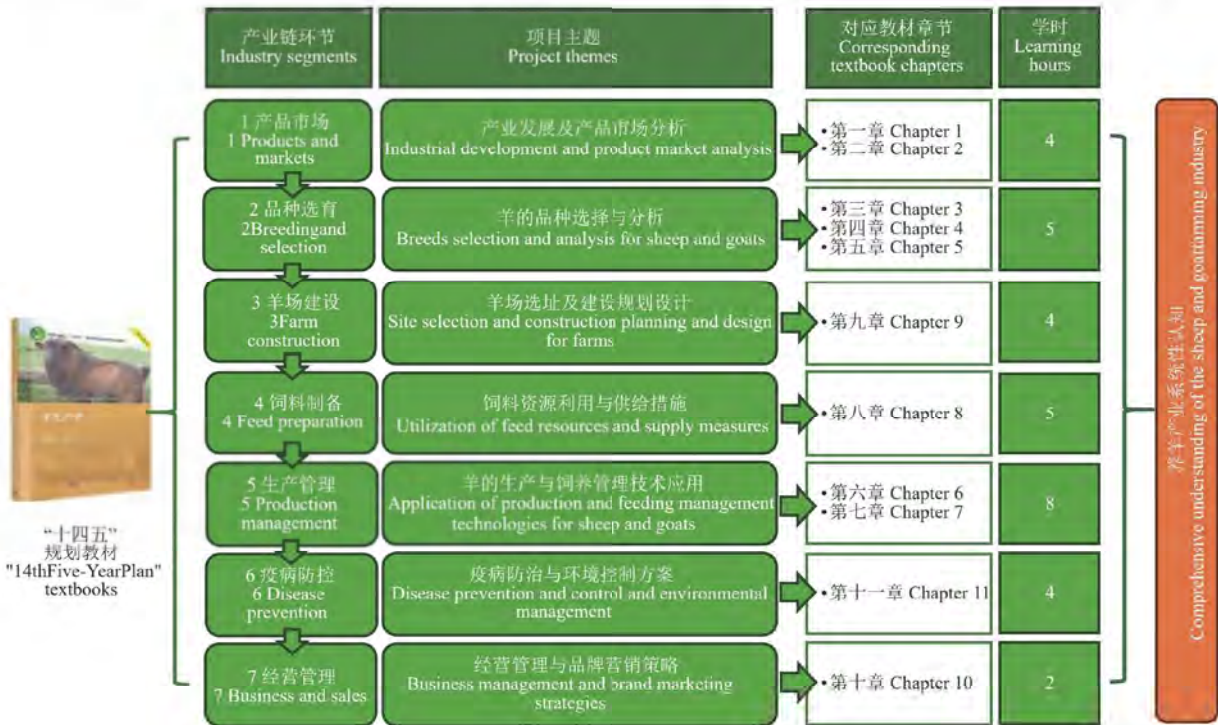


图 2 养羊学课程“项目驱动”教学内容安排

Figure 2 “Project-driven” teaching content arrangement for sheep and goat production course

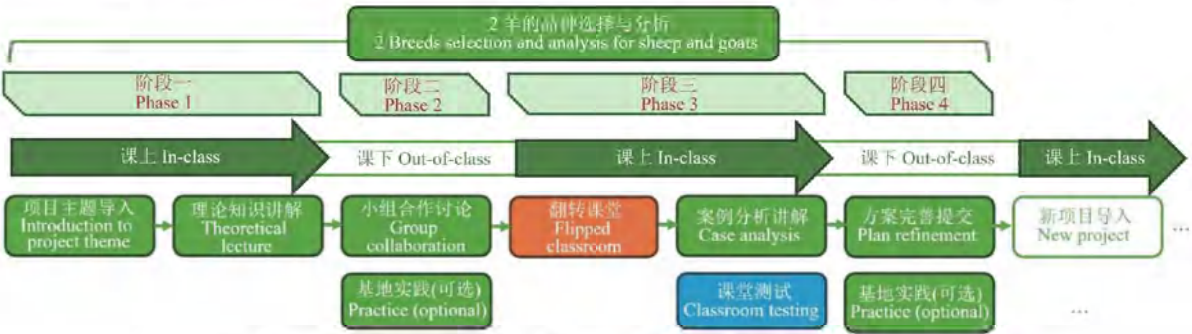


图 3 以“项目二：羊的品种选择与分析”为例展示项目驱动教学流程

Figure 3 Using “project 2: sheep breed selection and analysis” as an example to demonstrate the “project-driven” teaching process

全面公平并有效地评价学生的综合能力^[9]。同时包含必选和可选考核项目,满足个性化学习目标需求(图4)。评估利用“雨课堂”等线上教学工具提高过程评价的效率,减少教师评估工作负担,增强评估反馈的及时性,帮助教师掌握学生学习进度并改进教学方法。多元化的评价体系不仅提高教学质量,还能激发学生的学习动力,促进其全面发展。

3.2 更新教学内容,建设产学研平台,为项目驱动教学提供丰富的教学资源

3.2.1 结合项目主题建立实践案例及视频库,拓展科技前沿内容,提升课程创新性

围绕养羊业发展实际情况,结合各项目主题,定期更新课程内容,特别是将生物技术、智慧养殖技术、可持续发展实践等科技前沿与产业发展趋势内容融入教学^[10]。对于实践性较强的内容,采用案例分析及制作教学视频等方式提高学生对生产实践的理解(图5)。通过案例与视频增强课程的实用性和吸引力,帮助学生紧跟行业发展,提高其适应未来产业需求的能力。

3.2.2 建设实践教学基地,完善实践教学条件

与当地养羊企业开展产学研合作,如广东领头羊智慧农业有限公司、雷州市状元黑山羊养殖有限公司等,共同建设山羊“科技小院”“永根科技站”等

产学研平台及实践教学基地。实践基地条件完善,配有实验室,可支持本科生与研究生一起开展项目研究,并定期聘请产业专家和企业导师进行指导,为养羊学课程提供实践教学条件。同时课程开放多种实践教学形式,满足学生个性化实践学习需求,包括生产实践课程、假期实习以及参加创新创业竞赛(图6),为学生课外的持续性学习需要提供支持^[11-13]。产学研平台及实践教学基地建设不仅能丰富教学资源,还可增强学生的实践与产业适应能力,促进校企共同发展。

3.2.3 加强教师生产实践培养,增加产业服务经验,促进教师全方位发展

养羊学课程作为与生产实践联系较为紧密的专业课程,不仅要求教师教学及科研能力的发展,同时需要重视教师生产实践能力的培养。华南农业大学动物科学学院养羊学教学团队教师通过参加农业科技特派员、养羊技术推广项目以及企业合作派遣等方式,开展产业服务工作,积累生产实践经验,并将实践经验服务于教学与科研工作,促进教师能力的全方位发展(图7)。

3.3 结合项目驱动教学强化综合素质教育

课程结合项目驱动教学模式,围绕项目主题挖掘与之契合的素质教育元素,并通过案例提高学生



图4 养羊学课程多元化评价体系

Figure 4 Diversified evaluation system for sheep and goat production course

<http://cykx.lzu.edu.cn>

各项目重点教学内容 Key teaching content for each project		案例举例 Case examples
1	产品特点 Product features	地区文化对养羊产业的发展影响 The influence of regional culture on the industry
	品种特征 Breed characteristics	地方品种与引进品种的比较案例 Comparison case between local breeds and introduced breeds
2	育种技术 Breeding methods	全基因组选择分子育种技术前沿 Advancements in whole genome selection breeding technology
	习性与饲养模式 Habits and feeding models	光伏养羊及智慧养殖羊场 Case study of photovoltaic farming and smart farming
3	羊场规划 Farm planning	规模化湖羊养殖场建设案例 Case study of large-scale Hu Sheep farm construction
	消化生理 Digestive physiology	微生物等生物技术前沿 Advancements in biotechnology such as microbial ecology
4	资源利用 Resource utilization	“种养循环”养殖模式案例 Case study of "integrated farming" livestock farming model
	繁殖生理 Reproductive physiology	现代繁殖技术前沿 Advancements in modern breeding technologies
5	饲养管理 Rearing management	标准化羊场生产计划案例 Case study of standardized sheep and goat farm production plan
	疫病防治 Disease prevention	山羊及绵羊常见病案例 Common disease cases in goats and sheep
6	粪污处理 Manure treatment	羊场粪污处理设备及实践案例 Manure treatment equipment and practical cases
	信息管理 Information management	企业大数据数字化平台案例 Case study of enterprise big data digitalization platform
7	品牌营销 Brand marketing	羊奶及羊肉产品品牌建设案例 Case study of brand building for goat milk and meat products

图 5 养羊学课程各项目重点教学内容案例举例

Figure 5 Examples of key teaching content for each project in the sheep and goat production course

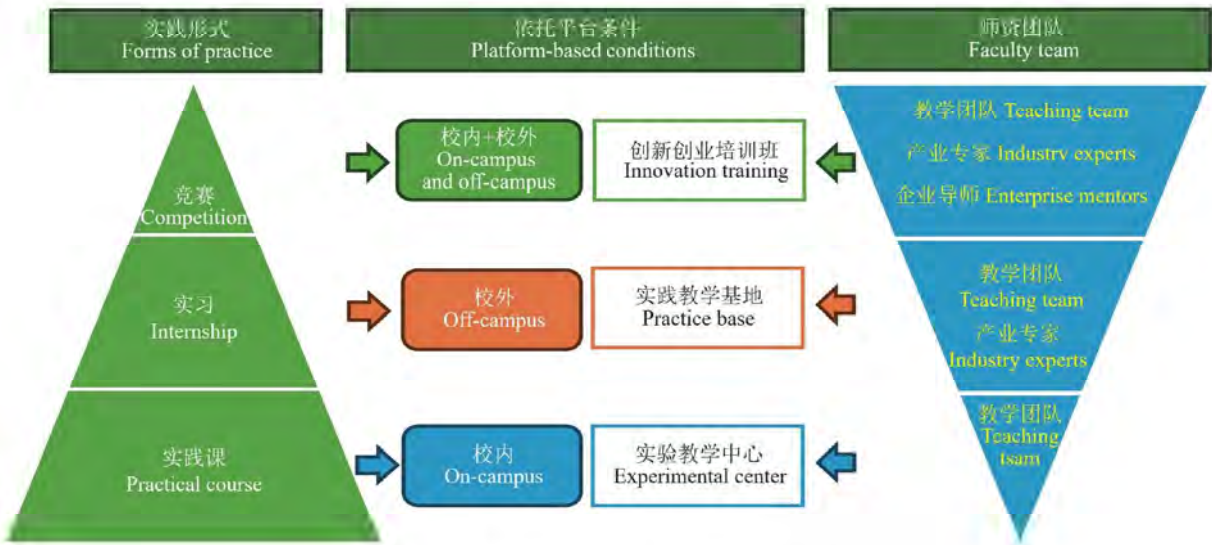


图 6 养羊学课程实践教学形式

Figure 6 Practical teaching methods in the sheep and goat production course

<http://cykx.lzu.edu.cn>



图7 养羊学课程教师能力全方位发展思路

Figure 7 Comprehensive development strategy for teachers in the sheep and goat production course

对乡村振兴及生态文明等理念的理解,完善对学生综合素质的培养(图8)^[14]。养羊学课程可从以下几个方面进行融入:

3.3.1 乡村振兴与社会责任

课程内容紧密结合乡村振兴,通过设计与乡村发展相关的项目,如“回到自己家乡通过养羊创业你会选择什么品种?”这样的项目背景设定,提高学生情感带入,达到情景式教学效果。并让学生在解决实际问题的过程中深刻理解农村社区发展的重要性,参与到乡村振兴实践中。同时,通过知名科

学家事迹及畜牧业法规的案例分享,提升学生的社会责任感和职业素养。这种方法不仅可培养学生的社会责任感,还能激发对农业和社会的热情,培养学生成为“知农爱农、强农兴农”的高素质畜牧人才。

3.3.2 生态文明与可持续发展

课程深度强调生态文明和可持续发展的重要性,特别是在绿色农业和乡村发展中的应用。通过实际产业案例,如将羊粪发酵成有机肥的“种养循环”养殖模式,以及将太阳能产业与养羊业相结合的“光农互补”养殖模式,增强学生的环境保护意识,并指导学生在项目中学习如何实现农业与环境的和谐共生。这种方式不仅可提高学生的环保意识,还能促使学生在未来的职业生涯中推动可持续农业的发展。

3.3.3 科技前沿与创新思维

课程内容紧密结合养羊产业的实际需求,引入科技前沿知识,培养学生的创新思维。在项目教学过程中,通过实践案例分析、视频展示和基地实习等方式,让学生接触最新的生物、信息和智慧养殖技术,激发他们探索和应用这些技术的兴趣。例如,通过分子育种技术和数字化信息管理系统在养羊业中的应用等,帮助学生在项目教学过程中将理论知识与实际应用相结合,提升他们的创新能力和

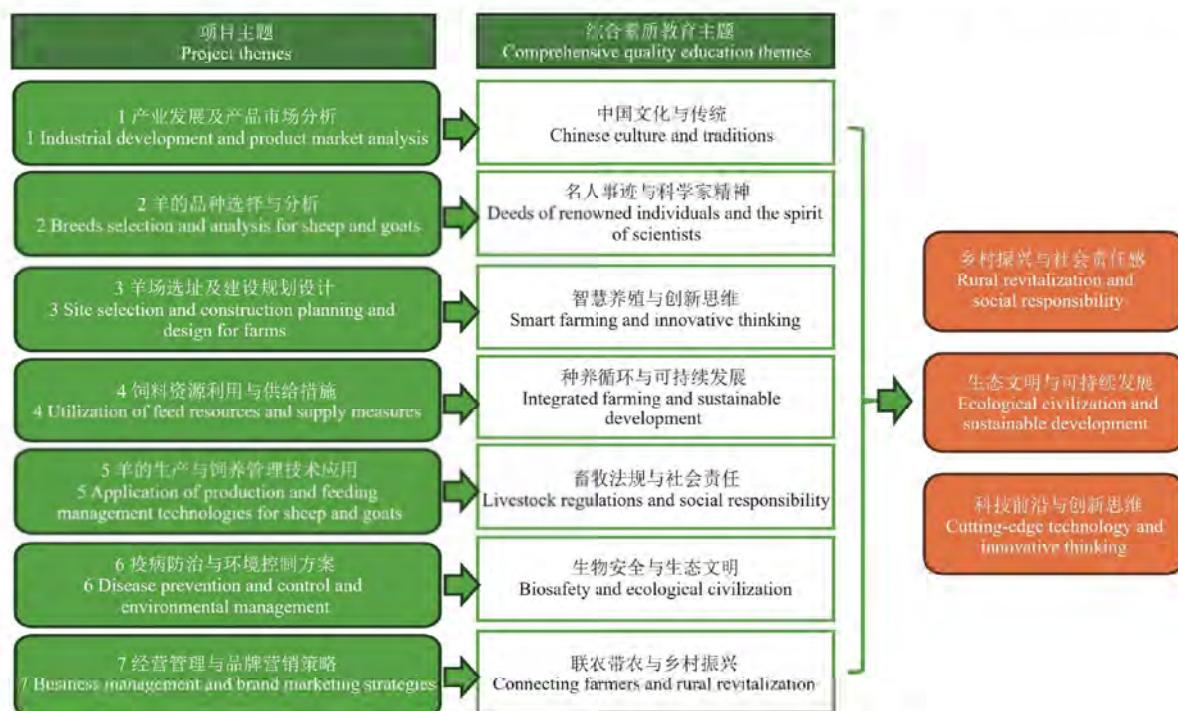


图8 基于“项目驱动”的养羊学课程综合素质教育主题

Figure 8 Comprehensive quality education themes in the “project-driven” sheep and goat production course

<http://cykx.lzu.edu.cn>

适应未来产业需求的能力。

4 课程改革成效与展望

通过教学改革的实施,养羊学课程在学生培养及课程建设的成效上得到了显著提升。在学生培养方面,“项目驱动”教学模式显著提高了学生的学习效果和实际应用能力,激发了学生的创新意识和创业精神。近 5 年,教学团队指导本科生发表论文 6 篇(包括北大核心期刊)^[15-20],指导学生参加创新创业训练项目 5 项、创新创业竞赛 5 项(包括国家级项目及竞赛)。通过项目驱动教学模式,学生能够将理论知识与实际问题相结合,提升了知识综合应用能力,并有学生通过课程的学习在毕业后在养羊产业进行创业,该典型案例被广东卫视《众创英雄会》节目播放,起到了良好的宣传示范作用,进一步证

明了课程改革对学生综合素质培养起到了良好的促进作用。在课程建设方面,近 5 年教学团队主持教改项目 5 项,建设产学研平台及实践教学基地 5 个。这些平台和基地不仅为学生提供了丰富的实践机会,还促进了校企合作,为项目驱动教学模式的实施提供了坚实的支撑作用,教师的教学水平也在实践中得到明显提升。基于“项目驱动”的养羊学课程改革成果获得了广东省教学成果创新大赛二等奖,可为其他动物生产类及与产业衔接较紧密的专业课程提供参考。

展望未来,养羊学课程的教学改革与实践工作将持续进行,以培养高素质人才为目标,进一步优化项目驱动的教学模式,深化产教融合,不断提升学生的综合素质和实践能力,为我国养羊业的高质量发展贡献力量。

参考文献 References:

- [1] 柳广斌,孙宝丽,刘德武.华南地区高等农林院校养羊学课程改革探索.学园,2020,13(29): 18-19.
LIU G B, SUN B L, LIU D W. Exploration of sheep and goat production course reform in higher agricultural and forestry institutions in south China. Academy, 2020, 13(29): 18-19.
- [2] 郝小燕,杨春合,李碧波,牟春堂,张春香.《羊生产学》教学的改革探索:以山西农业大学为例.畜禽业,2022,33(10): 47-49.
HAO X Y, YANG C H, LI B B, MU C T, ZHANG C X. Concept renewal and practice innovation: Reform and exploration of the teaching of sheep and goat production: A case study of Shanxi Agricultural University. Livestock and Poultry Industry, 2022, 33(10): 47-49.
- [3] 权凯,哈斯,李君.基于市场需求导向羊生产学课程教学改革思考.畜牧与饲料科学,2020,41(2): 121-124.
QUAN K, Hasi, LI J. Thoughts on the teaching reform of sheep and goat production based on market demand. Animal Husbandry and Feed Science, 2020, 41(2): 121-124.
- [4] 马海玉,刘玲玲,刘武军.“羊生产学”课程智慧教学实践创新探索.大学,2021(31): 158-160.
MA H Y, LIU L L, LIU W J. Innovation and exploration of wisdom teaching practice of “Sheep production science” course. University, 2021(31): 158-160.
- [5] 仲庆振,赵元,杜东菊,杨春馥,孙泽威.新农科背景下动物科学专业大学生创新能力的培养策略.教育教学论坛,2022(28): 181-184.
ZHONG Q Z, ZHAO Y, DU D J, YANG C F, SUN Z W. Cultivation strategy of innovative ability of college students majoring in animal science under the background of new agricultural science. Education Forum, 2022(28): 181-184.
- [6] 张建斌,杨华,杨升,蒲蕾,洪亮,李留安,秦顺义.新农科背景下动物科学一流专业建设研究.畜牧与兽医,2023,55(8): 138-144.
ZHANG J B, YANG H, YANG S, PU L, HONG L, LI L A, QIN S Y. Construction of first-class specialty in animal science under the background of agricultural science. Animal Husbandry and Veterinary Medicine, 2023, 55(8): 138-144.
- [7] 段春辉,丁亚伟,张英杰,刘月琴,纪守坤.以“学生为中心”的教学理念改革创新:以羊生产学教学为例.黑龙江动物繁殖,2022,30(5): 56-59.
DUAN C H, DING Y W, ZHANG Y J, LIU Y Q, JI S K. Reform and innovation of “student - centered” teaching idea: Taking sheep and goat production course as an example. Heilongjiang Journal of Animal Reproduction, 2022, 30(5): 56-59.
- [8] 段春辉,刘月琴,张英杰.基于生产需求的《羊生产学》课程考核改革现状及方式.畜牧兽医科学(电子版),2021(13): 196-197.

- DUAN C H, LIU Y Q, ZHANG Y J. Current situation and mode of evaluation reform of sheep and goat production based on production demand. *Graziery Veterinary Sciences (electronic version)*, 2021(13): 196-197.
- [9] 莫放, 李胜利, 刘佳, 张微. 创新和创业型人才培养的牛生产学课程考核方法的完善. *畜牧与饲料科学*, 2019, 40(11): 84-87.
- MO F, LI S L, LIU J, ZHANG W. Perfection of evaluation method of cattle production for innovation and entrepreneurial talent training. *Animal Husbandry and Feed Science*, 2019, 40(11): 84-87.
- [10] 孙宝丽. 产学研结合视角下动物生产类课程教学创新探索: 评《动物生产学》. *中国饲料*, 2022(7): 154.
- XUN B L. Exploration of teaching innovation of animal production course from the perspective of combination of production, education and research: Comment on animal production science. *China Feed*, 2022(7): 154.
- [11] 张微, 莫放, 杨春合, 贾志海. 基于重视实践能力培养的《羊生产学》课程考核改革与实践. *家畜生态学报*, 2019, 40(7): 94-96.
- ZHANG W, MO F, YANG C H, JIA Z H. Course assessment reform and practice on Sheep Production based on practical ability training. *Journal of Domestic Animal Ecology*, 2019, 40(7): 94-96.
- [12] 陈磊. 畜牧学专业课程《羊生产学》创新与实践教学探索. *现代畜牧科技*, 2020(12): 17-18.
- CHEN L. Innovation and practice teaching exploration of sheep and goat production course. *Technical Advisor for Animal Husbandry*, 2020(12): 17-18.
- [13] 李耀坤, 刘德武, 孙宝丽, 柳广斌, 郭勇庆, 邓铭. “互联网+赛教融合”养牛学课程改革与实践. *草业科学*, 2022, 39(10): 2237-2244.
- LI Y K, LIU D W, SUN B L, LIU G B, GUO Y Q, DENG M. Reformation and practice of cattle science via “Internet Plus Integration of Competition and Teaching” mode. *Pratacultural Science*, 2022, 39(10): 2237-2244.
- [14] 段春辉, 高立杰, 张英杰, 刘月琴. 《羊生产学》课程思政案例库建设的探索与实践. *畜禽业*, 2021, 32(7): 86-87.
- DUAN C H, GAO L J, ZHANG Y J, LIU Y Q. The exploration and practice of the construction of ideological and political case base of sheep and goat production course. *Livestock and Poultry Industry*, 2021, 32(7): 86-87.
- [15] 郭烁媛, 邝圆融, 伍翠莹, 柳广斌. 微生态制剂在羊生产中的应用研究进展. *饲料研究*, 2024, 47(5): 145-149.
- GUO L Y, KUANG Y R, WU C Y, LIU G B. Research progress in application of microecological preparation in sheep production. *Feed Research*, 2024, 47(5): 145-149.
- [16] 陈晓敏, 李沂霖, 何炜诺, 卢焕聪, 廖嘉龙, 黄正, 柳广斌. 分子标记技术在山羊育种中的研究进展. *中国草食动物科学*, 2023, 43(6): 45-51.
- CHEN X M, LI Y L, HE W N, LU H C, LIAO J L, HUANG Z, LIU G B. Research progress of molecular marker technology in goat breeding. *China Herbivore Science*, 2023, 43(6): 45-51.
- [17] 张芯, 侯碧巍, 邓铭, 柳广斌. 南方地区奶山羊养殖常见问题及改善建议. *中国畜禽种业*, 2023, 19(3): 74-77.
- ZHANG X, HOU B W, DENG M, LIU G B. The common problems and improvement suggestions of dairy goat farming in southern regions. *The Chinese Livestock and Poultry Breeding*, 2023, 19(3): 74-77.
- [18] 郭思乐, 邱金戈, 柳广斌, 刘德武, 邓铭. 华南地区规模化羊场建设存在的问题与建议. *黑龙江畜牧兽医*, 2021(21): 69-73.
- GUO S L, QIU J G, LIU G B, LIU D W, DENG M. Problems and suggestions of large - scale goat farm construction in south China. *Heilongjiang Animal Science and Veterinary Medicine*, 2021(21): 69-73.
- [19] 黄强, 陈智成, 练志全, 邓铭, 蔡丹凤, 侯云飞, 杨健华, 李耀坤. MAPK 信号通路基因在雷州山羊不同生长发育阶段表达规律研究. *甘肃畜牧兽医*, 2020, 50(2): 49-53.
- HUANG Q, CHEN Z C, LIAN Z Q, DENG M, CAI D F, HOU Y F, YANG J H, LI Y K. Study on the expression patterns of MAPK signaling pathway genes at different growth and development stages in Leizhou goats. *Gansu Animal Husbandry and Veterinary*, 2020, 50(2): 49-53.
- [20] 张秋萍, 田汉晨, 黄泽林, 凌翀, 池周颖, 郭勇庆, 孙宝丽, 邓铭. 荔枝加工副产物的青贮工艺研究. *饲料研究*, 2020, 43(4): 63-65.
- ZHANG Q P, TIAN H C, HUANG Z L, LING C, CHI Z Y, GUO Y Q, SUN B L, DENG M. Study on silage technology of by-products from lychee processing. *Feed Research*, 2020, 43(4): 63-65.

(责任编辑 苟燕妮)

ISSN 1001-0629
CN 62-1069/S

邮发代号：54-51
定价：20.00元



ACADEMY

中国知网(CNKI)系列数据库收录期刊
中国核心期刊(遴选)数据库收录期刊

学园

2020·29

第13卷·总第346期

高职艺术院校校本创新创业基础课程开发研究/胡霞 001

例谈课题研究内容的分解/金付栓等 015

华南地区高等农林院校养羊学课程改革探索/柳广斌等 018

在区域活动中培养中班幼儿自主性的策略/黄毓敏 052

幼儿教师职业能力提升路径探究/徐广宇 066

ISSN 1674-4810



9 771674 481204



目录 Contents

◎学理考辨

- 高职艺术院校校本创新创业基础课程开发研究/胡霞 1
- 立德树人背景下“家—校—社会”协同育人现状调查分析/胡晓雪 杨树保 肖云玲 5
- 高校辅导员科研能力提升困境与突破路径的探索/王阿凡 邵娜娜 张慧 10
- 艺术类高职院校课程思政科研项目的培育途径/邱倩 13
- 例谈课题研究内容的分解/金付栓 魏丽华 15

◎教学前沿

- 华南地区高等农林院校养羊学课程改革探索/柳广斌 孙宝丽 刘德武 18
- 项目式教学法在非英语专业学术英语写作课堂的应用/李蕊 20
- 小学语文中年级作文教学策略/王军英 22
- 透析新课程下冷思考的本质/范留琴 24
- 人工智能技术在高校英语教学中的应用/林森 26
- 分层作业在初中数学教学中的应用/耿春萍 28
- UMU 学习平台中考题本的应用探索/王燕 30

主管：云南出版集团有限责任公司
主办：云南出版传媒（集团）有限责任公司
出版：云南大家杂志社有限公司
印刷：云南国方印刷有限公司

发行方式：邮发
发行单位：昆明市报刊发行局
邮发代号：64-53
定价：30.00 元

出版人：李维
编委会主任：田大余
编委：李银和 杨文虎 李锦雯 庞远燕
习强 尹林涛 邱倩 孔凡斌
苏燕 马庆
主编：李银和
执行主编：庞远燕
值期编辑：陈泽晖
责任编辑：庞远燕 张雪荣 沈雯涓 郑菲菲
陈泽晖 赵峰

美术编辑：魏诚成
投稿电话：0871-64149191 64112768
投稿邮箱：xueyuan2016@189.cn
地址：昆明市环城西路 577 号
邮编：650034
出版日期：2020 年 10 月 15 日
国际标准连续出版物号：ISSN 1674-4810
国内统一连续出版物号：CN 53-1203/C

期刊基本参数：CN 53-1203/C*2008*t*A4*112*zh*P*30.00*500*50*2020-10

篮球教学中培养学生攻守技巧的策略/李 旭	32
课前“命题演讲”对高一学生英语口语能力提高效果的研究/李军芳	34
初中化学实验教学中学生环保意识的培养/周文杰	36
混合现实技术在中职美术设计类课程教学中的应用/周鹏程	38
小学数学教学中数学实验的应用策略/胡彦科	40
高校英语专业写作课程智慧课堂模式的构建/路博政	42
思维导图在小学数学单元复习教学中的应用/盛贤丽	44
小学高年级语文比较阅读教学策略/温银英	46
语篇分析法在日语阅读教学中的应用/程 青 彭瑞雪	48
Web 前端开发课程教学中学生审美能力培养的路径/朱 军	50

◎学苑新锐

在区域活动中培养中班幼儿自主性的策略/黄毓敏	52
中职英语教学中口语交际能力的培养/肖婷婷	54
中职数学的教学现状及改进策略/金学嘉	56
幼儿美术泥工教学策略/史倩倩	58
南通红色文化融入高中政治课堂探究/缪建美	60
初中语文名著导读教学策略/刘祥鹤	62
博物馆中初中英语课程资源的开发与利用 ——以苏州教育博物馆为例/陈 丽	64

◎管理视野

幼儿教师职业能力提升路径探究/徐广宇	66
积极心理学视域下高校辅导员的心理健康管理/倪莉彬	68
学生研学讲座活动的融合教育实践研究/王 杨	70
高校学生教学信息员工作的探索/刘红梅	72

C 目录 Contents

- 职业教育集团平台下校企共建产业学院研究/石玉杰 74
- 产学研结合背景下校企合作协同育人的实践研究/张占平 高丽莉 76
- 信息化时代下大学生思想政治教育新思路/贾宛航 78
- 高职院校企业管理课程中大学生职业素质模型的构建/贺伟艺 80
- 新工科背景下师范院校创新创业教育模式研究/王 静 82
- 混合式教学背景下基于打卡的大学英语行动研究/王艳素 84
- 产出导向法在独立学院特殊用途英语课堂使用的满意度调查/韦艳梅 86
- 重视隐性课程,推进小学思想政治教育/刘 卓 88
- 生涯教育视角下的后进生转化/余雪松 陈小敏 90
- 高职微积分教学中命题的形成与推理论证的逻辑思考/蒋 辉 罗 戎 张 燕 92
- 社会心理咨询服务专业化的路径探索/何丽娜 95
- 初中生时间管理倾向与创新心理品质的关系探析/李秋丽 梁剑伟 97

◎学人对话

- 优秀民族传统体育文化对大学生思想政治教育的影响研究/纪素艳 种法力 99
- 基于幼儿园艺术教育活动培养幼儿的自信心/赵 钰 101
- 中华优秀传统文化在幼儿教育中的运用/郑 荣 103
- 如何让高三作文教学有趣并有用/牟晓津 105
- 小学音乐教学中培养学生音乐核心素养的策略探究/黄海燕 107

华南地区高等农林院校养羊学课程改革探索

柳广斌 孙宝丽 刘德武

(华南农业大学 动物科学学院, 广东 广州 510642)

【摘要】养羊学课程是高等农林院校动物科学专业开设的专业课, 该课程对养羊业人才培养具有重要意义。就华南地区高等农林院校养羊学课程在教学过程中存在的问题进行分析, 结合华南农业大学动物科学学院教学改革经验提出改进措施, 以期华南地区高等农林院校养羊学课程的教学效果提高提供借鉴。

【关键词】养羊学; 教学改革; 华南地区

中图分类号: G642

文献标识码: A

文章编号: 1674-4810(2020)29-0018-02

随着我国畜牧产业结构不断的优化与调整, 近几年养羊业的发展得到了各地政府的大力支持, 产业正从农户原始的放养模式向公司科学的规模化、集约化养殖逐步转变, 在这个过程中行业对高质量技术及管理人才的需求也在不断增加, 因此培养符合养羊业发展需求的高素质人才成为农林院校的重要任务。养羊学课程是高等农林院校动物科学专业开设的专业课, 在人才培养课程体系设置中占有重要地位。就华南地区养羊学课程在教学过程中存在的问题进行分析, 结合华南农业大学动物科学学院教学改革经验提出改进措施, 以期华南地区养羊学课程的教学效果提高提供借鉴, 为华南地区养羊业的发展培养符合要求的专业人才。

一 华南地区养羊学课程教学过程中存在的问题

1. 教学内容设计不能满足华南地区养羊业发展需要

在教学内容的设计上, 目前依然主要参考北方养羊的经验与特点, 并不完全符合当前华南地区养羊业发展的实际情况。例如课程教学中多以绵羊为例进行讲解, 山羊较少, 但实际上山羊养殖在华南地区更为普遍。此外, 随着养羊业的快速发展, 一些新的生产技术及饲养管理模式已被开发出来并在实践中得到应用, 而这些新知识在目前养羊学课程中涉及较少, 无法让学生了解到行业发展的最新状况。

2. 教学素材相对陈旧不能吸引学生

在教学内容的呈现上, 目前的养羊学课程虽然采用了多媒体(幻灯片课件及视频)教学方式, 但内容的制作较为简陋, 并不能很好地体现目前生产技术的先进性和羊场生产的实际情况。这不仅不能达到教学

的目的, 反而会造成学生对如今养羊业发展的错误认识, 降低学生投入到行业中的积极性。

3. 教学方法比较单一, 教学效果不佳

当前养羊学以课堂讲授为主, 教师按照课程大纲的安排对各知识点进行逐一讲解, 互动性不强。由于该课程的专业性较强, 单一的填鸭式教学方式往往造成课堂气氛沉闷, 使学生感到学习内容枯燥乏味, 参与性不强, 学习兴趣不高, 最终教学效果并不理想。因此对养羊学的教学方式方法进行创新, 提高教学效果是十分有必要的。

4. 教师缺乏实践经验, 综合素质有待提高

授课教师的知识储备与实践经验会直接影响教学内容的设计与知识传递的效果, 而部分教师特别是青年教师往往缺乏养羊的实践经验, 这导致在养羊学的教学过程中, 教师并不能很好地突出生产实际中出现的问题及解决办法, 对于一些生产技术难以抓住重点与难点, 同时也无法就羊场生产管理细节进行案例的拓展与讲解, 使得教学过程过于照本宣科, 不生动形象, 让一些知识点难以理解, 甚至可能产生一些偏离实际的错误。

二 华南地区养羊学课程改进措施

1. 完善内容设计

目前养羊学课程的主要参考教材有赵有璋主编的《羊生产学》及张英杰主编的《羊生产学》, 这两部教材内容较为全面, 是养羊学课程的优秀教材。但需要注意的是, 养羊生产受地理环境条件的影响较大, 各地在羊的饲养品种、饲料资源利用及生产管理模式上存在一定差异。^[1]在养羊学课程教学内容的设计上, 应该根据当地养羊业发展的实际情况, 因地制宜地拓展

基金项目: 华南农业大学教育教学改革与研究项目 “‘养羊学’课程内容优化及微课、翻转课堂在教学实践中的应用研究”(JG19069)。

作者简介: 柳广斌(1982—), 男, 博士, 硕士生导师, 讲师, 研究方向: 动物健康养殖与安全生产; 孙宝丽(1981—), 女, 博士, 副教授, 研究方向: 动物健康养殖与安全生产; 刘德武(1966—), 男, 博士, 博士生导师, 教授, 研究方向: 动物健康养殖与安全生产。

通信作者: 刘德武。

相关知识内容,让学生更全面具体地了解养羊生产的实际情况,掌握适合当地养羊业的养羊生产技术。

此外,由于养羊业近些年的快速发展,其生产技术与饲养管理模式也发生了新的变化。例如全舍饲圈养技术已经在湖羊养殖中得到广泛应用,并取得了良好的效果。养羊学课程的教学内容应该在现有理论知识的基础上,更新对技术开发应用及行业发展状况的介绍,保持与时俱进,让学生掌握最新的生产技术及行业发展动态,只有这样才能培养出符合养羊业发展需求的技术人才。

2. 更新教学素材

教学素材的质量是影响教学效果的重要因素,随着计算机与网络技术的快速发展,多媒体作为信息传递的重要载体在信息承载量与展示能力上都有了长足进步。作为呈现形式的图片与视频的质量在现代电子与光学技术的推动下更是达到了新的高度,其中视频更是成为目前信息传播的主流形式。养羊学课程是一门强调技术与实践的课程,许多知识与生产技术的细节可以通过图片与视频来进行展示,帮助学生进行理解。例如在介绍羊的分娩过程时,如果配有视频进行讲解,将起到更好的教学效果。

目前养羊学课程的教学素材较为匮乏,部分已有素材质量较差,不能满足当下教学的需求,因此应对养羊学教学素材进行必要的更新及制作。一方面,目前互联网发达,已有大量的免费或付费资源,可以利用网络资源扩增养羊学课程的教学材料;另一方面,教师也可自己制作教学素材。这要求教师掌握一定的拍摄及视频制作技能。这种方法更有利于制作出课程需要的教学材料,达到更好的教学效果,是现代教师需要学习的一项技能。

3. 丰富教学方法

在养羊学课程教学中,可以采用微课、慕课及翻转课堂相结合的方式丰富教学方法,提高学生的积极性,进而改善教学效果。

微课是简短的课程视频,可以由教师自己制作也可以使用网络资源,其内容可以是与课程内容相关的知识,或是知识的拓展内容,可以安排在课前预习也可以作为课后的复习或拓展资料。^[2]由于微课视频时长

较短,一般为10分钟左右,学生可以利用碎片时间通过手机、平板或电脑等设备进行观看。

慕课可以作为课外的学习资料,安排学生学习一些其他学校或行业内专家开设的网络公共课程,通过不同学校老师或专家的讲解,开拓学生的视野,让学生掌握更全面丰富的知识。^[3]

翻转课堂是以学生为主的课堂教学形式。^[4]上课前可先安排学生进行微课或慕课内容的学习并引出问题,让学生通过自己的努力寻找答案。在课堂中以学生为主进行问题的讨论,教师主要进行内容的引导与节奏的把控,最终通过学生自身的主动学习将问题解决。这种教学方式可充分调动学生的积极性,通过教学过程的高度参与,加深学生对知识的理解,并形成深刻的印象,同时还可锻炼学生的信息收集分析及语言组织表达能力,提升学生的综合能力。

4. 建设实践基地

养羊学是一门实践科学,其中包含大量生产技术的实际应用,因此建立实践基地对教学效果的提升具有莫大帮助。^[5]可以通过与当地养羊企业合作的方式来联合建设实践基地,一方面可以让学生了解羊场的运作方式以及行业的发展状况,加深对知识的理解与认识;另一方面也可为企业输送技术人才,促进企业及养羊业的发展,达到双赢的目的。

同时还可与养羊企业展开产学研合作,让教师投入到产业技术的研发过程中,让青年教师有更多机会接触生产实践并从中积累经验,再将自身的经验转化为教学内容,提高教学质量。通过与企业合作,企业可以提高生产技术,教师可以积累经验,学生可以开阔视野,最终达到企业、教师及学生的共同成长。

三 总结

养羊学是一门研究养羊生产技术以提高养羊业经济效益为目的的科学,它既产生于养羊生产实践,又服务于养羊生产实践。因此养羊学课程的教学内容应该充分贴近生产实际,保持与时俱进,充分考虑地域差异对养羊技术的影响,因地制宜地优化教学内容,改进教学方式,提高教学质量,只有这样才能培养出符合行业需求的专业技术人才,促进养羊业的发展。

参考文献

- [1] 周多恩,刘德武,廖迎新,等.湖羊和川中黑山羊在华南地区适应性研究[J].华南农业大学学报,2016(5)
- [2] 黄浩平,蒋超男.基于微课的大学英语翻转课堂教学设计[J].黑龙江教育(高教研究与评估),2020(7)
- [3] 董世魁,苏德荣,纪宝明,等.基于慕课+SPOC+腾讯会议平台的“草原生态修复学”在线教学[J].草学,2020(3)
- [4] 王蕾.基于实时互动的“MOOC+SPOC+翻转课堂”混合教学模式研究[J].河北软件职业技术学院学报,2020(2)
- [5] 孙宝丽,谢青梅,付晓兰,等.动物科学类专业实践教学体系的改革与实践[J].家畜生态学报,2014(6)

(责编:赵 峰)

主管 云南出版集团有限责任公司
主办 云南出版传媒（集团）有限责任公司
出版 云南大家杂志社有限公司
主编 李银和
地址 昆明市环城西路 577 号
邮编 650034
电话 0871-64149191
邮箱 xueyuan2016@189.cn
印刷 云南国方印刷有限公司
国内发行 昆明市报刊发行局
邮发代号 64-53



ISSN 1674-4810
CN 53-1203/C

定价：30.00 元



第九届广东省教育教学成果奖
(高等教育)

获奖证书

获奖成果：以实践创新能力为导向的动物生产
类课程教学模式改革与实践

获奖者：孙宝丽、张永亮、刘德武、罗庆斌、
李耀坤、邓铭、郭勇庆、柳广斌

获奖等级：一等奖

证书号：GJ20191028





获奖成果: 以实践创新能力为导向的动植物生产模式改革与实践教学

获奖者: 孙宝丽、刘德武、罗庆斌、李耀坤、邓铭、郭勇庆、柳广斌、付晓兰

获奖等级: 一等奖

证书编号: JXCG19008





第四届全国高校教师教学创新大赛广东分赛
暨广东省高校教师教学创新大赛

获奖证书

主讲教师：柳广斌

团队成员：孙宝丽、刘德武、李耀坤

主讲课程：养羊学

获奖等级：新农科 二等奖

证书编号：GDTIC2024042

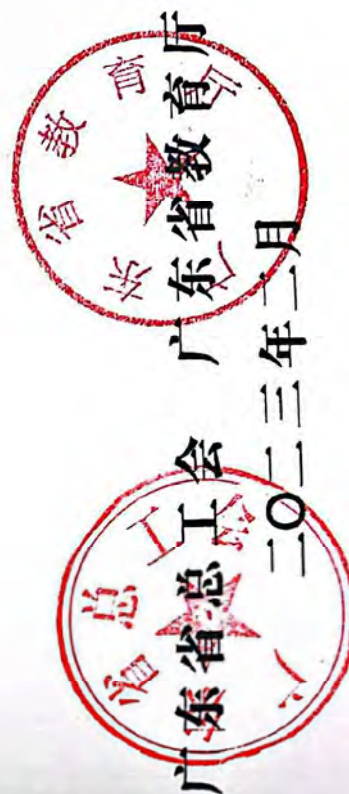


荣誉证书

柳广斌同志：

在广东省第六届高校（本科）青年教师教学大赛
（工科组）小组中表现优异，荣获三等奖。

特发此证，以资鼓励。



荣誉证书

HONORARY CREDENTIAL

柳广斌 老师：

荣获华南农业大学 2022 年度“本科课
堂教学十佳教师”。

特发此证，以资鼓励！

华南农业大学

2023 年 6 月 28 日

荣誉证书

动物科学学院 柳广斌 老师：

在华南农业大学2020-2021学年青年教师
教学优秀奖评选中荣获“一等奖”。

特发此证，以资鼓励！





第三届全国高校教师教学创新大赛广东分赛
暨广东省高校教师教学创新大赛

获奖证书

主讲教师：孙宝丽

团队成员：张永亮、柳广斌、李耀坤

主讲课程：动物生产学

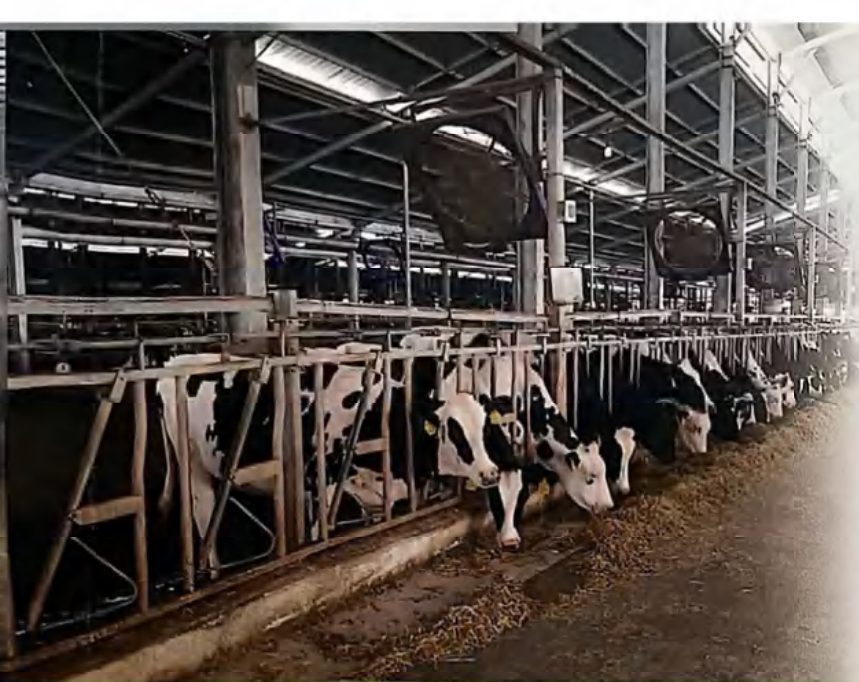
获奖等级：二等奖

证书编号：GDTIC2023060



广东省高等教育学会


二〇二三年四月



NAINIU
SHENGCHAN
SHIJIAN
JIAOCHENG

奶牛 生产实践教程

主编 李耀坤 邓铭 柳广斌

 吉林科学技术出版社

奶牛生产实践教程

主 编：李耀坤 邓 铭 柳广斌
副 主 编：刘德武 孙宝丽 郭勇庆
参编人员：张家豪 牛群皓 杨江鸿
 冼振宇 孔少芬 陈丽怡
 陆子涛 叶峻宁 赵秀慧

图书在版编目 (CIP) 数据

奶牛生产实践教程 / 李耀坤, 邓铭, 柳广斌主编

. -- 长春 : 吉林科学技术出版社, 2021. 6

ISBN 978-7-5578-8306-5

I. ①奶… II. ①李… ②邓… ③柳… III. ①乳牛-
饲养管理-教材 IV. ①S823. 9

中国版本图书馆 CIP 数据核字 (2021) 第 122292 号

奶牛生产实践教程

NAINIU SHENGCHAN SHIJIAN JIAOCHENG

主 编 李耀坤 邓铭 柳广斌
出 版 人 宛 霞
责任编辑 孟 盟
封面设计 王振华
制 版 北京壹滴水文化传播有限公司
幅面尺寸 185mm×260mm 1/16
字 数 180 千字
印 张 10
印 数 1-1 000 册
版 次 2021 年 6 月第 1 版
印 次 2021 年 6 月第 1 次印刷

出 版 吉林科学技术出版社
发 行 吉林科学技术出版社
地 址 长春市净月区福祉大路 5788 号
邮 编 130118
编辑部电话 0431-81629514
印 刷 长春市华远印务有限公司

书 号 ISBN 978-7-5578-8306-5
定 价 40.00 元

版权所有 翻印必究 举报电话: 0431-81629508

目 录

第一章 奶牛场的概况	1
第一节 基础条件	1
第二节 常见奶牛场生产规模及其示意图	7
第三节 饲养方式	9
第四节 人力资源配置	10
第五节 环境保护措施	16
第二章 饲养饲料	18
第一节 常见饲料原料	18
第二节 饲料配方制作	20
第三节 TMR 制作流程	22
第四节 不同阶段饲料的对比	25
第五节 饲料质量评定及方法	28
第六节 舍内舒适度评估	32
第七节 牛体评估	39
第三章 繁育	50
第一节 种公牛精液选择	50
第二节 青年牛初配标准及分析	55
第三节 人工授精流程	55
第四节 同期发情	57
第五节 发情鉴定	60
第六节 孕检	62
第四章 兽医 (常见疾病)	65
第一节 乳房炎的检测治疗	65
第二节 干奶牛干奶流程	68
第三节 修蹄、常见蹄病及治疗	71
第四节 产后护理流程	75

第五节 疾病症状及治疗	80
第六节 传染病的防控与免疫	86
第七节 场内消毒防疫措施	90
第五章 产房	93
第一节 围产期后期饲养要点	93
第二节 临产征兆	94
第三节 接助产操作及记录	97
第四节 初乳的质量鉴定及饲喂操作	101
第五节 犊牛的饲养管理	104
第六章 奶厅	110
第一节 奶厅建筑	110
第二节 挤奶操作流程	111
第三节 出奶	119
第四节 设备定期检查	120
第五节 设备维护	121
第七章 环保	133
第一节 奶牛场废弃物处理流程	133
第二节 废弃物处理设施（粪污固液分离设施）	138
第三节 废弃物处理效果	139
第八章 DHI 报告解读与分析	140
第一节 参数解读	140
第二节 DHI 技术实施的关键点	143
第二节 DHI 报告的应用范围	144
第九章 热应激评估	145
第一节 热应激	145
第二节 降温设施种类及使用	149
第三节 饲粮对热应激的改善	152
参考文献	154

责任编辑 孟 盟

封面设计 王振华

NAINIU 奶 牛
SHENGCHAN
SHIJIAN
JIAOCHENG 生产实践教程

ISBN 978-7-5578-8306-5



9 787557 883065 >

定价：40.00元

密级：公开

广东省农业科研类及技术推广示范类 项目（专项）任务合同书

项目（课题）名称：清远黑山羊标准化养殖技术与全产业链产品开发综合研究

承担单位：华南农业大学

项目负责人：孙宝丽

起止年限：2024 年 12 月 29 日至2025 年 12 月 28 日

广东省农业农村厅制

填写说明

1. 项目（课题）任务合同书甲方为广东省农业农村厅，乙方为项目（课题）承担单位。

2. 项目（课题）密级由项目（课题）承担单位提出建议，广东省农业农村厅认定。

3. 任务合同书正文须用“仿宋四号”字体填写，表格须用“宋体五号”字体填写。

4. 任务合同书书 A4 纸打印，一式陆份，广东省农业农村厅保存肆份，项目（课题）牵头承担单位贰份。

5. 该格式为广东省农业农村厅农业科研类及技术推广示范类项目（课题）任务合同的基本信息，不得随意增加或减少有关内容。

6. 《广东省农业科研类及技术推广示范类项目（专项）申报书》是《任务合同书》填报的重要依据，任务合同书填报不得降低考核指标，不得自行对主要研究内容作大的调整。任务合同书将作为项目（课题）过程管理、验收和监督评估的重要依据。

广东省农业农村厅农业科研类及技术推广示范类 项目（课题）信息表

项目（课题）名称		清远黑山羊标准化养殖技术与全产业链产品开发综合研究			
项目（课题）编号					
密 级		<input type="checkbox"/> 绝密 <input type="checkbox"/> 机密 <input type="checkbox"/> 秘密 <input checked="" type="checkbox"/> 公开			
牵头承担单位	名 称	华南农业大学			
	单位所在地	广东省广州市天河区		代码	440106
	通讯地址	广东省广州市天河区五山路 483 号		邮编	510642
	开户银行	中国工商银行股份有限公司广州五山支行			
	银行帐号	3602002609000310520			
	单位性质	<input type="checkbox"/> 事业型研究单位 <input type="checkbox"/> 其他事业单位 <input checked="" type="checkbox"/> 大专院校 <input type="checkbox"/> 转制为企业的科研院所 <input type="checkbox"/> 国有企业 <input type="checkbox"/> 集体所有制企业 <input type="checkbox"/> 合资企业 <input type="checkbox"/> 外商投资企业 <input type="checkbox"/> 港、澳、台投资企业 <input type="checkbox"/> 其他企业		代码	12440000455 4165634
	上级行政主管部门	广东省教育厅		代码	11440000006 940116U
	国务院国资委企业	<input type="checkbox"/> 是 <input checked="" type="checkbox"/> 否	“双一流”大学	<input checked="" type="checkbox"/> 是 <input type="checkbox"/> 否	
参与单位	序号	单 位 名 称			
	1	广东科贸职业学院			
	2	广东领头羊智慧农业有限公司			
项目（课题）负责人	姓 名	孙宝丽		性 别	<input type="checkbox"/> 男 <input checked="" type="checkbox"/> 女
	学 位	<input checked="" type="checkbox"/> 博士 <input type="checkbox"/> 硕士 <input type="checkbox"/> 学士 <input type="checkbox"/> 其他		出生日期	1981.9.24
	职 称	<input checked="" type="checkbox"/> 高级 <input type="checkbox"/> 中级 <input type="checkbox"/> 初级 <input type="checkbox"/> 其他		专 业	动物科学
	所在单位	华南农业大学			
	身份证件	身份证	证件号码	411024198109241823	
	联系电话	13802405625		E-mail	baolisun@scau.edu.cn

参加项目（课题） 人员情况	16 人。	高级 12 人，中级 2 人，初级 0 人，其他 2 人；	
	其中：	博士 12 人，硕士 3 人，学士 1 人，其他 0 人。	
投入人月总数	114 人月		
起始时间	2024 年 12 月	终止时间	2025 年 12 月
主要内容 (200 字以内)	<p>项目通过建立清远黑山羊标准化养殖技术体系和良种快速扩繁关键技术，提升养殖效率和产品质量，防止品种退化，保持优良性状。同时，制定产品标准化加工与质量控制标准，确保产品的一致性和高品质。开发预制菜产品，打造清远黑山羊品牌，提升市场竞争力，促进产业链的延伸和增值。项目的实施将推动清远黑山羊产业的持续健康发展，助力乡村振兴和区域经济发展。</p>		
预期成果	<input type="checkbox"/> 专利 <input checked="" type="checkbox"/> 技术标准 <input checked="" type="checkbox"/> 新产品（或农业新品种） <input checked="" type="checkbox"/> 新工艺 <input type="checkbox"/> 新装置 <input type="checkbox"/> 新材料 <input type="checkbox"/> 计算机软件 <input type="checkbox"/> 论文论著 <input checked="" type="checkbox"/> 研究报告 <input type="checkbox"/> 推广受益面积 <input checked="" type="checkbox"/> 推广受益人群 <input checked="" type="checkbox"/> 其他		
预期知识产权	获得国外发明专利 0 项，国内发明专利 0 项，其他 0 项。		
预期技术标准制定	<input type="checkbox"/> 国际标准 <input type="checkbox"/> 国家标准 <input type="checkbox"/> 行业标准 <input type="checkbox"/> 企业标准		
预算	300 万元，其中省级财政资金 300 万元，其他资金 0 万元		

一、目标与任务

(①项目(课题)研究/推广示范内容及任务分解:要解决的主要技术难点和问题,技术方案和创新点等;②项目(课题)研究/推广示范目标;③主要示范和产业化内容及相关技术路线)

(一)要解决的主要问题

1. 清远黑山羊标准化养殖技术缺乏:解决目前养殖模式传统、管理粗放、羔羊损失率高的问题,建立科学的标准化养殖技术体系,特别是母羊围产期的饲养管理,提高养殖效率和产品质量。

2. 良种繁育效率低下:通过开发人工受精、同期发情、胚胎移植等关键技术,建立高效的良种快速扩繁体系,防止近亲繁殖,保持和提升品种的优良性状。

3. 产品加工与质量控制薄弱:制定标准化的加工流程和质量控制标准,提升产品的一致性和安全性,满足市场对高品质羊肉产品的需求。

4. 品牌建设与市场竞争力不足:通过开发预制菜产品和打造品牌形象,提升清远黑山羊的市场知名度和美誉度,增强市场竞争力,促进产业升级。

(二)研究目标

本项目旨在建立清远黑山羊标准化养殖技术体系和良种快速扩繁关键技术,提升清远黑山羊的养殖效率和产品质量。通过制定产品标准化加工与质量控制标准,开发预制菜产品,打造清远黑山羊品牌,提升其市场竞争力,促进产业链的延伸和增值,推动清远黑山羊产业的持续健康发展。

(三)研究内容

1. 研究一:清远黑山羊标准化养殖配套技术建立与示范

(1) 清远黑山羊半放牧半舍饲养模式的技术开发

承担单位：华南农业大学

任务负责人：孙宝丽

依据清远黑山羊的生物学特性和广东省的饲养条件，建立适合其半放牧半舍饲养模式的技术方案。涵盖饲料加工、营养调控、种羊繁育、饲养管理、环境控制、疫病防控等各环节的规范化、标准化生产技术。通过构建一套优质、高效、健康的清远黑山羊养殖技术体系并进行示范，为良种清远黑山羊的养殖推广提供完善的配套技术支持。

(2) 清远黑山羊围产期母羊及羔羊饲养管理技术开发

承担单位：广东科贸职业学院

任务负责人：毕雪

针对清远黑山羊围产期母羊和初生羔羊成活率较低的问题，开发围产期母羊及羔羊的饲养管理技术。通过研究围产期母羊的营养需求，优化饲料配方和饲喂管理，增强母羊健康，降低产科疾病发生率。制定初生羔羊护理措施，包括助产、初乳摄取、温度控制和疫病防控等，提高羔羊成活率和生长速度。建立一套围产期母羊及羔羊饲养管理技术规范，形成标准化操作流程，为清远黑山羊养殖效益的提升提供技术支持。

2. 研究二：清远黑山羊良种快速扩繁关键技术建立

承担单位：华南农业大学

任务负责人：柳广斌

系统开发适用于清远黑山羊良种的快速扩繁技术，深入研究人工受精、早期妊娠诊断、同期发情、超数排卵、胚胎移植等关键繁殖技术，

建立高效的繁殖体系。旨在迅速提升清远黑山羊核心育种群的数量和质量，为产业链后续环节提供充足的优质种羊资源，促进品种优化升级。

3. 研究三：清远黑山羊产品标准化加工与质量控制研究

承担单位：华南农业大学

任务负责人：孙宝丽

为确保清远黑山羊加工产品的均一性和高品质，制定一系列标准化的加工流程和严格的质量控制标准。从原料选择、加工工艺、产品检验到包装储存，各环节严格执行标准化管理，确保每一份产品均达到行业最高质量标准。通过完善的流程控制，为消费者提供安全、健康、美味的清远黑山羊加工产品，提升产品市场信誉度。

4. 研究四：清远黑山羊预制菜开发与品牌建设

承担单位：广东领头羊智慧农业有限公司

任务负责人：王帅彬

为满足现代消费者对便捷、健康食品的需求，依托标准化生产的优质羊肉，研发清远黑山羊预制菜产品。通过精选原料、优化烹饪工艺、严格控制食品安全标准，为消费者提供多样化的优质选择。同时，打造清远黑山羊的品牌形象，整合全产业链资源优势，提升品牌影响力，增强市场竞争力，推动清远黑山羊产业的持续健康发展。

二、预期成果及考核指标

(①主要技术指标:如形成的知识产权、技术标准、新技术、新产品、新装置、论文专著等数量、指标及其水平等;②主要经济指标:如技术及产品应用所形成的市场规模、效益等;③项目(课题)实施中形成的示范基地、中试线、生产线及其规模等;④人才队伍建设;⑤其他应考核的指标。)

1. 形成 1 套清远黑山羊半放牧半舍饲养殖技术方案和 1 套良种高效繁殖技术体系,并在相关企业进行应用示范;
2. 制定清远黑山羊养殖、加工、产品质量等标准各 1 套;
3. 研发 5 款预制菜产品;
4. 加强清远黑山羊品牌宣传,省级媒体报道不少于 10 次。
5. 举办养殖技术培训班 2 次,培训人数达 100 人次。

成果名称	成果类型	考核指标 ¹			考核方式 (方法)及 评价手段 ²
		指标名称	立项时已有指标值/ 状态	完成时 指标值/ 状态	
1. 清远黑山羊半放牧半舍饲养殖技术方案	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input checked="" type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input type="checkbox"/> 其他_____	数量指标	无	1 套	技术方案及应用报告
2. 良种高效繁殖技术体系	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input checked="" type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input type="checkbox"/> 其他_____	数量指标	无	1 套	技术方案及应用报告
3. 清远黑山羊养殖、加工、产品质量等标准	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input checked="" type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input type="checkbox"/> 其他_____	数量指标	无	各 1 套	标准文件

4. 研发预制菜产品	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input checked="" type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input type="checkbox"/> 其他_____	数量指标	无	5 款	产品报告
		质量指标	无	产品满意度 $\geq 90\%$	调研报告
5. 省级媒体报道	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input checked="" type="checkbox"/> 其他_宣传推广_____	数量指标	/	≥ 10 次	媒体报道
6. 举办技术培训班	<input type="checkbox"/> 新理论 <input type="checkbox"/> 新原理 <input checked="" type="checkbox"/> 新产品 <input type="checkbox"/> 新技术 <input type="checkbox"/> 新方法 <input type="checkbox"/> 数据库 <input type="checkbox"/> 关键部件 <input type="checkbox"/> 软件 <input type="checkbox"/> 标准 <input type="checkbox"/> 应用解决方案 <input type="checkbox"/> 工程工艺 <input type="checkbox"/> 论文 <input type="checkbox"/> 实验装置/系统 <input type="checkbox"/> 发明专利 <input checked="" type="checkbox"/> 其他_技术培训_____	数量指标	/	举办 2 次	培训记录
		数量指标	/	培训人数 100 人次	培训记录
		质量指标	/	学员合格率 $\geq 98\%$	培训记录

1. “考核指标”，指相应成果的数量指标、技术指标、质量指标、应用指标和产业化指标等，其中，数量指标可以为论文、专利、产品等的数量；技术指标可以为关键技术、产品的性能参数等；质量指标可以为产品的耐震动、高低温、无故障运行时间等；应用指标可以为成果应用的对象、范围和效果等；产业化指标可以为成果产业化的数量、经济效益等。同时，对各项考核指标需填写立项时已有的指标值/状态以及项目（课题）完成时要到达的指标值/状态。同时，考核指标也应包括支撑和服务其他重大科研、经济、社会发展、生态环境、科学普及需求等方面的直接和间接效益。如对国家重大工程、社会民生发展等提供了关键技术支撑，成果转让并带动了环境改善、实现了销售收入等。若某项成果属于开创性的成果，立项时已有指标值/状态可填写“无”，若某项成果在立项时已有指标值/状态难以界定，则可填写“/”。

2. “考核方式方法”，应提出符合相关研究/推广示范成果与指标的具体考核技术方法、测算方法等。

三、项目（课题）年度计划及年度目标

年度	年度任务	年度预算		年度考核指标	重要任务的时间节点
		省级财政资金	其他渠道资金		
2024 年	建立清远黑山羊标准化养殖技术体系和良种快速扩繁关键技术,提升清远黑山羊的养殖效率和产品质量。通过制定产品标准化加工与质量控制标准,开发预制菜产品,打造清远黑山羊品牌,提升其市场竞争力,促进产业链的延伸和增值,推动清远黑山羊产业的持续健康发展。	300 万	0	1. 形成 1 套清远黑山羊半放牧半舍饲养殖技术方案和 1 套良种高效繁殖技术体系,并在相关企业进行应用示范; 2. 制定清远黑山羊养殖、加工、产品质量等标准各 1 套; 3. 研发 5 款预制菜产品; 4. 加强清远黑山羊品牌宣传,省级媒体报道不少于 10 次。 5. 举办养殖技术培训班 2 次,培训人数达 100 人次。	2024 年 12 月- 2025 年 1 月: 项目启动与基础调研; 2025 年 6 月- 2025 年 8 月: 技术完善与标准制定; 2025 年 9 月- 2025 年 12 月: 技术推广与项目总结。

四、任务分工情况

任务名称	承担单位	任务负责人	在任务中具体承担的情况	目标	研究/推广示范内容	考核指标	重要任务的时间节点	资金(万元)
研究一之(1): 清远黑山羊半放牧舍饲养殖模式的技术开发	华南农业大学	孙宝丽	技术研发	建立适合其半放牧舍饲养殖模式的技术方案	构建一套优质、高效、健康的清远黑山羊养殖技术体系并进行示范	形成1套清远黑山羊半放牧舍饲养殖技术方案;举办养殖技术培训2次,培训人数达100人次。	2024年12月:项目启动; 2025年6月:技术完善; 2025年9月:技术推广。	55
研究一之(2): 清远黑山羊围产期母羊及羔羊饲养管理技术开发	广东科贸职业学院	毕雪	技术研发	开发围产期母羊及羔羊的饲养管理技术	建立一套围产期母羊及羔羊饲养管理技术规范,形成标准化操作流程	形成1套清远黑山羊围产期母羊及羔羊饲养管理技术方案	2024年12月:项目启动; 2025年6月:技术完善; 2025年9月:技术推广。	15
研究二: 清远黑山羊良种快速扩繁关键技术建立	华南农业大学	柳广斌	技术研发	建立高效的繁殖体系	研究人工受精、早期妊娠诊断、同期发情、超数排卵、胚胎移植等关键技术	形成1套良种高效繁殖技术体系	2024年12月:项目启动; 2025年6月:技术完善; 2025年9月:技术示范。	100
研究三: 清远黑山羊产品标准化加工与质量控制研究	华南农业大学	孙宝丽	技术研发	制定一系列标准化和严格的流程控制标准	建立从原料选择、加工工艺、产品检验到包装储存,各环节严格执行的标准化操作规程	制定清远黑山羊养殖、加工、产品质量等标准各1套	2024年12月:项目启动; 2025年6月:	45

研究四：清远黑山羊预制菜开发与品牌建设	广东领头羊智慧农业有限公司	王帅彬	产品开发与示范推广	研发清远黑山羊预制菜产品并打造清远黑山羊品牌形象	通过精选原料、优化烹饪工艺、严格控制食品安全标准开发预制菜产品并对项目成果示范推广	研发5款预制菜产品；加强清远黑山羊品牌宣传，省级媒体报道不少于10次。	技术完善及标准制定； 2025年9月：技术示范。 2024年12月：项目启动与基础调研； 2025年6月：产品完善； 2025年9月：技术推广与项目总结。	85
---------------------	---------------	-----	-----------	--------------------------	---	-------------------------------------	---	----

五、项目（课题）资金来源与支出预算

（一）项目（课题）预算

单位：万元（保留两位小数）

序号	预算科目名称	合计	省级财政专项资金	其他渠道资金
1	一、资金支出	300.00	300.00	
2	（一）直接费用	288.00	288.00	
3	1. 设备费			
4	2. 材料费	101.56	101.56	
5	3. 测试化验加工费	111.50	111.50	
6	4. 燃料动力费			
7	5. 出版/文献/信息传播/知识产权事务费	29.30	29.30	
8	6. 会议/差旅/国际合作交流费	19.80	19.80	
9	7. 培训费			
10	8. 劳务费	18.84	18.84	
11	10. 专家咨询费	7.00	7.00	
12	11. 基本建设费			
13	（1）房屋建筑物构建			
14	（2）专用设备购置			
15	（3）基础设施建设			
16	（4）大型修缮			
17	（5）信息网络建设			
18	（6）其他基本建设支出			
19	12. 其他费用			
20	（二）间接费用	12.00	12.00	
21	二、项目（课题）资金来源			
22	（一）省级财政专项资金	300.00	300.00	/
23	（二）其他渠道资金		/	
24	1. 单位自筹资金		/	
25	2. 其他资金		/	

(二) 项目(课题)预算(承担单位)

承担单位 1: 华南农业大学

序号	预算科目名称	合计	省级财政专项资金	其他渠道资金
1	一、资金支出	200.00	200.00	
2	(一) 直接费用	188.00	188.00	
3	1. 设备费			
4	2. 材料费	58.56	58.56	
5	3. 测试化验加工费	75.50	75.50	
6	4. 燃料动力费			
7	5. 出版/文献/信息传播/知识产权事务费	17.30	17.30	
8	6. 会议/差旅/国际合作交流费	18.80	18.80	
9	7. 培训费			
10	8. 劳务费	15.84	15.84	
11	10. 专家咨询费	2.00	2.00	
12	11. 基本建设费			
13	(1) 房屋建筑物构建			
14	(2) 专用设备购置			
15	(3) 基础设施建设			
16	(4) 大型修缮			
17	(5) 信息网络建设			
18	(6) 其他基本建设支出			
19	12. 其他费用			
20	(二) 间接费用	12.00	12.00	
21	二、项目(课题)资金来源			
22	(一) 省级财政专项资金	200.00	200.00	/
23	(二) 其他渠道资金		/	
24	1. 单位自筹资金		/	
25	2. 其他资金		/	

承担单位 2: 广东科贸职业学院

序号	预算科目名称	合计	省级财政专项资金	其他渠道资金
1	一、资金支出	15.00	15.00	
2	(一) 直接费用			
3	1. 设备费			
4	2. 材料费	6.00	6.00	
5	3. 测试化验加工费			
6	4. 燃料动力费			
7	5. 出版/文献/信息传播/知识产权事务费	3.00	3.00	
8	6. 会议/差旅/国际合作交流费	1.00	1.00	
9	7. 培训费			
10	8. 劳务费	3.00	3.00	
11	10. 专家咨询费	2.00	2.00	
12	11. 基本建设费			
13	(1) 房屋建筑物构建			
14	(2) 专用设备购置			
15	(3) 基础设施建设			
16	(4) 大型修缮			
17	(5) 信息网络建设			
18	(6) 其他基本建设支出			
19	12. 其他费用			
20	(二) 间接费用			
21	二、项目(课题)资金来源			
22	(一) 省级财政专项资金	15.00	15.00	/
23	(二) 其他渠道资金		/	
24	1. 单位自筹资金		/	
25	2. 其他资金		/	

承担单位 3: 广东领头羊智慧农业有限公司

序号	预算科目名称	合计	省级财政专项资金	其他渠道资金
1	一、资金支出	85.00	85.00	
2	(一) 直接费用			
3	1. 设备费			
4	2. 材料费	37.00	37.00	
5	3. 测试化验加工费	36.00	36.00	
6	4. 燃料动力费			
7	5. 出版/文献/信息传播/知识产权事务费	9.00	9.00	
8	6. 会议/差旅/国际合作交流费			
9	7. 培训费			
10	8. 劳务费			
11	10. 专家咨询费	3.00	3.00	
12	11. 基本建设费			
13	(1) 房屋建筑物构建			
14	(2) 专用设备购置			
15	(3) 基础设施建设			
16	(4) 大型修缮			
17	(5) 信息网络建设			
18	(6) 其他基本建设支出			
19	12. 其他费用			
20	(二) 间接费用			
21	二、项目(课题)资金来源			
22	(一) 省级财政专项资金	85.00	85.00	/
23	(二) 其他渠道资金		/	
24	1. 单位自筹资金		/	
25	2. 其他资金		/	

六、项目（课题）单位提供的技术与条件保障

（包括现有技术基础和承诺提供的支撑条件，如仪器设备、水电、燃料、环保等条件）

1. 组织方式和机制

项目将成立由高校和企业负责人组成的项目领导小组，负责项目的整体规划和协调。邀请行业内知名专家组成技术专家组，提供技术指导和咨询，确保项目的技术水平和创新性。建立健全的项目管理制度，明确职责分工，确保项目按计划高效推进。

2. 产学研结合

充分发挥高校的科研优势 and 企业的市场资源，开展深度的产学研合作。依托“广东连山山羊科技小院”“永根科技站”等产学研合作平台，促进技术研发、成果转化和产业推广。通过产学研结合，加速科技成果的转化应用，提升产业竞争力。

3. 创新人才队伍的凝聚和培养

项目实施过程中，将注重创新人才队伍的凝聚和培养。通过项目研究，培养一批高水平的专业技术人才，提升团队的科研和创新能力。加强团队建设，促进成员之间的合作与交流，形成稳定、高效的创新团队。为清远黑山羊产业的持续发展提供人才保障。

4. 预算资金管理情况

统筹安排建设资金，严格按照有关财务制度及有关规定，科学、合理使用项目资金，确保资金使用效益。设立本项目资金专用账户，按照“统一规划、专账核算、专款专用”的原则，确保项目建设进度和预期目标。另外，加强财务管理和预算管理，建立科学化、精细化经费预算管理机制，专款专用，独立核算；严格控制经费超支，确保经费使用规范、安全、有效。

七、参与人员

(一) 项目(课题)负责人

姓 名	性 别	年 龄	职务职称	业务专业	为本项目 (课题)工 作时间(人 月)	所在单位	职责分工
孙宝丽	女	43	教授	动物科学	10	华南农业大 学	项目管理, 生产技术研究

(二) 主要研究人员

姓 名	性 别	年 龄	职务职称	业务专业	为本项目 (课题)工 作时间 (人月)	所在单位	职责分工
柳广斌	男	42	讲师	动物科学	6	华南农业大 学	讲师
邓铭	男	38	高级实验师	动物科学	10	华南农业大 学	高级实验师
刘德武	男	58	教授	动物科学	2	华南农业大 学	教授
陈永晴	男	40	副教授	农技推广	8	华南农业大 学	副教授
贾坤	男	43	副教授	动物医学	6	华南农业大 学	副教授
李耀坤	男	38	副教授	动物科学	2	华南农业大 学	副教授
郭勇庆	男	43	副教授	动物科学	6	华南农业大 学	副教授
田兴国	男	48	副教授	食品科学	6	华南农业大 学	副教授
毕雪	女	40	讲师	动物科学	10	广东科贸职 业学院	讲师
王亚欣	女	39	副教授	动物科学	6	广东科贸职 业学院	副教授
方心灵	男	39	副教授	动物科学	6	广东科贸职 业学院	副教授
张君	男	42	副教授	动物科学	6	广东科贸职 业学院	副教授

(三) 主要管理及其他支撑服务人员

姓 名	性 别	年 龄	职务职称	业务专业	为本项目 (课题)工 作时间 (人月)	所在单位	职责分工
王帅彬	男	30	高级工程师 /总经理	经营管理	10	广东领头羊 智慧农业有 限公司	参与单位项 目组织与实 施
尚帅	男	35	副总经理	产品开发	10	广东领头羊 智慧农业有 限公司	产品开发及 项目实施
周多恩	男	34	场长	养殖	10	广东领头羊 智慧农业有 限公司	生产管理及 项目实施

八、项目（课题）牵头承担单位与参与单位间的合作研究、知识产权分享、产业化等协议情况

联合申报项目协议书

甲方：华南农业大学

乙方：广东科贸职业学院

丙方：广东领头羊智慧农业有限公司

甲方、乙方、丙方经友好协商决定联合申报 2024 年度 省级种业振兴行动和科技兴农专项资金 项目，项目名称：清远黑山羊标准化养殖技术与全产业链产品开发综合研究。并达成如下合作协议：

第一条：项目研究工作详细分工：

甲方(主持方)：负责项目的主持申报与管理；建立适合清远黑山羊半放牧半舍饲养殖模式的技术方案；开发并建立清远黑山羊良种高效繁殖技术体系；制定清远黑山羊养殖、加工、产品质量等标准；开展技术指导与培训工作，负责项目报告撰写及上传。

乙方(参与方)：协助甲方进行项目申报；协助甲方开展清远黑山羊养殖技术的开发工作，重点负责清远黑山羊围产期母羊及羔羊饲养管理技术开发并形成技术方案；开展技术指导与培训工作；协助甲方完成项目报告撰写。

丙方(参与方)：负责实验羊群饲养管理，协助甲方和乙方开展技术开发及示范工作，提供必要的工作条件；重点负责预制菜产品开发及清远黑山羊品牌宣传工作。

第二条：经费分配：

1、项目申报政府资助经费 300 万元，甲方、乙方、丙方同意此经费按甲方 200 万元、乙方 15 万元、丙方 85 万元进行分配。如果政府下达的财政资助经费高于或低于 300 万元，则按相应的比例调增或调减。

2、甲方在收到 省财政资金 下达的资助经费后的一个月内将乙方、丙方所占经费支付给乙方、丙方指定帐户。

乙方账户信息：

账 户：广东科贸职业学院

帐 号：44067901040004185

开户行：农行广州石井支行

丙方账户信息：

账 户：广东领头羊智慧农业有限公司

帐 号：2018022609200017071

开户行：中国工商银行股份有限公司连山支行

第三条：企业配套经费比例： 无。

第四条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有；各方共同完成的由各方共享，具体按照各方的贡献大小进行分配或各方另行商定。

②共同完成的项目成果的转让，须在各方同意的前提下进行，任何一方不得私自转让或许可实施。

2. 项目成果申报各级奖项，各方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第五条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

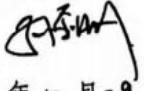
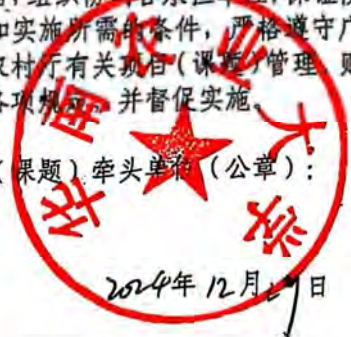
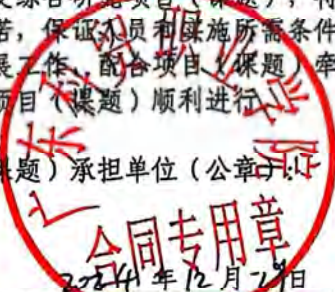
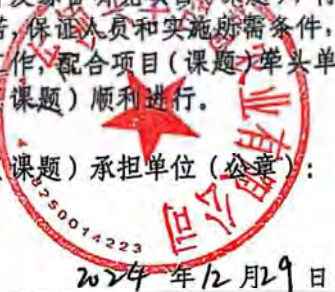

第六条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第七条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第八条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第九条：本协议自各方签字盖章之日起生效，至项目完成之日起终止；若合作申请未获资助，本协议自动废止。

九、任务合同书签订各方签章

项目 (课题) 负责人和 牵头承担 单位	<p>我同意主持广东省农业农村厅清远黑山羊标准化养殖技术与全产业链产品开发综合研究项目(课题),将按照申请书、项目立项通知和任务合同书组织实施本项目(课题),严格遵守广东省农业农村厅关于项目(课题)管理、财务管理等各项规定,按时报送有关材料,及时报告重大情况变动。</p> <p>项目(课题)负责人(签章):  2024 年 12 月 29 日</p>	<p>我单位同意组织实施广东省农业农村厅清远黑山羊标准化养殖技术与全产业链产品开发综合研究项目(课题),将履行有关承诺,组织协调各承担单位,保证队伍的稳定和实施所需条件,严格遵守广东省农业农村厅有关项目(课题)管理、财务管理等各项规定,并督促实施。</p> <p>项目(课题)牵头单位(公章):  2024 年 12 月 29 日</p>
参与 单位	<p>我单位同意共同承担广东省农业农村厅清远黑山羊标准化养殖技术与全产业链产品开发综合研究项目(课题),将履行有关承诺,保证人员和实施所需条件,按计划开展工作,配合项目(课题)牵头单位保障项目(课题)顺利进行。</p> <p>项目(课题)承担单位(公章):  2024 年 12 月 29 日</p>	<p>我单位同意共同承担广东省农业农村厅清远黑山羊标准化养殖技术与全产业链产品开发综合研究项目(课题),将履行有关承诺,保证人员和实施所需条件,按计划开展工作,配合项目(课题)牵头单位保障项目(课题)顺利进行。</p> <p>项目(课题)承担单位(公章):  2024 年 12 月 29 日</p>
参与 单位	<p>我单位同意共同承担广东省农业农村厅**项目(课题)(编号:**),将履行有关承诺,保证人员和实施所需条件,按计划开展工作,配合项目(课题)牵头单位保障项目(课题)顺利进行。</p> <p>项目(课题)承担单位(公章): 年 月 日</p>	<p>我单位同意共同承担广东省农业农村厅**项目(课题)(编号:**),将履行有关承诺,保证人员和实施所需条件,按计划开展工作,配合项目(课题)牵头单位保障项目(课题)顺利进行。</p> <p>项目(课题)承担单位(公章): 年 月 日</p>
广东 省农 业农 村厅 填写	<p>广东省农业农村厅审核意见:  同意按计划执行</p> <p>(盖章): 2025 年 2 月 6 日</p>	

凭证信息					
凭证内码:	25050108035	分录编号:	12	摘要:	划拨转/零B745-清远黑山羊标准化技术开子卡F25028
部门编号:	4300	部门名称:	动物科学学院		
项目编号:	F25028	项目名称:	转/零B745-清远黑山羊快速良种快速扩繁关键技术建立		
科目编号:	6001020201	科目名称:	其他农业支出 (213-01-99)		
经济分类科目编号:		经济分类科目名称:			
借金额:	0.00	贷金额:	1,000,000.00		
银行账号:		结算单号:		对方单位:	
支付状态:		打卡人:			

打印时间: 2025-07-14 16:28:42
打印单位: 华南农业大学

华南农业大学科研经费入账通知单（纵向）

单号：L20250134

财务处：

单位：元

由我校 动物科学院

柳广斌 老师负责的纵向科研课题，现有经费到账。

请按照下表的具体情况给予办理经费入账并开具相应的票据。

来款金额（元）	3000000	来款日期	20241230	经费卡号	F25 028
项目名称	清远黑山羊良种快速扩繁关键技术建立				
来款单位	广东省财政厅零余额				
到位金额	1000000	学校管理费（4%）		性质备注	混合经费
外拨金额	0	学院管理费（2%）			40000
可支配金额	1000000	课题组			20000
票据类型	开纵向新卡				办理日期
					20250519

注：

审核：

请依次到以下科室办理214

华南农业大学
South China Agricultural University

受理编号: c21140500002195

项目编号: 2021A1515010636

文件编号: 粤基金字(2021)4号

广东省基础与应用基础研究基金项目

合同书

项目名称: miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究

项目类别: 广东省自然科学基金-面上项目

项目起止时间: 2021-01-01 至 2023-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 柳广斌

联系电话: 18802085530



(广东科技微信公众号)



(查看合同书信息)



(受理纸质材料二维码)

广东省基础与应用基础研究
基金委员会
二〇二〇年制

填写说明

- 一、项目合同书/任务书内容原则上要求与申报书相关内容保持一致，不得无故修改。
- 二、项目承担单位通过广东省科技业务管理阳光政务平台下载项目合同书/任务书，按要求完成签名盖章后提交至省科技厅受理窗口。
- 三、签名盖章说明。请分别在单位工作分工及经费分配情况页、人员信息页、签约各方页等地方按要求签字或盖章，签章不合规或错漏将不予受理。其中，人员信息页要求所有参与人员本人亲笔签名，代签或印章无效，漏签将不予受理。
- 四、本合同书/任务书自签字并加盖公章之日起生效，各方均应负本任务书的法律责任，不应受机构、人事变动影响。

2021A1515010636

一、主要研究内容和要达到的目标

1. 研究目标

- (1) 验证miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用。
- (2) 验证miR-128及miR-450与候选靶基因的靶向关系。
- (3) 验证miR-128及miR-450借由靶基因对细胞功能及相关通路活性调控的作用。

2. 研究内容

(1) miR-128及miR-450对卵泡颗粒细胞增殖及凋亡的调控作用研究

合成miR-128及miR-450过表达mimics及抑制表达inhibitor, 利用细胞转染分别在KGN细胞(人卵泡颗粒细胞癌细胞)及山羊卵泡颗粒细胞中过表达及抑制表达miR-128及miR-450, 通过对细胞生长周期、增殖与凋亡的表型及标志物的检测, 验证miR-128及miR-450对卵泡颗粒细胞增殖及凋亡的调控作用。

(2) miR-128及miR-450靶基因验证研究

对前期研究预测筛选的候选靶基因, 构建3' UTR双荧光素酶报告载体, 通过miRNA mimics与靶基因3' UTR双荧光素酶报告载体的共转染及酶标检测, 在细胞中验证miR-128及miR-450与候选靶基因的靶向关系。同时通过在KGN细胞中过表达及抑制表达miRNA的方式, 利用Western Blot验证miR-128及miR-450对靶基因蛋白水平的调控作用。

(3) miR-128及miR-450借由靶基因对卵泡颗粒细胞及相关信号通路进行调控的验证研究

构建靶基因表达载体及并合成siRNA, 通过靶基因过表达、抑制表达(RNAi)及补偿表达实验, 以及对细胞表型及相关标志物的检测, 验证靶基因对细胞增殖、凋亡以及相关信号通路活性的调控功能。再通过miRNA过表达, 配合内源性靶基因3' UTR突变以及靶基因补偿实验, 进一步在卵泡颗粒细胞中验证miR-128及miR-450借由靶基因对细胞增殖、凋亡及相关通路活性的调控作用, 揭示miR-128及miR-450在卵泡颗粒细胞中的调控机制。

3. 拟解决的关键问题

- (1) miR-128及miR-450对卵泡颗粒细胞增殖及凋亡具有怎样的调控作用?
- (2) miR-128及miR-450的靶基因是什么? 通过哪条信号通路对卵泡颗粒细胞发挥调控作用?

二、项目预期获得的科研成果及形式

论文及专著情况	国家统计局刊物以上刊物 发表论文（篇）		2		科技报告（篇）		1	
	其中被SCI/EI/ISTP收录 论文数（篇）		1		培养人才（人）		1	
	专著（册）				引进人才（人）			
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权

2021A1515010636

三、项目进度和阶段目标

(一) 项目起止时间: 2021-01-01 至 2023-12-31		
(二) 项目实施进度及阶段主要目标:		
开始日期	结束日期	主要工作内容
2021-01-01	2021-12-31	合成miRNA mimic及inhibitor, miRNA过表达及抑制表达细胞实验, 细胞周期、增殖及凋亡表型及标志物活性检测, 验证miRNA对细胞功能的调控作用; 双荧光素酶报告系统及Western Blot验证靶基因。
2022-01-01	2022-12-31	靶基因表达载体及siRNA合成, 靶基因过表达及RNAi细胞实验, 细胞周期、增殖及凋亡表型及标志物活性检测, 信号通路活性标志物检测, 验证靶基因对细胞功能及相关信号通路活性的调控作用。
2023-01-01	2023-12-31	CRISPR/Cas9点突变载体构建, 靶基因点突变细胞系建立, miRNA过表达实验, 细胞周期、增殖及凋亡表型及标志物活性检测, 信号活性标志物检测, 验证miRNA借由靶基因对细胞功能及相关信号通路活性的调控作用。撰写研究报告及论文。

四、项目总经费及省基金委经费预算

1. 省基金委经费下达总额：（大写）壹拾万圆整；（小写）10万元；

2. 省基金委经费年度下达计划：

年度	2021 年	年	年	年	年
经费(万元)	10.00				

3. 总经费及省基金委经费开支预算计划：

经费筹集情况：					(单位：万元)
省基金委经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
10.00				0	10.00

政府部门、境外
资金及其他资金
投入情况说明：

与本项目相关的其他经费来源

(单位：万元)

其他计划资助经费：

单位配套经费：

其他经费资助：

其他经费来源合计：

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
柳广斌	210103198204261211	39	男	讲师	博士研究生	项目负责人	华南农业大学	柳广斌

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
李耀坤	410423198607084036	35	男	副教授	博士研究生	实验设计及数据分析	华南农业大学	李耀坤
邓铭	430726198601203910	35	男	实验师	硕士研究生	实验准备及管理	华南农业大学	邓铭
刘德武	42010619660711563X	55	男	教授	博士研究生	项目设计指导及数据分析	华南农业大学	刘德武
孙宝丽	411024198109241823	40	女	副教授	博士研究生	实验设计及数据分析	华南农业大学	孙宝丽
郭勇庆	13042119810616031X	40	男	讲师	博士研究生	材料采集及实验实施	华南农业大学	郭勇庆
江声伟	340811199705135514	24	男	未取得	硕士研究生	分子及细胞实验实施	华南农业大学	江声伟
邱金戈	441781199510120038	26	男	未取得	硕士研究生	分子及细胞实验实施	华南农业大学	邱金戈

六、工作分工及经费分配

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省基金委经费分配 (万元)
华南农业大学	负责项目实验设计、实验实施、实验结果及数据分析、研究报告撰写及论文发表工作。	10.00	10.00
	合计	10.00	10.00

2021A1515010636

七、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定，为顺利完成（2021）年 miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究 专项项目（文件编号：粤基金字（2021）4号）经协商一致，特订立本合同，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 乙方是项目资金管理的责任主体，应当建立健全科研项目资金管理制度，严格按照省科技经费使用范围和有关规定管好用好财政资金；应当按合同书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 实施“包干制”的面上项目及青年基金项目，依托单位应参照国家杰出青年科学基金试点项目经费使用“包干制”要求，制定经费使用“包干制”内部管理规定。项目经费支出应实际用于研发活动相关支出，使用范围限于设备费、材料费、测试化验加工费、燃料动力费、差旅/会议/国际合作与交流费、出版/文献/信息传播/知识产权事务费、劳务费、专家咨询费、依托单位管理费用、绩效支出以及其他合理支出。依托单位管理费用由依托单位根据实际管理支出情况与项目负责人协商确定。绩效支出由项目负责人根据实际科研需要和相关薪酬标准自主确定，依托单位按照现行工资制度进行管理。其余用途经费无额度限制，由项目负责人根据实际需要自主决定使用。项目验收时应提交经费决算表。
4. 项目负责人是项目资金使用的直接责任人，对资金使用的合规性、合理性、真实性和相关性承担法律责任。
5. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
6. 项目合同任务完成后，或合同书规定的任务、指标及经费投入等提前完成的，乙方可按照《广东省省级科技计划项目结题管理实施细则（试行）》提出验收结题申请，并按甲方要求做好项目验收结题工作。
7. 若项目发生需要终止结题的情况，乙方须按照《广东省省级科技计划项目结题管理的实施细则（试行）》提出终止结题申请，并按甲方要求做好项目终止结题工作。
8. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
9. 按照国家和省有关规定，提交科技报告及其他材料。
10. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。

11. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。

12. 确保本项目开展的研究工作符合我国科研伦理管理相关规定。

第四条 在履行本合同的过程中，如出现广东省政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本合同的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第八条 本合同一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目合同监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目合同的履行进行审核、进度调查，对项目合同变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

2. 委托代理人签订本合同书的，应出具合法、有效的委托书。

八、本合同签约各方

管理单位（甲方）：

广东省基础与应用基础研究基金委员会（盖章）



法定代表人（或法人代理）：

曾路

（签章）

2021 年 03 月 24 日

依托单位（乙方）：

华南农业大学

（盖章）

法定代表人（或法人代理）：

刘雅斌

（签章）

联系人（项目主管）姓名：
（签章）

安沛（基金类基础科、实验室平台办、海外名师人事处）

安沛

Email: 414484016@qq.com

电话: 020-85283435 / 13535577893

开户单位名称：

华南农业大学

开户银行名称：

广东广州工行五山支行

开户银行帐号：

3602002609000310520

2021年 04月 13日

年 月 日

联系人（项目负责人）姓名：

柳广斌

（签名）柳广斌

Email: ghliu@scau.edu.cn

电话: 18802085530

2021年 4月 12日

项目编号:	2021A1515010636
资助类别:	广东省自然科学基金-面上项目
文件编号:	粤基金字〔2021〕4号

广东省基础与应用基础研究基金项目 验收书

项目名称:	miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究		
项目负责人:	柳广斌	财政经费:	10 (万元)
计划完成时间:	2021-01-01 至 2023-12-31		
实际完成时间:	2021-01-01 至 2023-12-31		
依托单位:	华南农业大学		
参与单位:			
验收形式:	材料验收		
联系人:	倪慧群	联系电话:	15920301530
填表日期:	2024-03-29		

广东省基础与应用基础研究基金委员会
二〇二〇年制



(广东科技微信公众号)



(查看验收书信息)



(受理纸质材料二维码)

一、项目人员信息表

项目负责人：				
姓名	证件号码	职称	承担任务	所在单位
柳广斌	210103198204261211	讲师	项目负责人	华南农业大学
主要研究人员：				
姓名	证件号码	职称	承担任务	所在单位
李耀坤	410423198607084036	副教授	实验设计及数据分析	华南农业大学
邓铭	430726198601203910	实验师	实验准备及管理	华南农业大学
刘德武	42010619660711563X	教授	项目设计指导及数据分析	华南农业大学
孙宝丽	411024198109241823	副教授	实验设计及数据分析	华南农业大学
郭勇庆	13042119810616031X	讲师	材料采集及实验实施	华南农业大学
江声伟	340811199705135514	未取得	分子及细胞实验实施	华南农业大学
邱金戈	441781199510120038	未取得	分子及细胞实验实施	华南农业大学

二、项目摘要

中文摘要:

卵泡发育是影响动物繁殖能力的重要因素,其主要通过控制每次生殖周期排出的可用于受精的成熟卵母细胞影响山羊的产羔数。颗粒细胞作为卵泡的组成细胞,其增殖凋亡直接影响着卵泡的发育与排卵数量。前期研究发现miR-128及miR-450在大卵泡颗粒细胞中显著上调表达,其可能对细胞增殖及凋亡的相关信号通路具有调控作用。

该研究利用细胞转染等技术分析miR-128-3p和miR-450-5p对卵泡颗粒细胞增殖凋亡的影响,通过双荧光素酶实验验证靶基因,并通过WB等实验分析miR-128-3p与miR-450-5p及其靶基因对颗粒细胞增殖凋亡相关信号通路的调控作用。

结果表明:(1)miR-128-3p靶向GHSR基因3' UTR区域影响PCNA、BCL2、BAX基因表达抑制KGN细胞的增殖并促进细胞凋亡。在增殖凋亡相关信号通路探究中,发现miR-128-3p能够激活MAPK-ERK通路和Notch信号通路。而GHSR抑制MAPK-ERK通路和Notch通路。(2)miR-450-5p靶向结合凋亡家族基因BAX的3' UTR区域抑制BAX基因表达影响BCL2和BAX蛋白表达,从而促进卵泡颗粒细胞增殖并抑制细胞凋亡。在增殖凋亡相关信号通路探究中,miR-450-5p能够激活AKT信号通路来促进颗粒细胞的增殖,沉默BAX基因同样可以激活AKT信号通路。

该研究揭示了miR-128及miR-450对卵泡颗粒细胞增殖凋亡的调控作用,为全面了解卵泡发育的分子调控机制提供了理论依据。

关键词:

山羊; 卵泡颗粒细胞; miR-128; miR-450; 基因调控

三、报告正文

报告正文:

1. 完成的主要内容、取得的主要成果、达到的目标及创新之处

(1) 完成的主要内容

①完成了miR-128及miR-450对卵泡颗粒细胞增殖及凋亡调控作用研究

通过在卵泡颗粒细胞中转染miR-128和miR-450过表达mimics以及inhibitor,发现miR-128可调节PCNA、BAX和BCL2基因的表达,进而抑制卵泡细胞增殖并促进细胞凋亡,miR-450可以调节BAX和BCL2基因表达,促进卵泡颗粒细胞增殖并抑制细胞凋亡。

②完成了miR-128及miR-450靶基因验证研究

通过对靶基因预测数据库及大小卵泡基因表达数据联合分析,筛选候选靶基因,并构建3' UTR双荧光素酶报告载体。通过miRNA mimics与靶基因3' UTR双荧光素酶报告载体的共转染及酶标检测,发现miR-128可以靶向GHSR,并抑制卵泡颗粒细胞的增殖并促进凋亡,miR-450可以靶向BMF基因促进卵泡颗粒细胞的增殖并抑制凋亡。

③完成了miR-128及miR-450借由靶基因对卵泡颗粒细胞及相关信号通路进行调控的验证研究

研究发现miR-128可激活MAPK-ERK通路和Notch通路;GHSR抑制MAPK-ERK通路和Notch通路;补偿表达GHSR基因能够消除miR-128对MAPK-ERK通路和Notch通路的激活调控。miR-450能够靶向抑制BMF基因从而激活AKT信号通路。

(2) 取得的主要成果

项目实施期间发表论文7篇,其中SCI论文4篇;培养硕士研究生2人;完成科技报告1份。

(3) 达到的目标

①验证了miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用。

②验证了miR-128及miR-450与候选靶基因的靶向关系。

③验证了miR-128及miR-450借由靶基因对细胞功能及相关通路活性调控的作用。

(4) 创新之处

研究验证了miR-128及miR-450对卵泡颗粒细胞增殖及凋亡的调控作用。筛选了miR-128及miR-450的靶基因及其可能参与调控的信号通路。该研究结果为进一步研究卵泡发育的调控机制提供参考依据。

2. 研究计划完成情况

本研究已按计划完成了预定研究工作。且超额完成了绩效指标:原计划发表2篇学术论文(其中1篇SCI论文),实际发表7篇学术论文(其中4篇SCI论文);原计划培养1名硕士研究生,实际培养2名。

3. 研究的应用前景

研究初步揭示了miR-128及miR-450对卵泡颗粒细胞增殖及凋亡的调控作用。研究结果为进一步研究卵泡发育的调控机制提供参考依据。

4. 人才培养方面

培养硕士研究生2人。

5. 成果推广及经济效益

本项目在实施期间共发表了7篇学术论文。

6. 进一步工作设想

在细胞水平上理解卵泡发育受到各种限制的阻碍。为了更好地了解miR-128和miR-450在卵泡发育中的作用及其分子机制,计划将miR-128导入动物卵巢或构建卵巢组织特异性miR-128或miR-450基因敲除动物模型。通过组织切片、免疫组织化学和激素测量来验证miR-128或miR-450对卵泡发育的影响。并利用多组学技术,如转录测序、核糖体分析、蛋白质组学和代谢组学来进一步研究miR-128或miR-450调节卵泡发育的分子机制。

实际参加研究人数	高级职称	中级职称	初级职称	博士后	博士生	硕士生	其他人员
	3	3	0	0	0	2	0
计划执行情况	时间方面		按原计划				
	内容方面		内容不变				

四、研究成果目录

序号	成果类型	成果或论文名称	主要完成者	成果说明	标注状况
1	论文	Transcriptome analysis reveals mRNAs and long non-coding RNAs associated with fecundity in the hypothalamus of high-and low-fecundity goat	Biwei Hou, Min Mao, Shucan Dong, Ming Deng, Baoli Sun, Yongqing Guo, Yaokun Li, Dewu Liu and Guangbin Liu	期刊论文 (Frontiers in veterinary science)	第二标注
2	论文	Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats	Shucan Dong, Biwei Hou, Chuang Yang, Yaokun Li, Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu, Guangb	期刊论文 (Genes)	第一标注
3	论文	miR-450-5p and miR-202-5p Synergistically Regulate Follicle Development in Black Goat. International Journal of Molecular Sciences	Guanghang Feng, Jie Liu, Zitao Lu, Yaokun Li, Ming Deng, Guangbin Liu, Baoli Sun, Yongqing Guo, Xian Zou	期刊论文 (Journal of Molecular Sciences)	第三标注
4	论文	Expression Pattern of Goat Uterine Fluids Extracellular Vesicles miRNAs during Peri-Implantation.	Yanshe Xie, Guangbin Liu, Xupeng Zang, Qun Hu, Chen Zhou, Yaokun Li, Dewu Liu and Linjun Hong.	期刊论文 (Cells)	第三标注
5	论文	不同预处理及分离方法提取山羊血浆外泌体效果的研究	毛敏, 林雅婷, 伍翠莹, 李耀坤, 刘德武, 柳广斌	期刊论文 (黑龙江畜牧兽医)	第一标注
6	论文	不同动物原代细胞的永生化方法及其应用	侯碧巍, 董书餐, 刘德武, 孙宝丽, 李耀坤, 郭勇庆, 邓铭, 柳广斌	期刊论文 (中国兽医杂志)	第一标注
7	论文	细胞自噬对动物卵泡发育调控的研究进展	董书餐, 侯碧巍, 邹炯, 李耀坤, 刘德武, 孙宝丽, 郭勇庆, 邓铭, 柳广斌	期刊论文 (中国畜牧兽医)	第一标注

8	硕士研究生学位论文	miR-128-3p调控山羊颗粒细胞增殖/凋亡的分子机制研究	江声伟	硕士研究生学位论文	标注
9	硕士研究生学位论文	miR-450-5p与miR-202-5p调控山羊卵泡颗粒细胞发育的分子机制研究	冯光航	硕士研究生学位论文	标注

五、科技成果统计表

期刊论文(含已录用)	论文数量及检索系统收录(篇)																						
	总数	中文期刊	SCI	EI	SSCI	ISTP	其他																
	7.00	3	4	0	0	0	0																
	JCR大类分区(篇)(中科院期刊分区)																						
	一区		二区			三区			其他														
	0		3			1			0														
	代表性论文(单篇最高影响因子)																						
	发表期刊名称						影响因子																
	Cells						4.30																
著作(不含论文汇编、成果汇编、文化艺术作品等)	数量(本)																						
	合计	专著			编著			译著															
	0	0			0			0															
	代表性著作名称	无																					
专利(件)	申请																						
	合计	发明专利		实用新型专利		外观设计专利		PCT国际专利															
	0	0		0		0		0															
	授权																						
	合计	发明专利		实用新型专利		外观设计专利		PCT国际专利															
	0	0		0		0		0															
标准(项)	总数	其中:		国标		行标		地标															
	0			0		0		0															
软件著作权(项)	0																						
人才培养	人数				按职称				人才(团队)计划(人次)														
	合计	博士后	博士	硕士	合计	正高	副高	中级	国家级	省部级	荣誉名称												
	2.00	0	0	2	0	0	0	0	0	0	无												
项目后续资助	国家级项目后续资助(经费单位:万元)																						
	国家自然科学基金						科技部项目(基础研究类)																
	项数		经费		项数		经费																
	0		0		0		0																

六、经费决算表

经费下达总额：（大写）	壹拾万圆整	（小写）	10
项目编号：	2021A1515010636	项目类型：	广东省自然科学基金-面上项目
项目名称：	miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究	项目负责人：	柳广斌
是否数学等纯理论基础研究项目： 否			
支出科目	经费支出(万元)		备注（说明）
1. 设备费	0.00		
2. 业务费	8.85		主要用于实验试剂耗材购买、差旅费及论文版面费
3. 直接人力资源成本	0.60		主要用于参与项目研究生的劳务费
4. 绩效支出	0.00		
5. 管理费用	0.50		用于项目承担单位管理费
6. 其他费用	0.00		
合计	9.95		

七、其他财务信息

1. 经费使用说明表

项目经费到位10万元，与预算经费相符。在项目执行期间，项目经费按计划有序支出，截止到2023年12月31日，支出经费累计9.95万元，支出进度为99.5%。

支出经费主要用于业务费8.85万元，占全部经费的88.5%，其中包括材料费5.46万元，测试费0.98万元，燃料动力费0.16万元，差旅费0.83万元，论文版面费1.43万元。其他部分包括：劳务费0.6万元，主要用于参与项目研究生的劳务费；管理费0.5万元。

2. 会计师事务所信息

会计师事务所名称	无
签字注册会计师	无
防伪报备编号	无

八、专家意见表

专家1评议表									
1. 项目基本信息									
负责人		柳广斌			项目名称		miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究		
项目编号		2021A1515010636			项目类别		广东省自然科学基金-面上项目		
项目金额		10（万元）			自筹金额		0（万元）		
项目承担单位		华南农业大学			项目参与单位				
2. 验收专家信息									
姓名		单位			职务职称		专家类别		
专家1		*****			副研究员		技术专家		
3. 任务书指标完成情况									
成果内容				任务书指标	负责人填写的完成数	专家核实完成数	完成率		
论文及专著情况	国家统计源刊物以上刊物发表论文（篇）			2	7	7	350%		
	被SCI/EI/ISTP收录论文数（篇）			1	4	4	400%		
	专著（册）			0	0	0	100%		
	科技报告（篇）			1	1	1	100%		
培养人才（人）				1	2	2	200%		
引进人才（人）				0	0	0	100%		
专利情况(项)	发明专利（件）	申请	0	0	0	100%			
		授权	0	0	0	100%			
	实用新型专利（件）	申请	0	0	0	100%			
		授权	0	0	0	100%			
	外观设计专利（件）	申请	0	0	0	100%			
		授权	0	0	0	100%			
	国外专利（件）	申请	0	0	0	100%			
		授权	0	0	0	100%			
其他	无			完成	完成	完成			
项目评价									
财务意见		经核查，本项目结余资金为0.05万元，违规使用的经费为0万元。							
验收结论		通过							
验收日期		2024-05-15							

2021A151501063029

专家2评议表

1. 项目基本信息

负责人	柳广斌	项目名称	miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究
项目编号	2021A1515010636	项目类别	广东省自然科学基金-面上项目
项目金额	10（万元）	自筹金额	0（万元）
项目承担单位	华南农业大学	项目参与单位	

2. 验收专家信息

姓名	单位	职务职称	专家类别
专家2	*****	副教授	技术专家

3. 任务书指标完成情况

成果内容			任务书指标	负责人填写的完成数	专家核实完成数	完成率
论文及专著情况	国家统计源刊物以上刊物发表论文（篇）		2	7	6	300%
	被SCI/EI/ISTP收录论文数（篇）		1	4	4	400%
	专著（册）		0	0	0	100%
	科技报告（篇）		1	1	1	100%
培养人才（人）			1	2	2	200%
引进人才（人）			0	0	0	100%
专利情况(项)	发明专利（件）	申请	0	0	0	100%
		授权	0	0	0	100%
	实用新型专利（件）	申请	0	0	0	100%
		授权	0	0	0	100%
	外观设计专利（件）	申请	0	0	0	100%
		授权	0	0	0	100%
	国外专利（件）	申请	0	0	0	100%
		授权	0	0	0	100%
其他	无		完成	完成	完成	

项目评价	有原创性 该项目揭示了miR-128及miR-450对卵泡颗粒细胞增殖凋亡调控机制，为卵泡发育的调控机制提供新认识。
财务意见	经核查，本项目结余资金为0.05万元，违规使用的经费为0万元。
验收结论	通过
验收日期	2024-05-16

专家3评议表

1. 项目基本信息

负责人	柳广斌	项目名称	miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究
项目编号	2021A1515010636	项目类别	广东省自然科学基金-面上项目
项目金额	10（万元）	自筹金额	0（万元）
项目承担单位	华南农业大学	项目参与单位	

2. 验收专家信息

姓名	单位	职务职称	专家类别
专家3	*****	副教授	技术专家

3. 任务书指标完成情况

成果内容			任务书指标	负责人填写的 完成数	专家核实完成 数	完成率
论文及 专著情 况	国家统计源刊物以上刊 物发表论文（篇）		2	7	7	350%
	被SCI/EI/ISTP收录论 文数（篇）		1	4	4	400%
	专著（册）		0	0	0	100%
	科技报告（篇）		1	1	1	100%
培养人才（人）			1	2	2	200%
引进人才（人）			0	0	0	100%
专利情 况(项)	发明专利 （件）	申请	0	0	0	100%
		授权	0	0	0	100%
	实用新型专利 （件）	申请	0	0	0	100%
		授权	0	0	0	100%
	外观设计专利 （件）	申请	0	0	0	100%
		授权	0	0	0	100%
	国外专利 （件）	申请	0	0	0	100%
		授权	0	0	0	100%
其他	无		完成	完成	完成	
项目评价						
财务意见		经核查，本项目结余资金为0.05万元，违规使用的经费为0万元。				
验收结论		通过				
验收日期		2024-05-15				

九、验收结论表

验收结论：

该项目专家验收结论为通过。

该验收结论经公示无异议。

广东省基础与应用基础研究基金委员会意见：

miR-128及miR-450对山羊卵泡颗粒细胞增殖及凋亡的调控作用研究项目（项目编号：2021A1515010636），经专家评审及公示后，最终的验收结论为通过。

广东省基础与应用基础研究基金委员会（盖章）



2024年07月19日

受理编号: c1530550100079

项目编号: 2015A020209122

文件编号: 粤科规财字[2015]150号



2015A020209122

广东省省级科技计划项目 合同书

项目名称: 广东湖羊高产耐热新品系多基因聚合育种技术开发与示范

计划类别: 农村科技领域

项目起止时间: 2015-07-01 至 2018-06-30

管理单位(甲方): 广东省科学技术厅

承担单位(乙方): 华南农业大学

乙方主管部门(丙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 柳广斌

联系电话: 18802085530

项目联系人: 柳广斌

联系电话: 020-85280283

广东省科学技术厅
二〇一四年制

一、项目实施内容

1. 湖羊在南方气候饲养条件下,其生产性能变化规律与环境温湿指数(THI)关系的研究

本项目以广东温氏食品集团股份有限公司羊场的湖羊群体(1000只能繁母羊)为研究对象。羊群采用全舍饲的饲养方式,自由采食及自由饮水,精粗饲料使用饲料搅拌机(TMR)混匀后每天2次统一定时投放。连续跟踪测定该羊群一年内在各个月中的群体干物质采食量、羔羊日增重及母羊受胎率等指标。比较不同月份间湖羊群体生产指标的变化情况,并结合全年舍内温度及相对湿度记录,分析湖羊生产性能与环境温湿指数(THI)关系,确定南方气候饲养条件下湖羊热应激反应的THI阈值范围,以方便指导在生产实际中及时采取降温措施来减少损失。

2. 湖羊热应激反应对其行为及生理生化指标的影响研究

以羊场一个栋舍的湖羊为研究对象(300只),测定该群体内每只羊在广东梅雨春季(3月)、高温夏季(8月)及凉爽秋季(11月)三个月中的生理指标,如呼吸频率、心跳数、肛温等。并在上述三个月中每个月随机抽取30只羊测定血液生化指标,如:总蛋白、血糖、尿素、胆固醇等基础生化指标,以及部分代谢酶的活力,例如钠钾ATP酶(Na^+ , K^+ -ATPase)、超氧化物歧化酶(SOD)、休克蛋白70(HSP 70)等。分析热应激反应对湖羊生理及血液生化指标的影响,确定湖羊热应激反应的特征性生理及生化指标,为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。

3. 湖羊耐热性基因筛选及多基因聚合分子育种技术开发

筛选羊群中夏季与秋季生理指标变化幅度最小和最大的极端个体组成高耐热组和低耐热组实验羊群。并在次年8月热应激环境条件下测定两组湖羊的血液生化指标。根据血液生化指标变化特征进一步从两组中选取耐热性最低和最高的极端个体,并在热应激环境条件下屠宰采集组织。通过高通量测序技术对肝脏组织进行基因表达谱扫描,比较高耐热性和低耐热性湖羊间肝脏组织的基因表达谱差异,获得高耐热性和低耐热性湖羊间的差异表达基因。利用生物信息学技术对获得的差异表达基因进行基因功能分析,并通过分子实验手段检测相关基因在羊群中的SNP基因型及分布情况,确定可用于湖羊耐热性能选育的SNP分子标记。结合与湖羊生产性能相关的分子标记,开发一套可用于培育湖羊高生长速度、高繁殖力、强耐热性肉用新品系的多基因聚合分子育种技术。

二、项目考核指标

1. 项目完成后提供的研究开发成果及形式 (须明确产品、专利、版权、标准等成果的类型及数量)

成果形式		成果数量	成果形式		成果数量
发明专利	申请		引进人才(人)		
	授权		培养人才(人)		3
实用新型专利	申请		科技人才奖励(人)		
	授权		技术标准制定	牵头(个)	
外观设计专利	申请			参与(个)	
	授权		科技报告(篇)		
国外专利	PCT受理		软件著作权(项)		
	授权		论文论著(篇)		3
获得国家级奖项(项)			其中: 被收录论文数(篇)	SCI	
获得省级奖项(项)				EI	
新服务(项)				ISTP	
新产品(或新材料、新装备、新品种(系))			新工艺(或新方法、新模式、新技术)		1
创新载体项目必填		技术服务数量(项)			
		服务企业数量(家)			
科技金融项目必填		开展培训宣讲活动场次(次)			
		服务企业数量(家)			
		帮助企业融资(万元)			
		引进专业机构(家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告(篇)(至少1篇)			
		研究总报告(篇)(至少1篇)			
		研究中期报告(篇)			
		研究分报告(篇)			
		调研报告(篇)			
		专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		核心期刊论文(篇)[以第一作者发表, 须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		培养人才(人)			

广东省科技计划项目合同书

	获国家级奖项(项)	
	获省级奖项(项)	
	其他(具体形式:用户填)	
其他成果及形式说明:		
2. 主要技术经济指标及社会效益		
累计新增销售收入(万元)		
累计新增利税(万元)		
其他主要技术经济指标及社会效益说明:		
项目负责人(签章): 柳广斌 2015年 10 月 30 日		

2015A020209122

三、项目进度和阶段目标

开始日期	结束日期	主要工作内容
2015-07-01	2016-06-30	湖羊群体生产性能数据收集；环境温湿指数数据收集；湖羊生理及生化指标测定；数据分析。
2016-07-01	2017-06-30	高耐热及低耐热湖羊筛选；组织样品采集及提取RNA；高通量测序；测序数据分析。
2017-07-01	2018-06-30	SNP检测；多基因聚合分子育种方案制定；撰写论文及项目报告。

四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
华南农业大学	本项目研究工作由申报单位独立组织实施完成。	15.00	15.00
	合计	15.00	15.00

五、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额：（大写）壹拾伍万圆整；（小写）15.00万元；						
2. 省科技厅经费年度下达计划：（第一期）15.00万元；（余额）0.00万元						
3. 总经费开支预算计划：						
经费筹集情况：						（单位：万元）
总投入经费：15.00						
	省科技厅经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费：						
新增经费：	15.00					15.00
政府部门、境外资金及其他资金投入情况说明：	<div style="position: relative; height: 300px;"> 2015A020209122 </div>					

新增经费预算:			(单位: 万元)	
	新增经费总额		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:				
1、直接费用:	14.25		14.25	
(1)设备费:				
(2)材料费:	3.55	购买实验羊及实验试剂及耗材	3.55	购买实验羊及实验试剂及耗材
(3)测试化验加工外协费:	4.60	高通量测序、血液生化指标检测、引物合成	4.60	高通量测序、血液生化指标检测、引物合成
(4)燃料动力费:				
(5)差旅费:	2.52	实验室与羊场之间的往返车费	2.52	实验室与羊场之间的往返车费
(6)会议费:				
(7)国际合作与交流费:				
(8)出版/文献/信息传播/知识产权事务费:	1.42	文献查新、论文版面费、研究生论文打印费	1.42	文献查新、论文版面费、研究生论文打印费
(9)租赁费:				
(10)人员费:	2.16	在读研究生补助	2.16	在读研究生补助
(11)专家咨询费:				
(12)直接费用其他支出:				
(13)科技金融服务体系其他费用:	0.00		0.00	
①信用评级补贴:				
②大赛场租:				
③特派员奖励与补贴:				
2、间接费用:	0.75		0.75	
科研管理费:	0.75	按项目经费5%支出	0.75	按项目经费5%支出
合计:	15.00		15.00	

六、人员信息

项目负责人情况

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
柳广斌	33	男	讲师	教师	博士研究生	项目主持, 羊场管理, 高通量测序, 数据分析	华南农业大学	柳广斌

主要研究开发人员

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
孙宝丽	34	女	副教授	教师	博士	生产指标测定, 数据分析	华南农业大学	孙宝丽
刘德武	49	男	教授	教师	博士	项目指导, 数据分析	华南农业大学	刘德武
马静云	41	女	副教授	教师	博士	生理生化指标测定, 数据分析	华南农业大学	马静云
王凯	25	男	未取得	在读研究生	硕士	SNP检测	华南农业大学	王凯
周多恩	25	男	未取得	在读研究生	硕士	生产性能测定, 羊场数据收集	华南农业大学	周多恩
廖迎新	23	女	未取得	在读研究生	硕士	高通量测序数据分析	华南农业大学	廖迎新

七、承担、参与单位合作协议（须与申报书中合作协议或意向书相一致）

无

2015A020209122

八、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定，为顺利完成（2015）年广东湖羊高产耐热新品系多基因聚合育种技术开发与示范专项项目（项目编号：2015A020209122）经协商一致，特订立本合同，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按合同书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
4. 项目实施完成或实施到一定程度，须按照《广东省省级科技计划项目结题管理的实施细则（试行）》提出验收或终止结题的申请，并按甲方要求做好项目结题工作。
5. 在每年1月向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的效果等。
6. 按照国家和省有关规定，每年须提交年度科技报告；项目验收时，须提交验收科技报告。

第四条 在履行本合同的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本合同过程中，需要对项目起止时间、项目经费使用（包括自筹经费、经费分配及经费支出预算等）、项目内容（包括研发内容、技术指标、经济指标及成果指标等）、项目名称、项目承担单位（包括承担单位更名、承担单位替换）、参与单位、项目负责人和成员等进行变更的，甲乙双方按照《广东省省级科技计划项目合同书管理的实施细则（试行）》有关规定执行。

第六条 在履行本合同的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第七条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第八条 属技术保密的项目，甲乙双方应另行订立技术保密条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第九条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第十条 本合同的争议应由双方本着协商一致的原则解决，如双方协商不成的，则应向甲方所在地法院提起诉讼。

第十一条 保密条款：

1. 本合同保密内容范围为：

/

2. 本合同保密期限为：

/

3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。

4. 各方应建立技术秘密保护制度。

5. 属技术保密的项目必须经省负责技术保密部门审查后，确定可否发表或用于国际合作和交流。

第十二条 甲方可根据具体情况决定乙方是否需要单位担保，若需要保证单位，应订立担保条款，作为本合同正式内容一部分。当乙方不履行或不完全履行本合同，以及没有或没有完全承担违约责任时，乙方的保证单位承担连带保证责任。

第十三条 本合同一式六份，各份具有同等效力。甲方存三份，乙方存二份，丙方存一份，本合同自签字之日起生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

说明：本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

九、本合同签约各方

管理单位（甲方）：广东省科学技术厅（盖章）

单位地址：广州市连新路171号大院信息大楼

法定代表人（或授权代表）：黄宁生（签章）

联系人（经办人）姓名：林振亮（签章）

Email: linzl@gdstc.gov.cn

电话：020-83163905



年 月 日

承担单位（乙方）：华南农业大学（盖章）

二级部门：华南农业大学动物科学学院

单位地址：广东省广州市天河区五山路483号

法定代表人（或法人代理）：陈晓阳（签章）

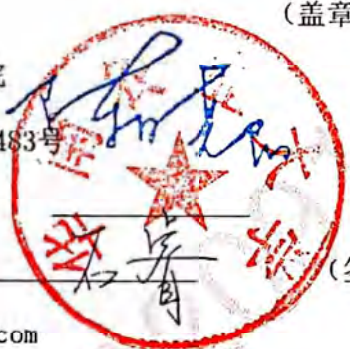
联系人（项目主管）姓名：石睿（签章）

Email: 77909213@qq.com

电话：020-85283435

开户单位名称：华南农业大学

开户银行及帐号：广东广州工行五山支行 3602002609000310520



年 月 日

乙方主管部门（丙方）：华南农业大学（盖章）

单位地址：广东省广州市天河区五山路483号

法定代表人（或法人代理）：陈晓阳（签章）

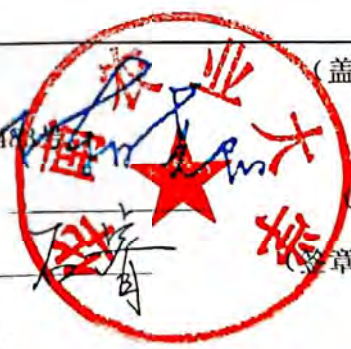
联系人（项目主管）姓名：石睿（签章）

Email: 77909213@qq.com

电话：020-85283435

开户单位名称：华南农业大学

开户银行及帐号：广东广州工行五山支行 3602002609000310520



年 月 日

项目编号: 2015A020209122



2015A020209122029

广东省科技计划项目验收书

项目名称:	广东湖羊高产耐热新品系多基因聚合育种技术开发与示范
下达文件编号:	粤科规财字[2015]150号
业务类别:	农业科技领域
承担单位(盖章):	华南农业大学
验收形式:	材料验收
组织验收单位:	华南农业大学
验收日期:	2019-03-28

广东省科学技术厅
二〇一五年制

二、研究开发主要内容(合同内容)

1. 湖羊在南方气候饲养条件下,其生产性能变化规律与环境温湿指数(THI)关系的研究

本项目以广东温氏食品集团股份有限公司羊场的湖羊群体(1000只能繁母羊)为研究对象。羊群采用全舍饲的饲养方式,自由采食及自由饮水,精粗饲料使用饲料搅拌机(TMR)混匀后每天2次统一定时投放。连续跟踪测定该羊群一年内在各个月中的群体干物质采食量、羔羊日增重及母羊受胎率等指标。比较不同月份间湖羊群体生产指标的变化情况,并结合全年舍内温度及相对湿度记录,分析湖羊生产性能与环境温湿指数(THI)关系,确定南方气候饲养条件下湖羊热应激反应的THI阈值范围,以方便指导在生产实际中及时采取降温措施来减少损失。

2. 湖羊热应激反应对其行为及生理生化指标的影响研究

以羊场一个栋舍的湖羊为研究对象(300只),测定该群体内每只羊在广东梅雨春季(3月)、高温夏季(8月)及凉爽秋季(11月)三个月中的生理指标,如呼吸频率、心跳数、肛温等。并在上述三个月中每个月随机抽取30只羊测定血液生化指标,如:总蛋白、血糖、尿素、胆固醇等基础生化指标,以及部分代谢酶的活力,例如钠钾ATP酶(Na^+ , K^+ -ATPase)、超氧化物歧化酶(SOD)、休克蛋白70(HSP70)等。分析热应激反应对湖羊生理及血液生化指标的影响,确定湖羊热应激反应的特征性生理及生化指标,为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。

3. 湖羊耐热性基因筛选及多基因聚合分子育种技术开发

筛选羊群中夏季与秋季生理指标变化幅度最小和最大的极端个体组成高耐热组和低耐热组实验羊群。并在次年8月热应激环境条件下测定两组湖羊的血液生化指标。根据血液生化指标变化特征进一步从两组中选取耐热性最低和最高的极端个体,并在热应激环境条件下屠宰采集组织。通过高通量测序技术对肝脏组织进行基因表达谱扫描,比较高耐热性和低耐热性湖羊间肝脏组织的基因表达谱差异,获得高耐热性和低耐热性湖羊间的差异表达基因。利用生物信息学技术对获得的差异表达基因进行基因功能分析,并通过分子实验手段检测相关基因在羊群中的SNP基因型及分布情况,确定可用于湖羊耐热性能选育的SNP分子标记。结合与湖羊生产性能相关的分子标记,开发一套可用于培育湖羊高生长速度、高繁殖力、强耐热性肉用新品系的多基因聚合分子育种技术。

三、研究开发主要内容(完成情况)

1. 湖羊在南方气候饲养条件下生产性能变化规律与环境温湿指数(THI)关系的研究

本研究以广东舍饲湖羊为研究对象。采用全舍饲的饲养方式,自由采食及自由饮水,精粗饲料使用饲料搅拌机(TMR)混匀后每天2次统一定时投放。连续跟踪测定该羊群一年内在各个月中的群体干物质采食量、羔羊日增重及母羊受胎率等指标。比较不同月份间湖羊群体生产指标的变化情况,并结合全年舍内温度及相对湿度记录,分析湖羊生产性能与环境温湿指数(THI)关系。

2. 湖羊热应激反应对其行为及生理生化指标的影响研究

研究以羊场湖羊为研究对象,测定该群体内每只羊在广东梅雨春季(3月)、高温夏季(8月)及凉爽秋季(11月)三个月中的生理指标,包括呼吸频率及肛温。根据测定结果将热应激期(8月)呼吸频率较快且肛温较高的湖羊归为A组,呼吸频率较平缓且肛温较正常的湖羊归为B组。对测定结果进行排序和筛选,选择A组表现极端的前15只湖羊为非耐热组,选择B组表现极端的前15只湖羊为耐热组,并对不同组湖羊进行标记。对筛选出来湖羊进行生理指标测定,同时记录测定时的舍内温度和湿度并估算温湿指数(THI)。并在8月和11月对选取的30只湖羊(非耐热组及耐热组)采集静脉血,测定血液生化指标。通过SAS等统计分析软件,综合分析热应激反应对湖羊生理及血液生化指标的影响。确定湖羊热应激反应的特征性生理及生化指标,为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。

3. 湖羊耐热性基因筛选及多基因聚合分子育种技术开发

(1) 湖羊耐热性基因筛选

在热应激(8月)及非热应激(11月)条件下,分别屠宰3只湖羊进行肝脏组织采集。在实验室条件下提取肝脏组织总RNA,并进行高通量表达谱测序。通过生物信息学分析,分析在热应激及非热应激条件下湖羊肝脏组织mRNA及LncRNA的表达变化情况,筛选出了可能与湖羊耐热性相关的候选基因。

(2) 湖羊多基因聚合分子育种技术开发

本研究在前人研究基础上进一步对绵羊高繁殖力候选基因BMPR-1B基因的多态性进行研究。研究结果发现其存在3个SNPs位点,且与湖羊的多胎性能有关联性,因此本研究进一步研究了该基因3个SNP位点的多基因聚合效应,筛选出了3个SNP位点的最佳组合基因型。

四、项目获得的成果

(主要指专利、论文及专著、动植物新品种、人才培养、新产品开发、工艺技术突破、运行机制等情况)

1. 揭示了湖羊在南方舍饲条件下的生产性能变化规律, 为湖羊在南方饲养及生产管理改进提供理论依据。
2. 揭示了湖羊在南方气候条件下热应激反应与环境THI变化的关系, 确定湖羊热应激反应的特征性生理及生化指标, 为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。
3. 揭示了湖羊在热应激条件肝脏组织的mRNA及lncRNA的变化规律, 同时挖掘了一批可能与绵羊热应激调控有关的候选基因及lncRNA, 为进一步的热应激机理研究打下基础。此外, 开了一种多基因聚合分子标记辅助选择方法, 通过对BMPR-1B基因的3个SNPs位点CCGGGA组合基因型的个体进行优选, 将可进一步提高湖羊的产羔数性状和加快湖羊的育种进程。
4. 发表学术论文3篇, 培养学生3人。

五、项目技术成果应用情况

项目研究探索湖羊热应激的生理生化变化规律，揭示了湖羊热应激反应与环境温湿度度的关系。同时研究还筛选了与湖羊耐热性能相关的基因以及提高湖羊生产性能的组合基因型。这些研究成果可指导湖羊生产并通过育种方法提高湖羊生产效率增加经济效益。

序号	应用单位	应用时间	应用效果
1	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
2	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
3	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
4	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
5	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
6	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
7	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
8	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
9	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
10	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
11	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
12	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
13	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
14	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
15	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
16	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
17	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
18	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
19	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能
20	广东省农业科学院畜牧研究所	2015.10-2016.12	筛选耐热基因，提高湖羊生产性能

六、项目考核指标

(一) 项目研究开发成果及形式情况					
合同签订的研究开发成果及形式			本项目实际获得的成果及形式		
成果形式		成果数量	成果形式		成果数量
发明专利	申请		发明专利	申请	
	授权			授权	
实用新型专利	申请		实用新型专利	申请	
	授权			授权	
外观设计专利	申请		外观设计专利	申请	
	授权			授权	
国外专利	PCT受理		国外专利	PCT受理	
	授权			授权	
获得国家级奖项 (项)			获得国家级奖项 (项)		
获省级奖项 (项)			获省级奖项 (项)		
新产品 (或新材料、新装备、新品种 (系))			新产品 (或新材料、新装备、新品种 (系))		
新工艺 (或新方法、新模式、新技术)		1	新工艺 (或新方法、新模式、新技术)		1
引进人才 (人)			引进人才 (人)		
培养人才 (人)		3	培养人才 (人)		3
科技人才奖励 (人)			科技人才奖励 (人)		
技术标准制定	牵头 (个)		技术标准制定	牵头 (个)	
	参与 (个)			参与 (个)	
软件著作权 (项)			软件著作权 (项)		
论文论著 (篇)		3	论文论著 (篇)		3
其中: 被收录论文数 (篇)	SCI		其中: 被收录论文数 (篇)	SCI	
	EI			EI	
	ISTP			ISTP	
新服务 (项)			新服务 (项)		
科技报告 (篇)			科技报告 (篇)		
创新载体项目必填	技术服务数量 (项)		创新载体项目必填	技术服务数量 (项)	
	服务企业数量 (家)			服务企业数量 (家)	

科技金融项目必填	开展培训宣讲活动场次(次)		科技金融项目必填	开展培训宣讲活动场次(次)	
	服务企业数量(家)			服务企业数量(家)	
	帮助企业融资(万元)			帮助企业融资(万元)	
	引进专业机构(家)			引进专业机构(家)	
院士工作站项目必填	引进院士及其团队科技成果转化数量		院士工作站项目必填	引进院士及其团队科技成果转化数量	
	院士开展的战略咨询和技术指导次数			院士开展的战略咨询和技术指导次数	
	院士年进站次数			院士年进站次数	
	院士及院士团队年进站时间			院士及院士团队年进站时间	
软科学项目必填	决策咨询报告(篇) (至少1篇)		软科学项目必填	决策咨询报告(篇) (至少1篇)	
	研究总报告(篇) (至少1篇)			研究总报告(篇) (至少1篇)	
	研究中期报告(篇)			研究中期报告(篇)	
	研究分报告(篇)			研究分报告(篇)	
	调研报告(篇)			调研报告(篇)	
	专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]	
	核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]			核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]	
	培养人才(人)			培养人才(人)	
	获国家级奖项(项)			获国家级奖项(项)	
	获省级奖项(项)			获省级奖项(项)	
	其他			其他	

其他成果及形式说明		其他成果及形式说明	
(二) 主要技术经济指标及社会效益			
合同经济指标		实际经济指标	
累计新增销售收入(万元)		累计新增销售收入(万元)	
累计新增利税(万元)		累计新增利税(万元)	
其它主要技术经济指标及社会效益:			
<div style="text-align: center;">2020209122</div>			
其它主要技术经济指标及社会效益完成情况:			
<div style="text-align: center;">2020209122</div>			
<div style="text-align: right;"> 项目负责人(签章): 柳斌 年 月 日 </div>			

七、承担/参与单位及工作分工

承担/参与单位名称	单位分工	分工完成情况
华南农业大学	本项目研究工作由申报单位独立组织实施完成。	项目负责人负责本项目所有资源的运用、员管理等，并已按合同要求完成相关研究工作。

八、项目经费及省科技厅经费使用情况

项目新增投资支出情况(万元)

省科技厅经费预算总额	壹拾伍万圆整		15.00
实际拨款经费	壹拾伍万圆整		15.00
支出经费	总经费	其中：省科技厅经费	其中：自筹资金
基建费	0		0
1、直接费用	14.25	14.25	0.00
(1)设备费	0	0	0
(2)材料费	3.46	3.46	0
(3)测试化验加工外协费	5.12	5.12	0
(4)燃料动力费	0	0	0
(5)差旅费	2.52	2.52	0
(6)会议费	0	0	0
(7)国际合作与交流费	0	0	0
(8)出版/文献/信息传播/知识产权事务费	0.99	0.99	0
(9)租赁费	0	0	0
(10)人员费	2.16	2.16	0
(11)专家咨询费	0	0	0
(12)直接费其他支出	0	0	0
(13)科技金融服务体系其他费用	0.00	0.00	0.00
①信用评级补贴	0	0	0
②大赛场租	0	0	0
③特派员奖励与补贴	0	0	0
2、间接费用	0.75	0.75	0.00
管理费	0.75	0.75	0
合计	15.00	15.00	0.00

项目负责人：柳广斌

财务负责人(并加盖财务公章)



已经投入资金(万元)	
合计:	15.00
其中政府资金(不含科技厅):	0
其中科技厅资金:	15.00
其中本企业资金:	0
其中贷款资金:	0
境外资金:	0
其他资金:	0

项目负责人: 柳斌

财务负责人(并加盖财务公章)



九、人员信息

参加项目工作人员	合计	7	
	按职称分类	高级职称:	3
		中级职称:	1
		初级职称:	
		其他人员:	3
	按学历分类	博士:	4
		硕士:	
		本科:	3
		大专:	
		其它:	
	其他分类	留学归国人员:	
		聘用外国专家:	

培养人员情况		
累计培养人才(个)	合计:	3
	取得博士学位:	
	取得硕士学位:	3
	取得副高以上技术职称:	

十、本申请项目所附附件清单

会计师事务所名称		
签字注册会计师		
防伪报备编号		
序号	附件名称	数量
1	项目下达文件	1
2	项目合同书	1
3	省科技计划项目实施工作总结报告	1
4	省科技计划项目技术总结报告	1
5	项目结题财务验收经费决算表	1
6	项目结题财务验收审计报告	0
7	产品测试报告	0
8	实物名称	0
9	用户使用报告	0
10	项目所获成果、专利一览表（含成果登记号、专利申请号、专利号等）	8
11	其他有关材料	0
12	广东省科技计划项目财务验收意见表	0
13	广东省科技计划项目验收意见表	6
14	科技报告收录证书	1

审核意见

1. 承担单位

同意验收组意见



2. 组织验收单位意见

同意验收组意见



3. 主管部门意见

同意验收组意见



(盖章) 年 月 日

4. 省科技厅管理部门意见

同意验收



(盖章) 年 月 日

附件2

广东省科技计划项目验收意见表

项目名称	广东湖羊高产耐热新品系多基因聚合育种技术开发与示范		
项目编号	2015A020209122	负责人	柳广斌
承担单位	华南农业大学		
验收专家成员			
姓 名	单 位	职务职称	签 名
黄运茂	仲恺农业工程学院	教授	
张辉华	佛山科学技术学院	教授	
刘小红	中山大学	研究员	

验收专家意见:

项目以湖羊为研究对象,研究湖羊在南方气候舍饲条件下的生产性能及生理生化指标变化。同时通过高通量测序及SNP检测等技术,挖掘与湖羊抗热性能及高生产性能相关的候选基因及SNP位点,为利用多基因聚合育种技术提高湖羊生产性能打下基础。

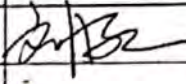
通过项目实施获得以下成果:(1)揭示了湖羊在南方舍饲条件下的生产性能变化规律,为湖羊在南方饲养及生产管理改进提供理论依据。(2)揭示了湖羊在南方气候条件下热应激反应与环境THI变化的关系,确定湖羊热应激反应的特征性生理及生化指标,为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。(3)揭示了湖羊在热应激条件肝脏组织的mRNA及lncRNA的变化规律,同时挖掘了一批可能与绵羊热应激调控有关的候选基因及lncRNA,为进一步的热应激机理研究打下基础。同时研究还对BMPR-1B基因的3个SNPs位点进行了多基因聚合分析,发现了CCGGGA的最佳基因型组合,为湖羊分子育种提供依据。

本项目经费使用合理,实施过程中发表了3篇学术论文并培养了3名研究生,达到了考核指标要求,因此验收合格。

验收结论: ☒ 通过 ☐ 不通过
验收等级: ☒ 合格 ☐ 良好

验收专家组组长签字:
日 期:

广东省科技计划项目验收意见表

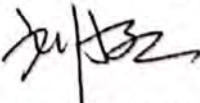
项目名称	广东湖羊高产耐热新品系多基因聚合育种技术开发与示范		
承担单位	华南农业大学		
项目编号	2015A020209122	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
刘小红	中山大学	教授	

该项目针对湖羊在广东地区集约化养殖过程中出现的问题，系统地研究在南方气候饲养条件下湖羊生产性能变化规律及与环境温湿度的关系，确定了引起湖羊热应激反应的环境温湿指数阈值范围，指导生产中及时采取降温措施，减少损失降低成本。同时该项目还研究了湖羊热应激反应对其生理及血液生化指标变化的影响，明确了可用于检测湖羊热应激反应的生化指标，为进一步开发预防及减少湖羊热应激反应的方法提供参考依据。此外本研究还将通过高通量测序技术对湖羊的耐热性基因进行筛选检测，挖掘可能与绵羊热应激调控有关的候选基因，同时检测与湖羊生产性能相关的 SNP 位点，为培育适合于广东地区集约化舍饲的湖羊品系打下基础。

该项目经费使用合理，且完成了合同规定的考核指标，因此考核合格。

验收结论：(✓) 通过 () 不通过
验收等级：(✓) 合格 () 良好

验收专家签字：
日期：


2019.3.20

广东省科技计划项目验收意见表

项目名称	广东湖羊高产耐热新品系多基因聚合育种技术开发与示范		
承担单位	华南农业大学		
项目编号	2015A020209122	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
张辉华	佛山科学技术学院	教授	

项目以湖羊为研究对象，研究湖羊在南方气候舍饲条件下的生产性能及生理生化指标变化。同时通过高通量测序及 SNP 检测等技术，挖掘与湖羊抗热性能及高生产性能相关的候选基因及 SNP 位点，为利用多基因聚合育种技术提高湖羊生产性能打下基础。

通过项目实施获得以下成果：(1) 揭示了湖羊在南方舍饲条件下的生产性能变化规律，为湖羊在南方饲养及生产管理改进提供理论依据。(2) 揭示了湖羊在南方气候条件下热应激反应与环境 THI 变化的关系，确定湖羊热应激反应的特征性生理及生化指标，为湖羊热应激个体的判断及未来预防与减少湖羊热应激反应的方法的开发提供参考依据。(3) 揭示了湖羊在热应激条件肝脏组织的 mRNA 及 lncRNA 的变化规律，同时挖掘了一批可能与绵羊热应激调控有关的候选基因及 lncRNA，为进一步的热应激机理研究打下基础。同时研究还对 BMPR-1B 基因的 3 个 SNPs 位点进行了多基因聚合分析，发现了 CCGGA 的最佳基因型组合，为湖羊分子育种提供依据。

本项目实施过程中发表了 3 篇学术论文并培养了 3 名研究生，达到了考核指标要求，因此验收通过。

验收结论：☒ 通过 () 不通过
验收等级：☒ 合格 () 良好

验收专家签字：

日期：

2019.3.19

广东省科技计划项目验收意见表

项目名称	广东湖羊高产耐热新品系多基因聚合育种技术开发与示范		
承担单位	华南农业大学		
项目编号	2015A020209122	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
黄运茂	仲恺农业工程学院	教授	

本项目以外省引进的湖羊为研究对象,研究其在广东地区舍饲条件下生产性能变化及热应激反应的规律,分析生产性能变化与温湿度的关系,为解决湖羊热应激问题提供依据。同时本项目还利用测序技术挖掘与湖羊热应激反应调控相关的候选基因,并通过多基因聚合分析寻找提高湖羊生产性能的最佳基因型组合,为进一步使用分子育种技术提高湖羊生产性能打下基础。本研究获得如下成果:


(1) 确定了在南方气候饲养条件下湖羊热应激反应的环境温湿指数阈值范围($THI < 75$ 无热应激),以指导羊场降温措施实施的时机,减少损失,降低成本。

(2) 研究了湖羊热应激反应对其生理及血液生化指标的影响,确定了湖羊热应激反应的特征性生理生化指标, T_4 可作为判断湖羊不同耐热能力的指标,为进一步研发预防及减少湖羊热应激反应的方法提供参考。

(3) 获得了一批对湖羊热应激调控相关的候选基因 *KCNMA1*、*VNN1*、*NR1H3*、*P4HB*、*NAT8* 及与之相关的 lncRNA,并发现了一种可提高湖羊繁殖性能的基因型组合,为分子育种技术在湖羊新品系培育中的应用打下基础。

(4) 发表学术论文 3 篇,培养学生 3 人,达到了项目考核要求。

验收结论: (✓) 通过 () 不通过
验收等级: (✓) 合格 () 良好

验收专家签字: 

日期:

2019.3.19

受理编号: c1730550100151

项目编号: 2017A020208050

文件编号: 粤科规财字[2017]50号

广东省省级科技计划项目

合同书

项目名称: 广东肉用黑山羊经济杂交配套方案开发

专项资金类别: 公益研究与能力建设

计划类别: 农村科技领域

项目起止时间: 2017-01-01 至 2019-12-31

管理单位(甲方): 广东省科学技术厅

承担单位(乙方): 华南农业大学

乙方主管部门(丙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 柳广斌

联系电话: 18802085530

项目联系人: 柳广斌

联系电话: 18802085530



(广东科技微信公众号)

广东省科学技术厅
二〇一七年制

(受理纸质材料二维码)

一、研发内容和关键技术

1. 研究目标

利用雷州黑山羊、川中黑山羊、努比亚黑山羊，进行不同组合的三元杂交，并对二元杂交子代及三元杂交子代的生长性能、繁殖能力、肉质等指标进行跟踪检测。通过不同组合之间配合力测定的结果比较，最终筛选出适合广东地区的肉用黑山羊经济杂交配套方案。

2. 研究内容

(1) 分别以雷州黑山羊、川中黑山羊、努比亚黑山羊为父本，以不同品种黑山羊为母本进行二元杂交。杂交的组合包括6种：1) 雷州黑山羊♀×川中黑山羊♂；2) 雷州黑山羊♀×努比亚黑山羊♂；3) 川中黑山羊♀×雷州黑山羊♂；4) 川中黑山羊♀×努比亚黑山羊♂；5) 努比亚黑山羊♀×雷州黑山羊♂；6) 努比亚黑山羊♀×川中黑山羊♂。对子代的生长性能、产肉性能及肉质等重要指标进行跟踪测定，同时培育二元杂交子代母羊做为三元杂交母本。

(2) 以不同杂交组合二元杂交子代母羊为母本，分别以雷州黑山羊、川中黑山羊、努比亚黑山羊为父本进行三元杂交。杂交组合包含6种：雷川♀×努比亚黑山羊♂；雷努♀×川中黑山羊♂；川雷♀×努比亚黑山羊♂；川努♀×雷州黑山羊♂；努雷♀×川中黑山羊♂；努川♀×雷州黑山羊♂。对子代的生长性能、产肉性能及肉质等重要指标进行跟踪测定。

(3) 整理全部二元杂交及三元杂交实验的相关数据，综合分析不同杂交组合下肉羊生长性能、产肉性能、繁殖性能及肉品质的综合配合力。比较不同杂交组合的差异及优劣势，最终确定出适合广东地区使用的肉用黑山羊经济杂交配套方案。

3. 拟解决的关键问题

利用杂交优势生产商品代是目前肉用畜禽生产的主要方式，但在肉用山羊生产中却少有成熟的杂交配套方案，这限制肉羊养殖生产的效率及肉羊养殖业的发展。为了解决这一问题，本项目选择国内优良的肉用黑山羊品种，包括广东本地雷州黑山羊、高繁型川中黑山羊及快大型努比亚黑山羊。通过对各品种间不同杂交组合方式的配合力测定，最终筛选出适合广东地区使用的肉用黑山羊经济杂交配套方案，为广东肉羊养殖业发展提供帮助。

4. 创新点

虽然我国黑山羊品种众多，但却少有成熟的用于生产肉用商品代的经济杂交配套方案。为了充分发挥广东本地黑山羊的品种优势，本项目以广东本地品种雷州黑山羊、高繁型川中黑山羊及快大型努比亚黑山羊为材料，通过不同的杂交组合方式将各种的优点集中于肉羊商品代，筛选出一套适合广东地区食用的黑山羊经济杂交配套方案。

二、项目考核指标

1. 项目完成后提供的研究开发成果及形式(须明确产品、专利、版权、标准等成果的类型及数量)

成果形式		成果数量	成果形式		成果数量
发明专利	申请		引进人才(人)		
	授权		培养人才(人)		
实用新型专利	申请		科技人才奖励(人)		
	授权		技术标准制定	牵头(个)	
外观设计专利	申请			参与(个)	
	授权		科技报告(篇)		
国外专利	PCT受理		软件著作权(项)		
	授权		论文论著(篇)		2
获得国家级奖项(项)			其中:被收录论文数(篇)	SCI	
获得省级奖项(项)				EI	
新服务(项)				ISTP	
新产品(或新材料、新装备、新品种(系))			新工艺(或新方法、新模式、新技术)		
创新载体项目必填		技术服务数量(项)			
		服务企业数量(家)			
科技金融项目必填		开展培训宣讲活动场次(次)			
		服务企业数量(家)			
		帮助企业融资(万元)			
		引进专业机构(家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告(篇)			
		研究总报告(篇)			
		研究中后期报告(篇)			
		研究分报告(篇)			
		调研报告(篇)			
		专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		培养人才(人)			

	获国家级奖项(项)	
	获省级奖项(项)	
	其他	
2. 其他成果及形式说明:		
1. 开发广东肉用黑山羊经济杂交配套方案1个。 2. 培养硕士研究生1名。		
3. 主要技术经济指标及社会效益		
累计新增销售收入(万元)		
累计新增利税(万元)		
4. 其他主要技术经济指标及社会效益说明:		
1. 开发广东肉用黑山羊经济杂交配套方案1个。 2. 发表学术论文1-2篇。 3. 培养硕士研究生1名。		
项目负责人(签章): <u>柳广斌</u> 2017年 6 月 20 日		

三、项目进度和阶段目标

开始日期	结束日期	主要工作内容
2017-01-01	2017-12-31	实验羊只筛选，并执行不同杂交组合方案的二元杂交。对子代的生产性能进行跟踪测定。
2018-01-01	2018-12-31	对二元杂交子代进行育成培育，并跟踪记录生长数据。根据不同杂交组合方案进行三元杂交。
2019-01-01	2019-12-31	对三元杂交子代进行生产性能测定。整理分析数据，选出最佳杂交配套组合方案。发表论文及撰写项目报告。

四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
华南农业大学	负责杂交配套方案的设计、生产性能测定及数据分析, 并对参与单位项目执行进行指导。	9.00	9.00
新兴县温氏新旺羊业有限公司	负责为项目实施提供场地、实验羊只、饲养人员, 并执行项目实施方案。并承担本项目除政府经费外额外资金的投入。	6.00	6.00
	合计	15.00	15.00



2017A020208050

五、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额：（大写）壹拾伍万圆整；（小写）15万元；						
2. 省科技厅经费拨付方式： 一次性拨款						
3. 省科技厅经费年度下达计划：（大写）壹拾伍万圆整；（小写）15万元；						
分期				经费(万元)		
第1期				15		
4. 总经费开支预算计划：						
经费筹集情况：						(单位：万元)
总投入经费：15.00						
	省科技厅经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费：						
新增经费：	15.00					15.00
政府部门、境外资金及其他资金投入情况说明： <div style="position: absolute; top: 50%; left: 50%; transform: translate(-50%, -50%) rotate(-45deg); opacity: 0.3; font-size: 2em; pointer-events: none;"> 2017A02020803 </div>						

新增经费预算:			(单位: 万元)	
	新增经费总额		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:	0	无		
1、直接费用:	14.25	无	14.25	无
(1) 设备费:	0	无		
(2) 材料费:	7.25	用于实验羊屠宰及试剂耗材购买	7.25	用于实验羊屠宰及试剂耗材购买
(3) 测试化验加工外协费:	4.20	用于部分肉质指标测定费用	4.20	用于部分肉质指标测定费用
(4) 燃料动力费:				
(5) 差旅费/会议费/国际合作与交流费:	1.08	用于实验室与羊场之间的往返车费	1.08	用于实验室与羊场之间的往返车费
(6) 出版/文献/信息传播/知识产权事务费:	1.00	论文版面费	1.00	论文版面费
(7) 劳务费:				
(8) 人员费:	0.72	用于在读研究生的补助	0.72	用于在读研究生的补助
(9) 专家咨询费:				
(10) 直接费用其他支出:				
(11) 科技金融服务体系其他费用:				
①信用评级补贴:				
②大赛场租:				
③特派员奖励与补贴:				
2、间接费用:	0.75	无	0.75	无
(1) 间接成本:				
(2) 管理成本:				
(3) 绩效支出:				
合计:	15.00	无	15.00	无

特别提醒: 2017年3月份, 广东省《关于进一步完善省级财政科研项目资金管理等政策的实施意见(试行)》(粤委办〔2017〕13号)出台, 对间接费用比例、劳务费开支范围、人员费用安排等进行了调整优化。为及时拨付2017年度科研经费, 平台直接提取申报书相关信息生成合同书并进行了预签订, 但各项目负责人、承担单位、主管部门须认真领会相关文件精神, 在合同书签订完成后2个月内通过平台提请项目经费变更或确认, 对相关经费开支进行细化完善, 否则, 将影响其科研信用评级或申报新的省级科技项目。

六、人员信息

项目负责人情况

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
柳广斌	35	男	讲师	教师	博士研究生	项目主持, 方案设计	华南农业大学	柳广斌

主要研究开发人员

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
李耀坤	30	男	讲师	教师	博士研究生	杂交方案设计	华南农业大学	李耀坤
孙宝丽	35	女	副教授	教师	博士研究生	数据分析	华南农业大学	孙宝丽
刘德武	50	男	教授	教师	博士研究生	方案设计, 项目指导	华南农业大学	刘德武
陈水龙	44	男	未取得	副总畜牧师	硕士研究生	项目实施管理	新兴县温氏新旺羊业有限公司	陈水龙
李和平	38	男	未取得	副经理	硕士研究生	项目实施管理	新兴县温氏新旺羊业有限公司	李和平
周多恩	26	男	未取得	无	硕士研究生	杂交方案执行	新兴县温氏新旺羊业有限公司	周多恩
何玉强	23	男	未取得	学生	硕士研究生	生产性能测定	华南农业大学	何玉强
杨新月	23	女	未取得	学生	硕士研究生	生产性能测定	华南农业大学	杨新月

七、承担、参与单位合作

甲方：华南农业大学

乙方：新兴县温氏新旺羊业有限公司

甲方与乙方经友好协商决定联合申报 2017年度广东省公益研究与能力建设专项项目，项目名称：广东肉用黑山羊经济杂交配套方案开发。达成如下合作协议：

第一条：项目分工：

甲方(主持方)：负责项目申报的各项具体工作；为乙方提供指导；负责项目各项管理和协调工作。

乙方(参与方)：承担除政府经费外额外资金的投入；负责提供本项目所需的场地和设备；配合甲方各项工作。

第二条：经费分配：

1、如果本申报项目获批立项，按政府下达的资助经费，甲方、乙方同意此经费分别按政府资助经费的甲方：60%、乙方：40%。

2、甲方在收到 政府下达的资助经费后的一个月将乙方所占经费支付给乙方指定帐户。

帐户：新兴县温氏新旺羊业有限公司；帐号：2020003209200136737；开户行：中国工商银行新兴支行。

第三条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有，对方有使用权；双方共同完成的由双方共享，具体按照双方的贡献大小进行分配或双方另行商定。

②项目成果的转让，须在双方同意的前提下进行，任何一方不得私自转让或许可实施，独立完成的除外。

2. 项目成果申报各级奖项，双方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第四条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第五条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第六条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第七条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第八条：本协议自双方签字盖章之日生效，若合作申请未获资助，本协议自动废止。

八、合同条款

第一条	甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定,为顺利完成(2017)年广东肉用黑山羊经济杂交配套方案开发专项项目(项目编号: 2017A020208050)经协商一致,特订立本合同,作为甲乙双方在项目实施管理过程中共同遵守的依据。
第二条	甲方的权利义务: 1. 按合同书规定进行经费核拨的有关工作协调。 2. 根据甲方需要,在不影响乙方工作的前提下,定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。 3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。
第三条	乙方的权利义务: 1. 确保落实自筹经费及有关保障条件。 2. 按合同书规定,对甲方核拨的经费实行专款专用,单独列账,并随时配合甲方进行监督检查。 3. 使用财政资金采购设备、原材料等,按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定,符合招标条件的须进行招标。 4. 项目实施完成或实施到一定程度,须按照《广东省省级科技计划项目结题管理的实施细则(试行)》提出验收或终止结题的申请,并按甲方要求做好项目结题工作。 5. 在每年1月向甲方如实提交上年度工作情况报告,报告内容包含上年度项目进展情况、经费决算和取得的效果等。 6. 按照国家和省有关规定,每年须提交年度科技报告;项目验收时,须提交验收科技报告。
第四条	在履行本合同的过程中,如出现广东省相关政策法规重大改变等不可抗力情况,甲方有权对所核拨经费的数量和时间进行相应调整。
第五条	在履行本合同过程中,需要对项目起止时间、项目经费使用(包括自筹经费、经费分配及经费支出预算等)、项目内容(包括研发内容、技术指标、经济指标及成果指标等)、项目名称、项目承担单位(包括承担单位更名、承担单位替换)、参与单位、项目负责人和成员等进行变更的,甲乙双方按照《广东省省级科技计划项目合同书管理的实施细则(试行)》有关规定执行。
第六条	在履行本合同的过程中,当事人一方发现可能导致项目整体或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。
第七条	本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享,除双方另有约定外,按国家和广东省有关法规执行。

第八条	属技术保密的项目，甲乙双方应另行订立技术保密条款，作为本合同正式内容的一部分，与本合同具有同等效力。
第九条	根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。
第十条	本合同的争议应由双方本着协商一致的原则解决，如双方协商不成的，则应向甲方所在地法院提起诉讼。
第十一条	保密条款： 1. 本合同保密内容范围为： / 2. 本合同保密期限为： / 3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。 4. 各方应建立技术秘密保护制度。 5. 属技术保密的项目必须经省负责技术保密部门审查后，确定可否发表或用于国际合作和交流。
第十二条	甲方可根据具体情况决定乙方是否需要单位担保，若需要保证单位，应订立担保条款，作为本合同正式内容一部分。当乙方不履行或不完全履行本合同，以及没有或没有完全承担违约责任时，乙方的保证单位承担连带保证责任。
第十三条	本合同一式六份，各份具有同等效力。甲方存三份，乙方存二份，丙方存一份，本合同自签字之日起生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。
说明：本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。	

九、本合同签约各方

管理单位（甲方）： 广东省科学技术厅 （盖章）

单位地址： 连新路171号

法定代表人（或授权代表）：

黄宁生



刘世伟

（签章）



2017-07-26
年 月 日

承担单位（乙方）： 华南农业大学 （盖章）

二级部门： 华南农业大学动物科学学院

单位地址： 五山路483号

法定代表人（或法人代理）： 陈晓阳

（签章）

联系人（项目主管）姓名： 石睿

（签章）

Email: kjcgxk@scau.edu.cn

电话： 020-85285390 / 13430350923

开户单位名称： 华南农业大学

开户银行及帐号： 广东广州工行五山支行 3602002609000310520

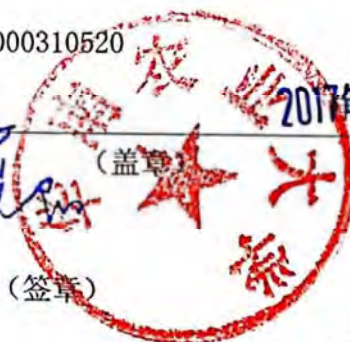
2017年 7月 2日

乙方主管部门（丙方）： 华南农业大学 （盖章）

单位地址： 五山路483号

法定代表人（或法人代理）： 陈晓阳

（签章）



2017年 7月 2日

项目编号: 2017A020208050



2017A020208050024

广东省科技计划项目验收书

项目名称:	广东肉用黑山羊经济杂交配套方案开发
下达文件编号:	粤科规财字[2017]50号
业务类别:	农村科技领域
承担单位(盖章):	华南农业大学
验收形式:	材料验收
组织验收单位:	华南农业大学
验收日期:	2020-08-01

广东省科学技术厅
二〇一五年制

说 明:

- 一、本验收表的各类信息必须如实填写,不得弄虚作假。
- 二、在验收时请同时提供广东省科技计划项目合同书及验收所需的具体材料。
- 三、如项目在验收的同时进行成果鉴定,验收专家组同时为成果鉴定专家组。
- 四、项目验收结束当年仍需填写“广东省科技计划项目年度执行情况调查表”。

一、项目基本信息

项目名称	广东肉用黑山羊经济杂交配套方案开发		
业务类别	农村科技领域		
承担单位	华南农业大学		
参与单位1	新兴县温氏新旺羊业有限公司		
参与单位2			
其他参与单位			
项目支持金额	15万元		
项目执行期	2017-01-01 至 2019-12-31		
项目负责人	柳广斌	联系电话	18802085530
项目联系人	柳广斌	电话	
传真		手机	18802085530
通信地址	广东省-广州市-天河区五山路483号		
邮政编码	510642		
验收日期	2020-08-01		

二、研究开发主要内容(合同内容)

1. 分别以雷州黑山羊、川中黑山羊、努比亚黑山羊为父本,以不同品种黑山羊为母本进行二元杂交。杂交的组合包括6种: 1) 雷州黑山羊♀X川中黑山羊♂; 2) 雷州黑山羊♀X努比亚黑山羊♂; 3) 川中黑山羊♀X雷州黑山羊♂; 4) 川中黑山羊♀X努比亚黑山羊♂; 5) 努比亚黑山羊♀X雷州黑山羊♂; 6) 努比亚黑山羊♀X川中黑山羊♂。对子代的生长性能、产肉性能及肉质等重要指标进行跟踪测定,同时培育二元杂交子代母羊做为三元杂交母本。
2. 以不同杂交组合二元杂交子代母羊为母本,分别以雷州黑山羊、川中黑山羊、努比亚黑山羊为父本进行三元杂交。杂交组合包含6种: 雷川♀X努比亚黑山羊♂; 雷努♀X川中黑山羊♂; 川雷♀X努比亚黑山羊♂; 川努♀X雷州黑山羊♂; 努雷♀X川中黑山羊♂; 努川♀X雷州黑山羊♂。对子代的生长性能、产肉性能及肉质等重要指标进行跟踪测定。
3. 整理全部二元杂交及三元杂交实验的相关数据,综合分析不同杂交组合下肉羊生长性能、产肉性能、繁殖性能及肉品质的综合配合力。比较不同杂交组合的差异及优劣势,最终确定出适合广东地区使用的肉用黑山羊经济杂交配套方案。

三、研究开发主要内容(完成情况)

1. 项目按任务书要求, 分别以雷州黑山羊、川中黑山羊、努比亚黑山羊为父本, 以不同品种黑山羊为母本进行二元杂交。杂交的组合包括6种: 1) 雷州黑山羊♀X川中黑山羊♂; 2) 雷州黑山羊♀X努比亚黑山羊♂; 3) 川中黑山羊♀X雷州黑山羊♂; 4) 川中黑山羊♀X努比亚黑山羊♂; 5) 努比亚黑山羊♀X雷州黑山羊♂; 6) 努比亚黑山羊♀X川中黑山羊♂。在实施过程中发现, 由于雷州黑山羊的体型比川中及努比亚黑山羊小, 导致在自然交配的情况下雷州黑山羊公羊爬跨川中及努比亚黑山羊母羊的成功较小, 因此在本研究中心以雷州黑山羊作为父本的杂交组合没有产生有效后代, 后续研究均以雷州黑山羊作为母本的杂交组合进行研究。研究对产生的子代生长性能、产肉性能及肉质等重要指标进行跟踪测定。
2. 以不同杂交组合二元杂交子代母羊为母本, 分别以川中黑山羊、努比亚黑山羊为父本进行三元杂交。交组合包含: 川雷♀X努比亚黑山羊♂, 努雷♀X川中黑山羊♂。对子代的生长性能进行跟踪测定。
3. 研究比较了不同杂交组合之间的效果, 筛选了适合广东地区使用的肉用黑山羊经济杂交配套方案。此外研究还统计分析不同黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律, 为舍饲黑山羊技术的开发与改进提供参考。

四、项目获得的成果

- (主要指专利、论文及专著、动植物新品种、人才培养、新产品开发、工艺技术突破、运行机制等情况)
1. 本研究统计分析不同黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律,结果显示川中黑山羊的胎均产羔数显著多于雷州黑山羊。造成舍饲山羊死亡的主要原因为肠炎及肺炎,且60日龄前羔羊死亡占总死亡数的50%以上。在未来的山羊舍饲过程中应着重关注羔羊阶段的饲养管理,防范肠炎及肺炎的发生。本研究的结果可为舍饲黑山羊技术的开发与改进提供参考。
 2. 本研究比较了雷州黑山羊、川中黑山羊及努比亚黑山羊之间不同杂交组合对子代生产性能的影响。结果显示无论是二杂交还是三元杂交,以川中黑山羊及努比亚黑山羊作为父本均可显著提高雷州黑山羊的生长速度和屠宰率,以川中和努比亚黑山羊作为父本的杂交之间差异不显著。但研究同时发现杂交也会降低雷州黑山羊肌肉的嫩度,因此利用川中或努比亚黑山羊杂交可以改善雷州黑山羊的生长及屠宰性能,但也会降低雷州黑山羊的肌肉品质。本研究结果可为进一步开发适合广东舍饲饲养的山羊新品种(品系)打下基础。
 3. 发表论文3篇,培养研究生1名。

五、项目技术成果应用情况

本研究揭示了不同黑山羊在华南地区舍饲条件下的繁殖性能及疫病发生规律，可为舍饲黑山羊技术的开发与改进提供参考。同时本研究明确了川中黑山羊及努比亚黑山羊作为父本对雷州黑山羊生产性能改良的效果，筛选了可用于雷州黑山羊改良的杂交组合方案，为进一步的雷州黑山羊新品系培育及养羊业发展打下基础。此外，在项目执行过程中培养了一名专业硕士研究生，为广东养羊业培养了专业人才。

2017A020208050024

六、项目考核指标

(一) 项目研究开发成果及形式情况					
合同签订的研究开发成果及形式			本项目实际获得的成果及形式		
成果形式		成果数量	成果形式		成果数量
发明专利	申请		发明专利	申请	
	授权			授权	
实用新型专利	申请		实用新型专利	申请	
	授权			授权	
外观设计专利	申请		外观设计专利	申请	
	授权			授权	
国外专利	PCT受理		国外专利	PCT受理	
	授权			授权	
获得国家级奖项 (项)			获得国家级奖项 (项)		
获省级奖项 (项)			获省级奖项 (项)		
新产品 (或新材料、新装备、新品种 (系))			新产品 (或新材料、新装备、新品种 (系))		
新工艺 (或新方法、新模式、新技术)			新工艺 (或新方法、新模式、新技术)		
引进人才 (人)			引进人才 (人)		
培养人才 (人)			培养人才 (人)		
科技人才奖励 (人)			科技人才奖励 (人)		
技术标准制定	牵头 (个)		技术标准制定	牵头 (个)	
	参与 (个)			参与 (个)	
软件著作权 (项)			软件著作权 (项)		
论文论著 (篇)		2	论文论著 (篇)		
其中: 被收录论文数 (篇)	SCI		其中: 被收录论文数 (篇)	SCI	
	EI			EI	
	ISTP			ISTP	
新服务 (项)			新服务 (项)		
科技报告 (篇)			科技报告 (篇)		
创新载体项目必填	技术服务数量 (项)		创新载体项目必填	技术服务数量 (项)	
	服务企业数量 (家)			服务企业数量 (家)	

科技金融项目必填	开展培训宣讲活动场次(次)		科技金融项目必填	开展培训宣讲活动场次(次)	
	服务企业数量(家)			服务企业数量(家)	
	帮助企业融资(万元)			帮助企业融资(万元)	
	引进专业机构(家)			引进专业机构(家)	
院士工作站项目必填	引进院士及其团队科技成果转化数量		院士工作站项目必填	引进院士及其团队科技成果转化数量	
	院士开展的战略咨询和技术指导次数			院士开展的战略咨询和技术指导次数	
	院士年进站次数			院士年进站次数	
	院士及院士团队年进站时间			院士及院士团队年进站时间	
软科学项目必填	决策咨询报告(篇) (至少1篇)		软科学项目必填	决策咨询报告(篇) (至少1篇)	
	研究总报告(篇) (至少1篇)			研究总报告(篇) (至少1篇)	
	研究中期报告(篇)			研究中期报告(篇)	
	研究分报告(篇)			研究分报告(篇)	
	调研报告(篇)			调研报告(篇)	
	专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]	
	核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]			核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]	
	培养人才(人)			培养人才(人)	
	获国家级奖项(项)			获国家级奖项(项)	
	获省级奖项(项)			获省级奖项(项)	
	其他			其他	

八、项目经费及省科技厅经费使用情况

项目新增投资支出情况 (万元)			
省科技厅经费预算总额	壹拾伍万圆整		15.00
实际拨款经费	壹拾伍万圆整		15.00
支出经费	总经费	其中：省科技厅经费	其中：自筹资金
基建费	0		
1、直接费用	13.89	13.89	
(1) 设备费	0	0	
(2) 材料费	9.24	9.24	
(3) 测试化验加工外协费	1.72	1.72	
(4) 燃料动力费	0	0	
(5) 差旅费/会议费/国际合作与交流费	0.79	0.79	
(6) 出版/文献/信息传播/知识产权事务费	1.42	1.42	
(7) 劳务费			
(8) 人员费	0.72	0.72	
(9) 专家咨询费	0	0	
(10) 直接费用其他支出	0	0	
(11) 科技金融服务体系其他费用	0.00	0.00	
①信用评级补贴	0	0	
②大赛场租	0	0	
③特派员奖励与补贴	0	0	
2、间接费用	0.75	0.75	
(1) 间接成本	0	0	
(2) 管理成本	0.75	0.75	
(3) 绩效支出	0	0	
合计	14.64	14.64	

项目负责人：柳子斌

财务负责人 (并加盖财务公章) 蔡红



已经投入资金(万元)	
合计:	14.64
其中政府资金(不含科技厅):	
其中科技厅资金:	14.64
其中本企业资金:	
其中贷款资金:	
境外资金:	
其他资金:	

项目负责人: 刘子斌

财务负责人(并加盖财务公章)



吴新

2017A02020805

九、人员信息

参加项目工作人员	合计		
	按职称分类	高级职称:	
		中级职称:	
		初级职称:	
		其他人员:	
	按学历分类	博士:	
		硕士:	
		本科:	
		大专:	
		其它:	
	其他分类	留学归国人员:	
		聘用外国专家:	
培养人员情况			
累计培养人才(个)	合计:		
	取得博士学位:		
	取得硕士学位:		
	取得副高以上技术职称:		

十、本申请项目所附附件清单

会计师事务所名称		
签字注册会计师		
防伪报备编号		
序号	附件名称	数量
1	项目下达文件	1
2	项目合同书	1
3	省科技计划项目实施工作总结报告	1
4	省科技计划项目技术总结报告	1
5	项目结题财务验收经费决算表	1
6	项目结题财务验收审计报告	0
7	产品测试报告	0
8	实物名称	0
9	用户使用报告	0
10	项目所获成果、专利一览表(含成果登记号、专利申请号、专利号等)	4
11	其他有关材料	1
12	广东省科技计划项目财务验收意见表	0
13	广东省科技计划项目验收意见表	6
14	科技报告收录证书	1

2017A0202002A

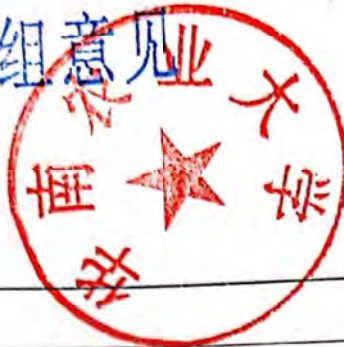


办证意见

审核意见

1. 承担单位

同意验收组意见



2020年9月28日 (盖章)

2. 组织验收单位意见

同意验收组意见



2020年9月28日 (盖章)

3. 主管部门意见

同意验收组意见



2020年9月28日 (盖章)

年 月 日 (盖章)

4. 省科技厅管理部门（专业机构）意见

同意验收



年 月 日 (盖章)

附件2

广东省科技计划项目验收意见表

项目名称	广东肉用黑山羊经济杂交配套方案开发		
项目编号	2017A020208050	负责人	柳广斌
承担单位	华南农业大学		

验收专家成员

姓 名	单 位	职务职称	签 名
黄运茂	仲恺农业工程学院	教授	
张辉华	佛山科学技术学院	教授	
赵志辉	广东海洋大学	教授	

本项目利用雷州黑山羊、川中黑山羊、努比亚黑山羊，进行不同组合的杂交，并对杂交子代的生长性能、肉质等指标进行测定，筛选适合广东地区的肉用黑山羊经济杂交方案。通过项目的实施获得以下成果：

1. 本研究统计分析不同品种黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律，为舍饲黑山羊技术的开发与改进提供参考。
2. 本研究比较了雷州黑山羊、川中黑山羊及努比亚黑山羊之间不同杂交组合对子代生产性能的影响，为进一步开发适合广东舍饲饲养的山羊新品种（品系）打下基础。
3. 发表论文3篇，培养研究生1名。

本项目经费使用合理，已达到考核指标要求，验收合格。

验收结论： ☒ 通过 ☐ 不通过
验收等级： ☒ 合格 ☐ 良好

验收专家组组长签字：
日 期：

广东省科技计划项目验收意见表

项目名称	广东肉用黑山羊经济杂交配套方案开发		
承担单位	华南农业大学		
项目编号	2017A020208050	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
黄运茂	仲恺农业工程学院	教授	

该项目统计分析不同品种黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律，为舍饲黑山羊技术的开发与改进提供参考。同时项目比较了雷州黑山羊、川中黑山羊及努比亚黑山羊之间不同杂交组合对子代生产性能的影响，开发山羊杂交方案，为进一步开发适合广东舍饲饲养的山羊新品种（品系）打下基础。

该项目经费使用合理，实施期间发表论文3篇，培养研究生1名。已达到考核要求，验收合格。

验收结论：(√) 通过 () 不通过
验收等级：(√) 合格 () 良好

验收专家签字：

日期：



广东省科技计划项目验收意见表

项目名称	广东肉用黑山羊经济杂交配套方案开发		
承担单位	华南农业大学		
项目编号	2017A020208050	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
张辉华	佛山科学技术学院	教授	张辉华

本项目利用雷州黑山羊、川中黑山羊、努比亚黑山羊，进行不同组合的杂交，并对杂交子代的生长性能、肉质等指标进行测定，筛选适合广东地区的肉用黑山羊经济杂交方案。通过项目的实施获得以下成果：

1. 本研究统计分析不同品种黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律，为舍饲黑山羊技术的开发与改进提供参考。

2. 本研究比较了雷州黑山羊、川中黑山羊及努比亚黑山羊之间不同杂交组合对子代生产性能的影响，为进一步开发适合广东舍饲饲养的山羊新品种（品系）打下基础。

3. 发表论文3篇，培养研究生1名。

本项目经费使用合理，已达到考核指标要求，验收合格。

验收结论：(✓) 通过 () 不通过
验收等级：(✓) 合格 () 良好

验收专家签字：

日期：

柳广斌
2020.8.8

广东省科技计划项目验收意见表

项目名称	广东肉用黑山羊经济杂交配套方案开发		
承担单位	华南农业大学		
项目编号	2017A020208050	负责人	柳广斌
验收组成员			
姓名	单 位	职务职称	签 名
赵志辉	广东海洋大学	教授	

项目以三个肉羊品种为材料进行杂交，通过对不同杂交组合的后代进行生产性能及肉品质测定，筛选适合于广东地区使用的肉用黑山羊经济杂交方案，为广东省养羊业的发展提供理论基础。项目经费使用合理，并获得以下成果：

(1) 项目比较了雷州黑山羊、川中黑山羊及努比亚黑山羊之间不同杂交组合对子代生产性能的影响。结果可为进一步开发适合广东舍饲饲养的山羊新品系或品种提供参考依据。

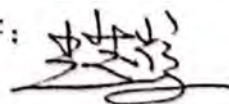
(2) 项目研究了不同黑山羊在华南地区舍饲条件下繁殖性能以及疫病发生情况的规律，为舍饲黑山羊技术的改进提供参考。

(3) 项目实施期间发表论文 3 篇，培养研究生 1 名，达到考核要求。

验收结论：(√) 通过 () 不通过

验收等级：(√) 合格 () 良好

验收专家签字：



日期：2020.9.7

广东省农村科技特派员帮扶工作协议书

甲方：清远市科学技术局

乙方：清远市连山壮族瑶族自治县小三江镇人民政府

丙方：华南农业大学农村科技特派员团队

为贯彻落实党中央实施乡村振兴战略决策部署，落实省委、省政府关于实施“百县千镇万村高质量发展工程”促进城乡区域协调发展有关部署要求，按照《广东省科技支撑“百县千镇万村高质量发展工程”促进城乡区域协调发展实施方案(试行)》《广东省农村科技特派员科技助力百县千镇万村高质量发展行动计划(2023-2026)》等有关规定，经友好协商，就广东省清远市连山壮族瑶族自治县小三江镇农村科技特派员帮扶工作达成如下协议，由各方共同遵守。

一、工作背景

1、乙丙双方已于2024年1月4日进行工作对接，对乙方科技需求进行了充分调研和了解。

2、丙方已于2022年6月-2024年8月在乙方地区开展了黑山羊养殖、腐竹加工、“稻豆菜”种植、鹅步山泉、麻

竹笋种植、鸡枞菌种植、鱼稻共生、乡村运营中心等方面的科技服务，与广东领头羊智慧农业有限公司、连山壮族瑶族自治县众创农贸发展有限公司、连山壮族瑶族自治县碧泉禾花鱼养殖专业合作社、中国移动连山分公司、连山全季文旅有限公司、鹅步山泉有限公司等具有较好的合作基础。

二、甲方职责和义务

1、开展农村科技特派员跟踪管理、考核评价、任务验收、培训交流等相关工作。

2、指导和督促农村科技特派员按要求落实有关帮扶任务，协调解决农村科技特派员在基层开展工作遇到的问题和困难。

3、按预算拨付农村科技特派员下乡服务经费到团队负责人派出单位。

三、乙方职责和义务

1、帮扶期间，乙方应协调相关资源，创造良好的科技帮扶工作环境，保障农村科技特派员团队在帮扶期间必要的工作条件。

2、帮扶期间，由乙方对丙方进行统一管理，乙方应指导丙方围绕重点帮扶产业、领域开展科技帮扶，对帮扶内容、时间和成效等进行管理和记录。

四、丙方职责和义务

1、帮扶期间，丙方应认真履行《广东省科学技术厅关于落实“百千万工程”开展新一轮农村科技特派员重点派驻任

务(2024-2026 年)选派管理工作的通知》工作职责。

2、帮扶期间，丙方应密切联系和协助乙方开展工作，全力完成乙方安排的科技帮扶任务。

3、帮扶期间，丙方应深入基层一线，实地调研、深度挖掘制约乡镇发展的关键科技问题，完成驻镇帮镇扶村重点派驻任务（“黑山羊养殖技术示范推广及科技小院平台建设”），并积极开展资源对接、技术指导、技能培训、成果转化、创业辅导、政策宣传、规划设计等“三农”科技服务，为乡镇产业发展、科技水平提升、关键共性问题解决等提供科技支撑。日常管理中建立必要的工作台账，收集报送农村科技成果和典型案例，开展宣传报道。

4、与乙方协商的具体帮扶任务包括：

（1）2024-2026 年开展的主要工作

1）政策宣传解读：农村科技特派员团队将致力于宣传解读党的“三农”政策、“百千万工程”政策及科技政策，以增强当地农民对政策的理解和认同。

2）科技资源集聚：加强广东连山山羊科技小院及华南农业大学永根科技站等产学研平台在当地的建设，促进人才、项目、资金和平台等科技创新要素在乡镇的集聚，并开展对外交流合作。

3）科技成果转化：发挥华南农业大学动物科学学院科研团队技术优势，推动黑山羊新品种、养殖、麻竹笋种植、鸡枞菌种植、鱼稻共生等新技术及相关新产品在当地的应用



和产业化，促进科技创新，培育乡村新产业新业态。

4) 知识技能培训：采用集中授课、现场交流、实地指导等多种方式，为当地种植户、养殖户提供相关知识和技能的辅导和培训，旨在培育高素质的农业人才队伍。

5) 技术难题攻关：针对当地养殖业、种植业发展中的技术难题，团队依托华南农业大学动物科学学院、科技小院及永根科技站等平台，开展联合科研技术攻关，解决当地养殖、种植技术难题。

6) 其他服务：开展与“三农”相关的各类科技活动，如技术示范、咨询服务等，以全面提升乡村农业科技水平。

(2) 团队成员分工

柳广斌：主要负责养羊技术攻关、成果转化及技能培训等工作。

刘德武：主要负责科技小院等产学研平台建设、人才培养及技术交流等工作。

陈永晴：主要负责政策宣传解读及技术推广等工作。

(3) 年度重点工作任务

2024 年，通过集中讲座、现场交流、实地指导等多种方式，为当地养殖户、种植户提供相关知识和技能的培训，提高种植、养殖技术，增加经济收益。

2025 年，依托广东省畜禽养殖标准化示范场，建立黑山羊种养循环生态养殖模式并进行推广，进一步解决企业及养殖户的技术问题，促进当地农业绿色发展。

2026 年，依托科技小院等产学研平台，培育具备优良生产性能和品质特色的清远黑山羊良种种羊，开展对外交流合作，树立清远黑山羊品牌。

（3）年度绩效目标

1）推广新技术或引进新品种不少于 1 项（种），服务带动企业或农户不少于 10 家（户），共帮助受援对象增收不少于 2 万元。

2）在开展现场技术指导或专题培训、政策宣讲等活动不少于 4 场，培训人次不少于 100 人次。为被帮扶镇解决三农相关技术问题不少于 5 个。

3）季度帮扶动态不少于 4 份，在县级及以上电视台、报刊、公众号等新闻媒体宣传报道不少于 1 次。

4）配合驻镇帮扶工作队开展科技帮扶工作。

五、协议履行和争议解决

1、三方应积极履行协议条款，如出现一方未按协议履行条款，影响帮扶工作开展的，另两方应督促提醒，以免产生严重后果。

2、乙丙双方在履行协议过程中发生争议的，应通过共同协商方式解决，产生严重后果或重大影响、要求变更或解除协议的，应及时报告甲方，在甲方和省科技管理部门指导和协调下进行争议事项处理。

六、其他

1、本协议应在三方充分沟通、实地调研、细致对接基

础之上签订，共一式叁份，三方各执壹份，协议有效期 3 年，自签订之日起生效。

2、其他未尽事宜，由三方友好协商解决。

甲方（盖章）：清远市科学技术局

主要负责人/委托人：

2024 年 9 月 28 日

乙方（盖章）：清远市连山壮族瑶族自治县小三江镇人

民政府

法定代表人/委托人/工作队长：

2024 年 9 月 23 日

丙方（盖章）：华南农业大学农村科技特派员团队（团
队负责人所在学院或科技处章）

主要负责人/委托人：

2024 年 9 月 18 日

受理编号:

项目编号:

文件编号:

河源市科技计划项目 合 同 书

项 目 名 称:	山羊繁殖及配套技术示范推广		
计划类别:	新技术推广示范		
项目起止时间	2022年8月—2023年7月		
管理单位(甲方):	河源市科学技术局		
承担单位(乙方):	华南农业大学		
通讯地址	广东省广州市天河区五山路483号		
邮政编码	510642	单位电话	020-85283435
项目负责人	柳广斌	联系电话	18802085530
项目联系人	孔君	联系电话	020-85283435
乙方主管部门(丙方):			

河源市科学技术局

二〇一七年制

一、项目实施内容（具体业务合同填写说明见合同填报界面）（详见附页）

繁育模式与繁殖技术是羊养殖中的重要环节，不仅影响养殖效益，甚至决定养殖成败。广东地区羊养殖中普遍存在母羊繁殖效率低，配种和产犊管理难度大等问题，导致能繁母畜数量持续下降，亟须通过诱导发情、人工授精等先进繁殖管理技术的研发和推广，以实现母羊 1 年 4 羔甚至更多的目的。本项目针对南方肉羊繁殖技术落后、应用程度低、饲养人员缺乏科学知识及相关培训等问题，以广东省农业技术推广一等奖成果“华南地区羊的繁育及配套技术研发与示范推广”为依托，积极开展羊的繁殖及配套技术示范与推广，主要包括人工授精及配套技术示范推广、常见繁殖疾病预防与治疗技术推广、山羊饲养管理及繁殖技术培训等。拟通过项目的实施改善山羊繁殖效率，提高繁殖技术应用程度，提升养殖人员技术水平，增加养羊经济效益，推动乡村振兴。



三、项目考核指标					
(一) 项目完成后提供的研究开发成果及形式 (须明确产品、专利、版权、标准等成果的类型及数量)					
成果形式		成果数量	成果形式		成果数量
发明专利	申请		引进人才 (人)		
	授权		培养人才 (人)		
实用新型专利	申请		科技人才奖励 (人)		
	授权		技术标准制定	牵头 (个)	
		参与 (个)			
			科技报告 (篇)		
外观设计专利	申请		软件著作权 (项)		
	授权		论文论著 (篇)		
国外专利	PCT 受理		其中: 被收录论文数 (篇)	SCI	
	授权			IE	
获国家级奖项 (项)				CA	
获省级奖项 (项)					
新产品 (或新材料、新装备、新品种 (系))					
新工艺 (或新方法、新模式、新技术)			新服务 (项)		
服务企业数量 (家)		技术服务数量 (项)			3
		服务企业数量 (家)			1
科技金融项目必填		开展培训宣讲活动场次 (次)			
		服务企业数量 (家)			
		帮助企业融资 (万元)			
		引进专业机构 (家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告 (篇) (至少 1 篇)			
		决策咨询报告 (篇) (至少 1 篇)			
		研究中期报告 (篇)			
		研究分报告 (篇)			
		调研报告 (篇)			
		专著 (篇) [须注明 “**市软科学研究计划项目 (项目编号:) 资助”]			

	专著（篇）[须注明“**市软科学研究计划项目（项目编号：）资助”]	
	培养人才（人）	
	获国家级奖项（项）	
	获省级奖项（项）	
	其他（具体形式：_____）	
其他成果及形式说明：		
无		

(二) 主要技术经济指标及社会效益	
累计新增销售收入 (万元)	
累计新增利税 (万元)	
其他主要技术经济指标及社会效益说明:	
<p>1. 技术指标: 繁殖技术覆盖示范羊场 2000 只能繁母羊, 提高示范场能繁母羊繁殖力, 预计每只母羊年平均产羔数提高 0.5 只。</p> <p>2. 经济效益: 按羊只出栏 1800 元/只计算, 每只能繁母羊年均产羔数提高 0.5 只, 即平均每只能繁母羊每年可增加销售额 $1500 \times 0.5 = 900$ 元/只/年。</p> <p>3. 社会效益: 至少开展技术培训 2 次, 每次预计培训 50 人, 总计培训 100 人次。本项目实施可改善母羊繁殖效率, 增加养户收入, 提高养殖信心。通过示范推广可带动农户养殖的积极性, 促进当地农产品结构的调整, 推动乡村振兴。</p>	

三、项目进度和阶段目标			
(一) 项目起止时间: 2022 年 8 月 — 2023 年 7 月			
(二) 项目实施进度及阶段主要目标			
开始日期—结束日期	主要工作内容 (限 500 字)		
2022 年 8 月—2022 年 12 月	进行山羊养殖状况调研, 开展羊的繁殖及配套技术示范, 对企业及农户进行技术指导与培训。		
2023 年 1 月—2022 年 7 月	进一步开展羊的繁殖及配套技术示范与推广, 对企业及农户进行技术指导与培训。		
—			
—			
四、承担/参与单位工作分工及经费分配情况			
承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	市科技局经费分配 (万元)
 华南农业大学	开展羊的繁殖及配套技术示范与推广	3	3
 广东华威洋畜牧有限公司	山羊养殖及技术示范	0	0
	合计	3	3

五、项目总经费及市科技局经费预算						
(一) 市科技局经费下达总额: (大写) 叁万元; (小写): 3 万元						
(二) 市科技局经费拨付方式:						
(三) 市科技局经费年度下达计划:						
年度	2022 年					
经费 (万元)	3					
(四) 总经费及市科技局经费开支预算计划:						
经费筹集情况:						(单位: 万元)
总投入经费: 3						
	市科技局 经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费						
新增经费	3					3
政府部门、境外资金及其他资金投入情况说明:						
新增经费预算:						(单位: 万元)
	新增经费总额			市科技局经费		
支出经费	经费额	用途说明		经费额	用途说明	
基建费						
1、直接费用	2.82			2.82		
(1) 设备费						
(2) 材料费	0.50	技术示范试剂耗材费		0.50	技术示范试剂耗材费	
(3) 测试化验加工 外协费	0.32	技术示范样品检测费		0.32	技术示范样品检测费	
(4) 燃料动力费						
(5) 差旅费	1.00	技术推广过程的差旅费		1.00	技术推广过程的差旅费	
(6) 会议费						
(7) 国际合作与交 流费						
(8) 出版/文献/信 息传播/知识产权事 务费	0.50	技术宣传材料制作印发		0.5	技术宣传材料制作印发	
(9) 租赁费						
(10) 人员费	0.50	参与项目的研究生补助		0.05	参与项目的研究生补助	
(11) 专家咨询费						

(12) 直接费其他支出				
(13) 科技金融服务体系其他费用				
①信用评级补贴				
②大赛场租				
③特派员奖励与补贴				
2、间接费用	0.18		0.18	
(1) 管理费	0.18	按项目总经费 6% 计算	0.18	按项目总经费 6% 计算
合计	3.00		3.00	

六、人员信息								
项目负责人:								
姓名	性别	年龄	职务	职称	学历	在项目中承担的任务	所在单位	签名
柳广斌	男	40	无	讲师	博士	技术指导与培训	华南农业大学	柳广斌
主要研究开发人员:								
姓名	性别	年龄	职务	职称	学历	在项目中承担的任务	所在单位	签名
刘德武	男	56	无	教授	博士	技术咨询	华南农业大学	刘德武
钟春梅	女	47	总经理	无	本科	山羊养殖与示范	广东华威洋畜牧有限公司	钟春梅
孙宝丽	女	41	无	副教授	博士	技术指导与培训	华南农业大学	孙宝丽
郭勇庆	男	41	无	讲师	博士	技术指导与培训	华南农业大学	郭勇庆
李耀坤	男	36	无	副教授	博士	技术指导与培训	华南农业大学	李耀坤
邓铭	男	36	无	实验师	硕士	技术指导与培训	华南农业大学	邓铭
吕建达	男	25	无	无	本科	技术示范与推广	华南农业大学	吕建达
侯碧巍	女	22	无	无	本科	技术示范与推广	华南农业大学	侯碧巍

七、承担、参与单位合作协议（须与申报书中合作协议或意向书相一致）

联合申报项目协议书

甲方：华南农业大学

乙方：广东华威洋畜牧有限公司

甲方与乙方经友好协商决定联合申报 2022 年河源市科技计划项目，项目名称：山羊繁殖及配套技术示范推广。并达成如下合作协议：

第一条：项目研究工作分工：

甲方(主持方)：开展羊的繁殖及配套技术示范与推广。

乙方(参与方)：负责山羊养殖及技术示范。

第二条：经费分配：如果本申报项目获批立项，政府下达的资助经费由甲方进行统筹管理。

第三条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有，对方有使用权；双方共同完成的由双方共享，具体按照双方的贡献大小进行分配或双方另行商定。

②项目成果的转让，须在双方同意的前提下进行，任何一方不得私自转让或许可实施，独立完成的除外。

2. 项目成果申报各级奖项，双方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第四条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第五条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第六条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第七条：合作一方在工作进行中有问题不及时报告，影响项目整体无法完成者，将承担相关责任，并报主管部门。

第八条：本协议自双方签字盖章之日生效，若合作申请未获资助，本协议自动废止。

八、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》和国家有关法规和规定,为顺利完成 2022 年河源市科技计划中 山羊繁殖及配套技术示范推广 项目(文件编号: _____)经协商一致,特订立本合同,作为甲乙双方在合同执行中共同遵守的依据。

第二条 甲方应:1.按合同规定进行经费核拨和工作协调。2.检查乙方项目实施和经费使用情况。3.在收到乙方项目验收申请书后一个月内组织验收。

第三条 乙方应:1.按合同规定的开支范围,对甲方核拨经费实行专款专用,单独列账,配合甲方进行监督检查。2.在每年十二月一日前向甲方如实提交本年度项目实施情况、年度经费使用的书面报告。3.项目完成后,乙方应在合同规定完成期限后一个月内向甲方提交书面报告(验收申请和结题报告),申请甲方进行验收。4.项目验收后,乙方须在验收日期完成后的一个月内向甲方提供完整的验收资料和结题报告。

第四条 在履行本合同的过程中,如遇到市财政计划改变等不可抗拒的特殊情况,甲方对所核拨经费的数量和时间可进行相应变更。

第五条 在履行本合同过程中,如乙方需要调整项目进度,需向甲方提出书面申请并阐明理由,甲方可对合同中经费年度下达计划、项目进度和阶段目标进行相应调整。

第六条 在履行本合同的过程中,当事人一方发现可能导致项目失败或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

第七条 在履行本合同的过程中,因出现在现有水平下无法克服的技术困难,致使项目失败或部分失败造成损失,1.乙方应及时通知甲方,提供相关证据并予以说明。2.甲方以已核拨的经费为最高限承担部分责任,乙方不得再要求甲方承担最高限额以外的其他任何责任。

第八条 乙方违反约定造成项目工作停滞、延误或失败,未能通过验收,应承担违约责任。

第九条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享,除双方另有约定外,按国家和省有关法规执行。

第十条 属技术保密的项目当事人双方订立技术保密条款,作为合同正式内容的一部分。

第十一条 根据项目具体情况,经双方协商订立的附加条款作为本合同正式内容的一部分。

第十二条 甲方可根据具体情况决定乙方是否需要单位担保,若需要保证单位,应订立担保条款,作为本合同正式内容一部分。

第十三条 本合同的争议应由双方本着协商一致的原则解决,当合同需要更改或解除时,双方应订立变更条款或协议。协商不成或不愿意协商解决的,任何一方均有权向甲方所在地人民法院提起诉讼解决。

第十四条 本合同一式六份,各份具有同等效力。甲方存三份,乙方存二份,保证单位存一份,本合同自签字之日起生效,有效期至项目验收后一年内。各方均应负合同的法律责任,不应受机构、人事变动而影响。

第十五条 违约责任:

违反本合同约定, 违约方应承担违约责任。

1. 违反本合同第三条约定, 经甲方催告后 5 日内仍未解决的, 乙方应当承担违约责任, 承担方式和违约金额如下: (1) 合同解除。 (2) 乙方应在违约事由发生后 7 日内全额退还甲方已核拨的经费, 并自行承担由此引起的损失。

2. 违反本合同第八条约定, 乙方应当承担违约责任, 承担方式和违约金额如下: (1) 在发生违约事由的 7 日内全额退还甲方已核拨的经费。 (2) 按已核拨经费的 20% 支付违约金。

第十六条 保密条款:

1. 本合同保密内容范围为: /

2. 本合同保密期限为: /

3. 乙方应与可解知悉保密内容的人员签订技术秘密保护协议。

4. 双方应建立技术秘密保护制度。

5. 属技术保密的项目必须经省负责技术保密部门审查后, 确定可否发表或用于国际合作和交流。

第十七条 保证条款(可由保证人和被保证人另行约定)

当乙方不履行或不完全履行本合同, 并没有或没有完全承担违约责任时, 保证人承担一般保证责任。

说明: 1. 本合同书中, 凡是当事人约定无需填写的条款, 在该条款的空白处划 (</>。

2. 委托代理人签订本合同书的, 应出具合法、有效的委托书。

九、本合同签约各方

管理单位（甲方）：河源市科学技术局（盖章）

单位地址：

法定代表人（或授权代表）：叶小段（签章）

联系人（经办人）姓名：伍文彬（签章）

E-mail:

电话：

承担单位（乙方）：华南农业大学

单位地址：

法定代表人（或法人代理）：刘雅红（签章）

联系人（项目主管）姓名：孔君（签章）

E-mail: kongjun@scau.edu.cn

电话：020-85283435

开户单位名称：华南农业大学

开户银行：广州工行五山支行

开户帐号：3602002609000310520

年 月 日

乙方主管部门（丙方）：（签章）

单位地址：

法定代表人（或法人代理）：（签章）

联系人（项目主管）姓名：（签章）

E-mail:

电话：

开户银行：

开户帐号：

年 月 日

受理编号: 190122104202697

项目编号: 2018032

文件编号: 阳科通〔2018〕60号



阳江市科技计划项目合同书

项目名称: 黑山羊杂交配套技术开发及新品系培育

计划类别: 产业技术创新专项

项目起止时间: 2018-07-11至2020-07-11

管理单位(甲方): 阳江市科学技术局

承担单位(乙方): 阳江市创富农业科学研究院

通讯地址: 阳江市阳西县织篢镇联安村委会牛创岭村10号

邮政编码: 529800 单位电话: 0662-5888688

项目负责人: 邵金回 联系电话: 18127275789

项目联系人: 邵金回 联系电话: 0662-5888688

乙方主管部门(丙方): 阳西县科技局

阳江市科学技术局
二零一八年制



一、项目实施内容

本项目拟在雷州黑山羊本品种选育基础上,进一步引进努比亚黑山羊。努比亚黑山羊具有繁殖性能高,泌乳能力强、体型大生长速度快的特点。项目通过不同的杂交组合方式,测试努比亚黑山羊与雷州黑山羊的杂交效果,开发适合本地生产的黑山羊杂交配套组合,并通过现代育种技术培育黑山羊肉羊新品系。

1. 主要研究内容:

(1) 利用雷州黑山羊、努比亚黑山羊进行不同父母本的二元杂交,并对后代的生长性能、繁殖能力、肉质等指标进行跟踪检测。通过不同组合之间配合力测定的结果比较,最终筛选出最佳杂交配套方案。

(2) 对优秀杂交组合后代进行横交固定。并通过BLUP育种值估计、分子标记辅助选择等方法选育提纯,形成生产性能较高的黑山羊肉羊新品系。

2. 拟解决的关键问题及技术路线:

广东本地黑山羊品种生产性能低下制约养羊业发展,本项目通过杂交技术开发及现代育种技术选育,将广东品种雷州黑山羊及外来品种努比亚黑山羊的优势进行整合,开发可提高黑山羊肉羊生产效率的杂交配套组合方案,并培育生产性能优化的新品系。

3. 创新点:

二元杂交配合力测定,开发最佳杂交方式;利用现代育种技术培育黑山羊肉羊新品系。



二、项目考核指标

(一) 项目完成后提供的研究开发成果及形式 (须明确产品、专利、版权、标准等成果的类型及数量)

成果形式		成果数量	成果形式	成果数量
发明专利	申请	0	引进人才 (人)	0
	授权	0	培养人才 (人)	1
实用新型专利	申请	0	技术标准制定	牵头 (个) 0
	授权	0		参与 (个) 0
科技人才奖励 (人)		0	科技报告 (篇)	0
外观专利	申请	0	软件著作权 (项)	0
	授权	0	论文论著 (篇)	2
获国家级奖项 (项)		0	SCI	0
获省级奖项 (项)		0	被收录论文数 (篇)	IE 0
新产品 (或新材料、新装备、新品种 (系))		0	CA	0
国外专利	PCT受理	0	新工艺 (或新方法、新模式、新技术)	0
	授权	0	新服务 (项)	0
服务企业数量 (家)		技术服务数量 (项)		0
		服务企业数量 (家)		0



科技金融项目必填	开展培训宣讲活动场次(次)	0
	服务企业数量(家)	0
	帮助企业融资(万元)	0
	引进专业机构(家)	0
院士工作站项目必填	引进院士及其团队科技成果转化数量	0
	院士开展的战略咨询和技术指导次数	0
	院士进站次数	0
	院士及院士团队年进站时间	0
软科学项目必填	决策咨询报告(篇)(至少1篇)	0
	研究总报告(篇)	0
	研究中期报告(篇)	0
	研究分报告(篇)	0
	调研报告(篇)	0
	专著(篇)[须注明“**市软科学研究计划项目(项目编号:)资助”]	
	培养人才(人)	0
	获国家级奖项(项)	0
	获省级奖项(项)	0
	其他(具体形式: 用户填)	



其他成果及形式说明:

本项目的实施将肉羊黑山羊品种资源,将雷州黑山羊和努比亚山羊杂交配套组合,培育出适合当地的品种,提升肉羊的生长性能、繁殖能力;将黑山羊杂交出来新品种进一步加大力度广泛宣传推广,对养殖户进行知识普及和技术培训,让更多养殖户能享受研究开发的成果,从而获得更好的经济效益。

(二) 主要技术经济指标及社会效益

累计新增销售收入(万元)

400

累计新增利税(万元)

10

其他主要技术经济指标及社会效益说明:

本项目拟开展的研究以及最后形成的技术成果,首先解决公司急需的关键技术问题,提高肉羊生产性能,降低饲养成本,同时改善羊肉品质。最直接的成果输出形式就是生产出具有本企业特色的羊肉产品,在肉质、口感、风味以及富含的微量营养方面,有别于市场上的同类产品,定位高端市场。成熟的共性技术和成果作为公益性研究的一部分,无偿对外公开发布,供同行其他企业借鉴参考,为我省以及华南地区肉用山羊养殖、农民脱贫致富以及精准扶贫提供支持。

项目负责人(签章):

邹金凤

年 月 日



三、项目进度和阶段目标

(一) 项目起止时间: 2018-07-11 至 2020-07-11

(二) 项目实施进度及阶段主要目标

开始日期--结束日期	主要工作内容 (限 500 字)
2018-07-11 至 2019-07-11	实验羊只筛选, 并执行不同杂交组合方案的二元杂交, 对于代的生产性能进行跟踪测定。
2019-07-11 至 2020-07-11	横交产生子代并进行生产性能测定及分子标记检测, 根据育种值及分子标记进行提纯选育。

190122104202691



四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	市科技局经费分配 (万元)
阳江市创兴农业科学研究院	负责项目实施提供场地、实验羊只、饲养人员，并执行项目实施方案	27	7
华南农业大学	负责杂交配套方案的设计、生产性能测定及数据分析，并对申报单位项目执行进行指导	3	3

190122104202697



五、项目总经费及市科技局经费预算

(一) 市科技局经费下达总额: (大写) 壹拾万元整; (小写): 10 万元

(二) 市科技局经费拨付方式: 一次性拨款

(三) 市科技局经费年度下达计划: 第一期10万元

(四) 总经费及市科技局经费开支预算计划:

经费筹集情况: (单位: 万元)

总投入经费: 30万元

	市科技局 经费	自筹资金				合计
		自有资金	贷款	地方政府 投入	其它	
已投入经 费	0	0	0	0	0	0
新增经费	10	20	0	0	0	30

其它政府部门、境外资金及其他资金投入情况说明:

(二) 新增经费预算 (单位: 万元)

支出经费	新增经费总额		其中: 市科技局经费	
	经费额	用途说明	经费额	用途说明
基建费	0			
1、直接费用	29.7	实验耗材、生化指标及肉质检测费等	9.7	实验耗材、生化指标及肉质检测费等
(1) 设备费	0		0	
(2) 材料费	9.6	实验耗材、试剂、	3	实验耗材、试剂、饲料等



		饲料等		
(3) 测试化验加工外协费	7.5	生化指标及肉质检测、基因鉴定等	2.5	生化指标及肉质检测、基因鉴定等
(4) 燃料动力费	0		0	
(5) 差旅费/会议费/国际合作与交流费	5.1	项目人员差旅费	1.7	项目人员差旅费
(6) 出版/文献/信息传播/知识产权事务费	3	论文版面费及文献资料购买	1	论文版面费及文献资料购买
(7) 劳务费	0		0	
(8) 人员费	4.5	人员劳务费	1.5	人员劳务费
(9) 专家咨询费	0		0	
(10) 直接费其他支出	0		0	
(11) 科技金融服务体系其他费用	0		0	
①信用评级补贴	0		0	
②大赛场租	0		0	
③特派员奖励与补贴	0		0	
2、间接费用	0.3	参与单位管理费	0.3	参与单位管理费
(1) 间接成本	0		0	
(2) 管理成本	0.3	参与单位管理费、按参与单位分配额10%计算	0.3	参与单位管理费，按参与单位分配额10%计算
(3) 绩效支出	0		0	
合计	30		10	



六、人员信息

项目负责人:								
姓名	性别	年龄	职务	职称	学历	在项目中承担的任务	所在单位	签名
邹金国	男	33	阳江市创富农业科学研究院 总经理	未取得	其他	负责项目的战略规划、运营管理 管理工作	阳江市创富农业科学 研究院	邹金国
主要研究开发人员:								
姓名	性别	年龄	职务	职称	学历	在项目中承担的任务	所在单位	签名
柳广斌	男	0	教师	讲师	博士研究生	参与单位项目负责人、育种 及杂交方案设计	华南农业大学	柳广斌
刘文江	男	24	在读研究生	未取得	硕士研究生	项目执行及数据测定	华南农业大学	刘文江
陈华明	男	0	生产主管	未取得	大专	羊场生产管理	阳江市创富农业科学 研究院	陈华明
梁仕聪	男	29	饲养员	未取得	其他	羊场饲养管理	阳江市创富农业科学 研究院	梁仕聪
曾华开	男	30	饲养员	未取得	其他	羊场饲养管理	阳江市创富农业科学 研究院	曾华开



七、承担、参与单位合作协议（须与申报书中合作协议或意向书相一致）

甲方：阳江市创兴农业科学研究院

乙方：华南农业大学

甲方与乙方友好协议决定联合申报2018年度阳江市科技计划项目，项目名称：黑山羊公安配套技术开发及新品系。并达成如下合作协议：

第一条：项目研究工作分工

甲方（主持方）：负责项目实施提供场地、实验羊只、饲养人员，并执行项目

实施方案；负责项目申报材料的组织和书写、申报及跟踪。

乙方（参与方）：负责项目实施方案的设计、数据的测定及分析、研究报告的

撰写，为乙方提供相关的技术咨询和信息服务，对甲方

项目执行进行指导。

第二条：经费分配：

1、如果本申报项目获批立项，按政府下达的资助经费，甲方、乙方同意此

经费分别按政府资助经费的甲方：70%、乙方：30%进行分配。

2、甲方在收到政府下达的资助经费后的一个月将乙方所占经费支付给乙方指定帐户

帐户：华南农业大学

帐号：3602002609001020

开户行：广州工行五山支行

第三条：知识产权归属：

1、项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有，对方有使用权；双方共同完成的由双方共享，具体按照双方的贡献大小进行分配或双方另行商定。

②项目成果的转让，须在双方同意的前提下进行，任何一方不得私自转让或许可实施，独立完成的除外。

2、项目成果申报各级奖项，双方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第四条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止

合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

就五条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第六条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出

审查报告，报项目主管部门审批。

第七条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停

拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第八条：本协议自双方签字盖章之日生效，若合作申请未获资助，本协议自动废止。



八、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定,为顺利完成2018年黑山羊杂交配套技术开发及新品系培育专项项目(文件编号:阳科通(2018)60号)经协商一致,特订立本合同,作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务:1.按合同书规定进行经费核拨的有关工作协调。2.根据甲方需要,在不影响乙方工作的前提下,定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。

第三条 乙方的权利义务:1.确保落实自筹经费及有关保障条件。2.按合同书规定,对甲方核拨的经费实行专款专用,单独列账,并随时配合甲方进行监督检查。3.使用财政资金采购设备、原材料等,按照有关规定,符合招标条件的须进行招标。4.项目实施完成或实施到一定程度,须提出验收或终止结题的申请,并按甲方要求做好项目结题工作。5.在每年1月向甲方如实提交上年度工作情况报告,报告内容包含上年度项目进展情况、经费决算和取得的效果等。6.按照国家、省和市、县(区)有关规定,提交科技报告或其他材料。

第四条 在履行本合同的过程中,如遇到市财政计划改变等不可抗力情况,甲方对所核拨经费的数量和时间可进行相应变更。

第五条 在履行本合同过程中,如项目完成的进度加快或延缓,经双方协商,可对合同中经费年度下达计划、项目进度和阶段目标进行相应变更。

第六条 在履行本合同的过程中,当事人一方发现可能导致项目整体或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

第七条 在履行本合同的过程中,因出现在现有水平下无法克服的技术困难,致使项目失败或部分失败造成损失,1.乙方应及时通知甲方,提供相关证据并予以说明。2.甲方以已核拨的经费为最高限承担部分责任。

第八条 乙方违反约定造成项目工作停滞、延误或失败,未能通过验收,应承担违约责任。

第九条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享,除双方另有约定外,按国家和有关法规执行。

第十条 属技术保密的项目当事人双方订立技术保密条款,作为合同正式内容的一部分。

第十一条 根据项目具体情况,经双方协商订立的附加条款作为本合同正式内容的一部分。

第十二条 甲方可根据具体情况决定乙方是否需要单位担保,若需要保证单位,应订立担保条款,作为本合同正式内容一部分。

第十三条 本合同的争议应由双方本着协商一致的原则解决,当合同需要更改或解除时,双方应订立变更条款或协议,仲裁和诉讼在甲方所在地进行。

第十四条 本合同一式四份,各份具有同等效力。甲方存二份,乙方存二份,本合同自签字之日起生效,有效期至项目验收后一年内。各方均应负合同的法律责任,不应受机构、人事变动而影响。

第十五条 违约责任:

违反本合同约定,违约方应承担违约责任。

1.违反本合同第三条第一项约定,乙方应当承担违约责任,承担方式和违约金额如下:

(1) 合同解除。

(2) 乙方退还甲方已核拨的经费,并自行承担由此引起的损失。

2.违反本合同第八条约定,乙方应当承担违约责任,承担方式和违约金额如下:

(1) 退还甲方已核拨的经费。

(2) 按已核拨经费的20%支付违约金。



第十六条 保密条款:

1. 本合同保密内容范围为:

无

2. 本合同保密期限为:

无

3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。

4. 各方应建立技术秘密保护制度:

5. 属技术保密的项目必须经市负责技术保密部门审查后, 确定可否发表或用于国际合作和交流。

第十七条 保证条款 (可由保证人和被保证人另行约定)

1. 当乙方不履行或不完全履行本合同, 以及没有或没有完全承担违约责任时, 保证人承担一般担保责任。
说明:

1. 本合同书中, 凡是当事人约定无需填写的条款, 应在该条款的空白处划 (/) 。

2. 委托代理人签订本合同书的, 应出具合法、有效的委托书。

190122104202691



九、本合同签约各方

管理单位（甲方）：阳江市科学技术局

(盖章)

单位地址：广东省阳江市江城区安宁路38号

法定代表人（或授权代表）：

谭坚华

(签章)

联系人（经办人）姓名：

陈琴

(签章)

E-mail: yj2830108@163.com

电话：13702816033

年 月 日

承担单位（乙方）：阳江市创富农业科学研究院

(盖章)

单位地址：阳江市阳西县织篢镇联安村委会牛创岭村10号

法定代表人（或法人代表）：

邵金回

(签章)

联系人（项目主管）姓名：

邵金回

(签章)

E-mail: 1037649367@qq.com

电话：0662-5888688

开户单位名称：阳江市创富农业科学研究院

开户银行：中国工商银行

开户帐号：2014002709000165380

年 月 日

乙方主管部门（丙方）：阳西县科技局

(盖章)

单位地址：阳西县政府综合办公楼6号一楼

法定代表人（或法人代表）：

梁永雄

(签章)

联系人（项目主管）姓名：

陈焱辉

(签章)

E-mail: cyh655@163.com

电话：13794754895

开户单位名称：

开户银行：

开户帐号：

年 月 日



联合申报项目协议书

甲方：阳江市创富农业科学研究院

乙方：华南农业大学

甲方与乙方经友好协商决定联合申报 2018 年度 阳江市科技计划项目，项目名称：黑山羊杂交配套技术开发及新品系培育。并达成如下合作协议：

第一条：项目研究工作分工：

甲方(主持方)：负责项目实施提供场地、实验羊只、饲养人员，并执行项目实施方案；负责项目申报材料的组织和书写、申报及跟踪落实；承担本项目除政府经费外额外资金的投入。

乙方(参与方)：负责项目实施方案的设计、数据的测定及分析，研究报告的撰写。为乙方提供相关的技术咨询和信息咨询服务，对甲方项目执行进行指导。

第二条：经费分配：

1、如果本申报项目获批立项，按政府下达的资助经费，甲方、乙方同意此经费分别按政府资助经费的甲方：70%、乙方：30%进行分配。

2、甲方在收到政府下达的资助经费后的一个月内将乙方所占经费支付给乙方指定帐户。

账 户：华南农业大学

帐 号：3602002609000310520

开户行：广州 工行 五山支行

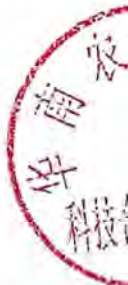
第三条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有，对方有使用权；双方共同完成的由双方共享，具体按照双方的贡献大小进行分配或双方另行商定。

②项目成果的转让，须在双方同意的前提下进行，任何一方不得私自转让或许可实施，独立完成的除外。

2. 项目成果申报各级奖项，双方单位排名根据具体情况另行商定，人员排



名原则上按贡献大小先后排名。

第四条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第五条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第六条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第七条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第八条：本协议自双方签字盖章之日生效，若合作申请未获资助，本协议自动废止。

甲方单位（盖章）：

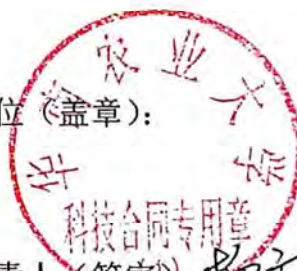


项目负责人（签字）：

邵金四

2018年7月13日

乙方单位（盖章）：



项目负责人（签字）：

柳子斌

2018年7月13日



阳西

阳江市科学技术局文件

阳科通〔2018〕60号

关于下达 2018 年度科技创新平台、 创新服务体系建设专项资金 项目计划的通知

各县（市、区）科技局，各有关单位：

2018 年度科技创新平台、创新服务体系建设专项资金项目已经公示无异议，现按规定下达给你们，并就有关事项通知如下：

一、本次下达的 2018 年度科技创新平台、创新服务体系建设专项资金项目共 10 项，经费 200 万元。

二、项目承担单位收到本通知后，须尽快按照《阳江市科学技术局关于市科技计划项目合同书管理的实施细则》

(阳科通〔2015〕51号)有关规定与市科技局签订项目合同书(合同书可在市科技局网站 <http://www.yjkj.gov.cn/> 表格下载栏下载)。

三、各级主管部门应履行项目的日常监督职责,督促项目承担单位做好项目的组织实施,并配合市有关部门组织开展的监督检查、绩效评价、验收结题、项目审计等相关工作。

四、各项目承担单位要抓紧项目的组织实施,严格按照科技经费的使用范围和有关规定管好用好财政资金,专款专用,确保按期完成科研任务,提升创新能力。项目完成后,要按照《阳江市科学技术局关于市科技计划项目结题管理的实施细则》(阳科通〔2015〕52号)有关规定进行结题。

附件:1、2018年度科技创新平台、创新服务体系建设
专项资金项目计划安排汇总表(不下发)

2、2018年度科技创新平台、创新服务体系建设
专项资金项目计划安排表(分发)



附件 2:

2018 年度科技创新平台、创新服务体系建设项目专项资金项目计划安排表

单位: 万元					
序号	项目名称	项目类别	项目编号	承担单位	立项经费
阳西县					
1	黑山羊杂交配套技术开发及新品系培育	农业攻关项目	2018032	阳江市创富农业科学研究院	10
合计: 1 项					10

HXKJHT 2022 1874

合同编号:

技术服务合同

项目名称: 韶关市仁化县牛羊产业园山羊养殖
技术服务

委 托 方: 韶关丹霞女农业科技有限公司
(甲 方)

受 托 方: 华南农业大学
(乙 方)

签订时间: 2022. 7. 28

签订地点: 广州

有效期限: 二年

中华人民共和国科学技术部印制

填 写 说 明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术服务合同

委托方（甲方）： 韶关丹霞女农业科技有限公司

住 所 地： 广东省韶关市仁化县大桥镇古洋村委会

法定代表人： 申丽萍

项目联系人： 申丽萍

联系方式： 13232066558

通讯地址： 广东省韶关市仁化县大桥镇古洋村委会

电 话： 13232066558 传真：

电子信箱：

受托方（乙方）： 华南农业大学

住 所 地： 广州市天河区五山路 483 号

法定代表人： 刘雅红

项目联系人： 柳广斌

联系方式： 188020855330

通讯地址： 广州市天河区五山路 483 号

电 话： 1880208550 传真：

电子信箱： gbliu@scau.edu.cn

本合同甲方委托乙方就韶关市仁化县牛羊产业园山羊养殖技术服务项目进行的专项技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：

(1) 协助产业园羊场在现有基础上建立良种繁育体系，制定育种方案。

(2) 协助产业园羊场在现有基础上建立标准化饲养管理体系，制定羊场标准化饲养管理方案。

(3) 为产业园企业提供技术支持与培训服务。

2. 技术服务的内容：

(1) 山羊良种繁育体系建立

协助羊场在现有基础上建立种羊性能评估体系，通过外貌评定、生产性能测定、乳品质及肉品质测定等指标对现有种羊群体进行评估筛选。建立山羊现代育种体系，为羊场制定选育种方案。

(2) 羊场标准化饲养管理体系建立

羊场生产状况诊断研究：系统调研羊场生产管理状况，用科学的角度分析羊场在生产管理中存在的问题，制定改进方案。

羊场生产过程关键指标监控体系建立：协助羊场确定生产过程关键监控指标，制定指标数据收集、记录、计算及报表制作的方案，建立生产过程关键技术指标监管体系。

羊场标准化饲养管理方案制定：制定生产管理流程优化方案，制定各环节标准化饲养管理作业指导书，包括：种公羊饲养管理、妊娠羊饲养管理、分娩哺乳羊饲养管理、羔羊饲养管理、后备羊饲养管理、育肥羊饲养管理、种羊配种、饲料加工配合、羊场卫生防疫等。

(3) 产业园技术指导服务

受甲方委托，专家团队对产业园企业项目的可行性、工程设备造价、项目合理合规性等提供工作建议和技术支持服务。为产业园企业提供技术咨询、指导及培训服务。

3. 技术服务的方式：方案制订、远程咨询、现场指导、技术培训、派遣研究生开展测定及实验研究。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：韶关市仁化县牛羊产业园

2. 技术服务期限：二年

3. 技术服务进度：

(1) 2022 年 8 月 1 日-2022 年 12 月 31 日：协助开展山羊生产性能评估测定，初步制定育种方案；开展羊场生产状况诊断研究，初步制定羊场标准化饲养管理方案；专家团队进行技术指导服务。

(2) 2023 年 1 月 1 日-2024 年 7 月 31 日：协助羊场建立育种体系，完善育种方案；协助羊场建立标准化饲养管理体系，完善羊场标准化饲养管理方案；专家团队进行技术指导服务。

4. 技术服务质量要求：完成技术服务目标。

5. 技术服务质量期限要求：二年

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1. 提供技术资料：

(1) 与本项目相关的生产数据及发展规划材料。

2. 提供工作条件：

(1) 为乙方专家和学生到场提供技术服务时所需的基础工作条件，如食宿和试验场地，协助开展调研、测定及试验研究工作，确保技术方案和各项技术管理措施的顺利实施。

(2) 其它临时出现可能影响合同执行的问题由甲乙双方本着友好合作的精神，共同协商解决。

3. 其他： 无。

4. 甲方提供上述工作条件和协作事项的时间及方式： 双方协商。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

1. 技术服务费总额为： 55 万元（伍拾伍万元）

2. 技术服务费由甲方 分三期 （一次或分期）支付乙方。

具体支付方式和时间如下：

(1) 第一期拨款总款额的 50%，于本合同签订之日起一周内拨付至乙方账户；

(2) 第二期拨款总款额的 30%，2022 年 10 月 31 日前拨付至乙方账户；

(3) 第三期拨款总款额的 20%，于项目验收后一周内拨付至乙方账户。

乙方开户银行名称、地址和帐号为：

开户名： 华南农业大学

开户银行： 广州工行五山支行

地址： 广州市天河区五山路

帐号： 3602002609000310520

第五条：双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）： 无。

2. 涉密人员范围： 无。

3. 保密期限： 无。

4. 泄密责任： 无。

乙方：

1. 保密内容（包括技术信息和经营信息）： 无。

2. 涉密人员范围： 无。

3. 保密期限： 无。

4. 泄密责任： 无。

第六条：本合同的变更必须由双方协商一致，并以书面形式确定。
但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在 7 日内予以答复；逾期未予答复的，视为同意：

1. 无。

第七条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1. 乙方完成技术服务工作的形式：方案制订、远程咨询、现场指导、技术培训、派遣研究生开展测定及实验研究。

2. 技术服务工作成果的验收标准：双方认可。

3. 技术服务工作成果的验收方法：双方协商。

4. 验收的时间和地点：双方协商。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归双（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归双（乙、双）方所有。

第十条：双方确定，在本合同有效期内，甲方指定申丽萍为甲方项目联系人，乙方指定柳广斌为乙方项目联系人。项目联系人承担以下责任：

1. 沟通协调推动项目顺利实施。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十一条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力;

第十二条: 双方因履行本合同而发生的争议, 应协商、调解解决。协商、调解不成的, 确定按以下第 1 种方式处理:

1. 提交 广州市 仲裁委员会仲裁;

2. 依法向人民法院起诉。

第十三条: 双方确定: 本合同及相关附件中所涉及的有关名词和技术术语, 其定义和解释如下:

1. 无。

第十五条: 双方约定本合同其他相关事项为: 未尽事宜, 双方可以协商, 签定补充协议等。

第十六条: 本合同一式 6 份, 具有同等法律效力。

第十七条: 本合同经双方签字盖章后生效。

甲方： 韶关丹霞女农业科技有限公司 (盖章)

法定代表人 / 委托代理人： (签名)

年 月 日

乙方： 华南农业大学 (盖章)

法定代表人 / 委托代理人： (签名)

年 月 日

卷之六

合同登记编号:

[illegible]

1. 申请登记人: _____

2. 登记材料: (1) _____

(2) _____

(3) _____

3. 合同类型: _____

4. 合同交易额: _____

5. 技术交易额: _____

技术合同登记机构（印章）

经办人：

年 月 日

大學

技术开发（合作）合同

项目名称：温氏优良肉用山羊及绵羊品种选育及配套
饲养技术研发

甲方：广东温氏食品集团股份有限公司

乙方：华南农业大学

丙方：新兴县温氏新旺羊业有限公司

签订时间：2015年9月21日

签订地点：广东温氏食品集团股份有限公司

有效期限：2018年6月30日

广东温氏食品集团股份有限公司
二〇一五年制

填写说明

1、甲方为科研项目下达单位，即广东温氏食品集团有限公司；乙方为项目牵头承担单位；丙方为项目参与单位（若无参与单位可不用填写）。

2、本合同主要适用于进行新技术、新产品、新工艺、新材料或者新品种及其系统的研究开发，科技成果推广和软科学研究所订立的技术开发合同。

3、合同条款中所有空项都需如实填写，确无此项的，请在该条款处注明“无”等字样。

4、单位盖章必须是单位公章，部门章无效。

5、本合同书未尽事项，可由当事人附页另行约定，并可作为本合同的组成部分。

技术开发（合作）合同

甲方：广东温氏食品集团股份有限公司

住 所 地：广东省云浮市新兴县广东温氏食品集团股份有限公司

法定代表人：温鹏程

项目联系人：陈丽

联系方式：0766-2986008

通讯地址：广东省云浮市新兴县广东温氏食品集团股份有限公司

电 话：0766-2986008 传 真：0766-2986003

电子信箱：chenlili0826@163.com

乙 方：华南农业大学

住 所 地：广东省广州市天河区五山 483 号

法定代表人：陈晓阳

项目联系人：柳广斌

联系方式：18802085530

通讯地址：广东省广州市天河区五山华南农业大学动物科学学院

电 话：020-85280275 传 真：020-85280740

电子信箱：gbliu@scau.edu.cn

丙 方：新兴县温氏新旺羊业有限公司

住 所 地：广东省云浮市新兴县温氏新旺羊业有限公司

法定代表人：谢应林

项目联系人：李和平

联系方式：18802085530

通讯地址：广东省云浮市新兴县簕竹镇榄根温氏旧总部

电 话：0766-2285332 传 真：0766-2285332

电子信箱：2660155257@qq.com

股份

国银
157
-2
新兴
北路9
005

本合同合作各方就共同参与研究开发温氏优良肉用山羊及绵羊品种选育及配套饲养技术研发项目事项,经过平等协商,在真实、充分地表达各自意愿的基础上,根据《中华人民共和国合同法》的规定,达成如下协议,并由合作各方共同恪守。

第一条 甲方下达项目,乙方和丙方必须按下列要求完成研究、开发任务,本合同合作研究开发项目的要求如下:

1. 技术目标:

(1) 针对温氏集团已引进的种羊群体,通过 BULP 及分子育种等方法,对群体进行进一步的提纯选育,培育高生长性能、高繁殖力及高耐热性的配套品系,并通过品系间杂交提高本品种肉羊的生产性能。

(2) 引进优良肉用绵羊及山羊父系品种,利用配合力测定制定出最佳肉羊杂交改良方案,并通过横交固定为温氏培育优良的肉用绵羊及山羊新品种。

(3) 开发适用于温氏肉羊群体的多基因聚合分子育种技术,并通过该技术提高新品种及品系培育效率。

(4) 通过肉羊生产性能测定及饲养管理方式摸索,开发一套适用于广东地区温氏羊场使用的肉羊集约化舍饲配套技术。

2. 技术内容:

(1) 优良肉用山羊及绵羊高生产性能耐热品系培育:以生产性能和繁殖性能为目标性状,对川中黑山羊及湖羊进行本品种内选育,培育山羊与绵羊各自的高生长性能品系与高繁殖性能品系。品系育成后利用品系间杂交培育同时具备高生长性能和繁殖力的肉用山羊及绵羊。同时对山羊及绵羊耐热性能进行选育,提高肉羊的抗热应激能力。

(2) 温氏优良肉用山羊及绵羊的杂交改良研究:引进努比亚山羊,波尔山羊及杜泊羊,利用努比亚山羊及波尔山羊改良川中黑山羊,利用杜泊羊改良湖羊。确定最佳的杂交组合方案,并通过横交固定培育适合于南方集约化养殖的肉羊新品种。

(3) 肉用山羊及绵羊多基因聚合分子育种技术开发:通过高通量测序技术,研究不同 SNP 基因型与山羊及绵羊生产性能等诸多性状的关系,确定可应用于温氏肉羊群体的 SNP 位点信息,定制低密度 SNP 基因芯片,并通过多基因聚合分子育种技术对山羊及绵羊进行辅助选育,提高品种及品系培育的效率。

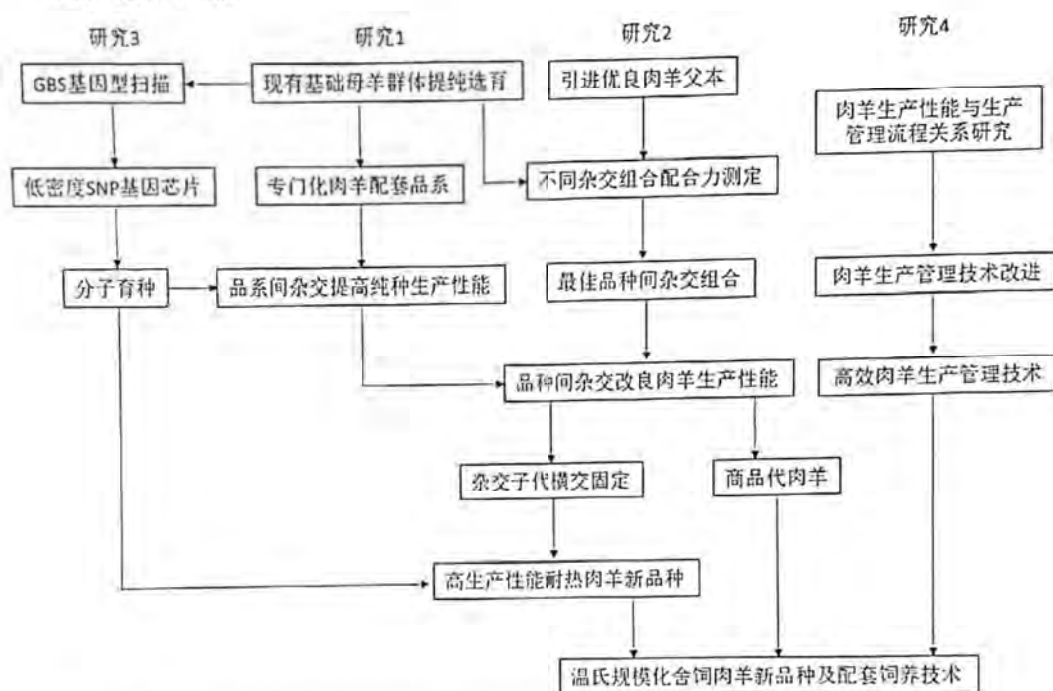
(4) 温氏肉用山羊及绵羊饲养管理技术研究:研究在集约化舍饲养殖条件下,山羊及绵羊的行为与精神状态、生理生化指标、疫病发生情况等指标与舍内温氏指数 (THI)、饲养密度、饲料营养、饲养管理流程等之间的关系。摸索一套适合于南方肉用山羊及绵羊集约化舍饲养殖的配套技术。

3. 技术方法和路线:

(1) 技术方法:

本项目所使用的研究方法涉及家畜育种学、生物统计学、分子生物学、生物信息学等方面。具体关键技术包括: BULP 育种值估计、品系培育、二元杂交实验、三元杂交实验、杂交配合力测定、肉羊生产性能测定、生理指标测定, 血液生化指标检测、DNA 提取、高通测序技术、SNP 芯片检测、生物信息数据挖掘与分析, PCR 技术, QPCR 技术, 电泳技术等分子实验技术。

(2) 技术路线:



第二条 本合同合作各方在研究开发项目中, 分工承担如下工作:

乙方:

1、 研究开发内容:

- (1) 高通量测序数据分析工作。
- (2) 测序数据分子生物实验验证工作。
- (3) 低密度 SNP 芯片设计及实验验证工作。

2、 工作进度:

项目时间段		项目进度安排 (各阶段达到的目标)
2015年6月1日	2016年6月30日	采集绵羊及山羊群体血样; 生产数据收集; 高通量测序
2016年7月1日	2017年6月30日	高通量测序数据分析, SNP 分子生物学验证, 低密度 SNP 芯片设计
2017年7月1日	2018年6月30日	SNP 芯片验证实验, 生产数据与 SNP 芯片数据分析

3、 研究开发期限:

2015年6月30日—2018年6月30日。

4、 研究开发地点：

广东省云浮市新兴县温氏新旺羊业有限公司良洞种羊场，华南农业大学动物科学学院实验室。

丙方：

1、 研究开发内容：

- (1) 种羊引进、育种方案制定及具体实施工作。
- (2) 实验样品采集、羊场生产数据及实验数据收集工作。
- (3) 羊场肉羊生产管理技术研究的具体实施工作。

2、 工作进度：

项目时间段		项目进度安排（各阶段达到的目标）
2015年6月1日	2016年6月30日	育种方案制定，引进种羊，品系选育，二元杂交实验，生产及实验数据收集
2016年7月1日	2017年6月30日	品系选育，三元杂交实验，生产及实验数据收集，生产流程优化
2017年7月1日	2018年6月30日	品系选育及品系杂交，杂交后代横交固定，生产及实验数据收集，生产流程优化，总结分析数据撰写报告

3、 研究开发期限：

2015年6月30日—2018年6月30日。

4、研究开发地点：

广东省云浮市新兴县温氏新旺羊业有限公司良洞种羊场。

第三条 合作各方确定，各自为本合同项目的研究开发工作提供以下技术资料

和条件：
乙方：提供分子实验场所，协助丙方开展相关试验研究，并为试验的顺利开展和完成提供相关的技术指导和协调工作。

丙方：开展具体的相关试验，为试验的开展提供人员和试验材料，并进行样品测定、数据整理和试验结果分析、总结，形成报告。

本合同履行完毕后，上述技术资料和条件按以下方式处理：由接受方自行处理。

第四条 本合同合作各方在研究开发项目中，乙方、丙方应明确经费预算。

经费预算：20.040 万元

经费使用单位：华南农业大学

经费投入预算		经费支出预算		备注
来 源	预算数（万元）	科 目	预算数（万元）	
丙方拨款	20.040	材料费	3.500	购买分子实验耗材及试剂
乙方自筹	0.000	测试费	10.000	高通量测序及血液生化指标检测
相关单位拨款	0.000	差旅费	2.376	实验路费及住宿费

其 他	0.000	人员费	2.160	在读研究生补助
		管理费	2.004	总经费的 10%，其中 5% 为学校管理费、1% 为所在单位管理费、4% 为课题组绩效奖励
合 计	20.040	合 计	20.040	
备注：无				

注：①主要（大中型）仪器设备清单及价格另附。

②其他须说明的事项，请在备注栏中注明。

第五条 合作双方确定，按如下方式提供或支付本合同项目的研究开发经费及其他投资：

乙方：

- 1、提供或支付方式： 无 。
- 2、支付或折算为技术投资的金额： 无 。
- 3、使用方式： 按经费支出预算及乙方相关规定自行决定 。

丙方：

1、提供或支付方式： 为乙方提供人民币贰拾万零肆佰元整（¥200400）。合同签订后两周内将全款拨到乙方账户。乙方应在丙方付款之日起两周内将发票交与丙方。

- 2、支付或折算为技术投资的金额： 无 。
- 3、使用方式： 无 。

第六条 以提供技术为投资的合作方应保证其所提供的技术不侵犯任何第三方的合法权益。如发生第三人指控合作一方或多方因实施该项技术而侵权的，提供技术应当 承担相应经济责任 。

第七条 在合同执行过程中，任何一方不得擅自修改合同内容，如确需要修改，应由甲、乙、丙三方共同商定，在修改条款上经三方签章确认，或重新签订合同。

第八条 甲、丙方中途无故撤销或不履行合同时，所拨经费不得追回；乙方如无正当理由不履行合同，或非不可抗拒的原因致使合同无法执行时，甲、丙方有权收回所拨经费。

第九条 未经其他合作方同意，合作一方或多方不得将本合同项目部分或全部研究开发工作转给第三人承担。

第十条 在本合同履行中，因出现在现有技术水平和条件下难以克服的技术困难，导致研究开发失败或部分失败，并造成合作一方或多方损失的，合作各方约定按以下方式承担风险损失：

- 1、 各自承担各自风险责任，互不追究责任 ；

2、无。

合作各方确定，本合同项目的技术风险按1方式认定。认定技术风险的基本内容应当包括技术风险的存在、范围、程度及损失大小等。认定技术风险的基本条件是：

- 1、本合同项目在现有技术水平条件下具有足够的难度；
- 2、乙、丙双方在主观上无过错且经认定研究开发失败为合理的失败。

一方发现技术风险存在并有可能导致研究开发失败或部分失败的情形时，应当在30日通知其他合作方并采取适当措施减少损失。逾期未通知并未采取适当措施而导致损失扩大的，应当就扩大的损失承担赔偿责任。

第十一条 合作各方确定按以下方式支付研究开发成果：

乙方：

1、研究开发成果支付的形式及数量：(1) 完成高通量测序数据分析及分子实验验证。(2) 开发一套肉羊育种低密度 SNP 芯片及多基因聚合分子育种方案。

2、研究开发成果支付的时间及地点：2018 年 6 月，于广东温氏食品集团股份有限公司提交。

丙方：

1、研究开发成果支付的形式及数量：(1) 通过品系培育，使肉羊新品系较原品种生产性能（生长速度）提高 10%。(2) 确定最佳的肉羊杂交组合方案，并使杂交子代生产性能（生长速度）较原品种提高 20%。(3) 制定一套温氏肉羊全舍饲集约化养殖技术标准。(4) 发表学术论文 3 篇。

2、研究开发成果支付的时间及地点：2018 年 6 月，于广东温氏食品集团股份有限公司提交。

第十二条 合作各方确定，按以下标准及方法对本合同最终完成的研究开发成果进行验收：由甲方组织验收。

第十三条 合同各方确定，因履行本合同所产生、并由合作各方共同完成的阶段性技术成果及其相关知识产权权利归属，约定如下：合作各方共享。

合同各方确定，因履行本合同所产生的最终研究开发技术成果及其相关知识产权权利归属，约定如下：合作各方共享。

第十四条 合作各方分别独立完成并与履行本合同有关的阶段性技术成果的研究开发人员，享有在有关此阶段性技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

合作各方应以协商方式确定最终研究成果的完成人员名单。此完成人员享有在有关最终技术成果文件上写明技术成果完成者的权利和取得有关荣誉证书、奖励的权利。

第十五条 为有效履行本合同，合作各方确定，在本合同有效期内，乙方指定 柳广斌 为乙方项目联系人，丙方指定 李和平 为丙方项目联系人。一方变更项目联系人的，应当及时以书面形式通知其他合作各方。未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十六条 乙方和丙方必须于每年12月前向甲方提交年度计划执行情况及经费使用情况说明和下一年度的计划实施方案。

第十七条 甲方有权对项目经费使用情况进行检查或审计。

第十八条 甲、乙、丙三方对技术经济资料负有保密责任。

第十九条 本合同一式 四 份，甲、乙方各执 1 份，丙方 2 份。

第二十条 本合同经各方签字盖章后生效。

甲方： 广东温氏食品集团股份有限公司 (盖章)
法定代表人/委托代理人 (签字) 李和平
开户银行：中国银行新兴支行
帐号：7045774 (盖章)
电话：0766-2291903
地址：云浮市新兴县新城镇东堤北路9号 (签名)

2015年10月9日

乙方： 华南农业大学 (盖章)
法定代表人/委托代理人 (签字) 李和平 (签名)

2015年10月8日

丙方： 新兴县温氏新旺羊业有限公司 (盖章)
法定代表人/委托代理人 (签字) 李和平 (签名)

2015年10月8日

HX KJ HT 2020023

合同编号:

技术服务合同

项目名称: 肉牛标准化饲养管理技术开发

委托方: 云浮市岭南肉牛科技有限公司
(甲方)

受托方: 华南农业大学
(乙方)

签订时间: 2020.10.29

签订地点: 广东云浮

有效期限: 2020.10.29-2022.10.31



中华人民共和国科学技术部印制

填写说明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术服务合同

委托方（甲方）： 云浮市岭南肉牛科技有限公司

住 所 地： 云浮市云安区石城镇迳心村 324 国道肉牛产业园

法定代表人： 王丹

项目联系人： 杨际帆

联系方式： 13929290707

通讯地址： 云浮市云安区石城镇迳心村 324 国道肉牛产业园

电 话： 13929290707 传真：

电子信箱：

乙 方： 华南农业大学

住所地： 广东省广州市天河区五山 483 号

法定代表人： 刘雅红

项目联系人： 柳广斌

联系方式： 18802085530

通讯地址： 广东省广州市天河区五山华南农业大学动物科学学院

电话： 020-85280275 传真： 020-85280740

电子信箱： gbliu@scau.edu.cn

本合同甲方委托乙方就 肉牛标准化饲养管理技术开发

项目进行的专项技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国合同法》的规定，达成如下协议，并由双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：提高肉牛营养配方和标准化饲养管理水平，提高肉牛养殖综合经济效益。

2. 技术服务的内容：(1) 提供肉牛饲料加工、饲养管理、环境控制等方面标准化生产技术服务；(2) 建立优质肉牛高效生产技术方案及胴体质量活体评价技术规程、优质肉牛高效饲养的全混日粮饲喂体系（TMR），形成肉牛优质高效健康养殖的标准化技术体系；(3) 提供相关技术信息咨询服务和现场生产指导；(4) 对申报项目进行指导。

3. 技术服务的方式：方案制订、现场指导和远程咨询。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：云浮市岭南肉牛科技有限公司

2. 技术服务期限：2 年

3. 技术服务进度：2020 年 11 月 - 2021 年 11 月，肉牛场现存问题的调研与整理；2021 年 11 月 - 2022 年 10 月，肉牛标准化饲养管理技术开发研究，制定饲养管理标准。

4. 技术服务质量要求：根据甲方需要，乙方定期组织相关专家到甲方进行现场指导和技术培训，提高甲方肉牛标准化饲养管理水平，提高肉牛养殖综合效益。

5. 技术服务质量期限要求：2 年

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提供下列工作条件和协作事项：

1. 提供技术资料：

(1) 肉牛场品种来源、饲料供应、营养配方等相关资料

(2) 肉牛场日常饲养管理制度和疾病综合防控技术规程

(3) 牛场发展规划、生产数据及其他相关信息

2. 提供工作条件:

(1) 提供乙方到现场工作的基本交通和食宿条件;

(2) 协助专家与相关技术管理人员的对接沟通工作,严格执行经双方确认的技术方案;

(3) 协助开展相关实验研究;如提供实验场地、实验动物和饲养人员等,具体试验需要双方另行商定。

3. 其他: 无。

4. 甲方提供上述工作条件和协作事项的时间及方式: 双方协商。

第四条: 甲方向乙方支付技术服务报酬及支付方式为:

1. 技术服务费总额为: 贰拾万元(¥200000)

2. 技术服务费由甲方 一次 (一次或分期) 支付乙方。

具体支付方式和时间如下: 合同签订后 10 天内,甲方将服务经费转入乙方指定账户,服务费用支出由乙方负责,做到专款专用。

乙方开户银行名称、地址和帐号为:

开户银行: 广州 工行 五山支行

地址: 广州市天河区五山路

帐号: 3602 0026 0900 0310 520

第五条：双方确定因履行本合同应遵守的保密义务如下：

甲方：

1. 保密内容（包括技术信息和经营信息）： 无。
2. 涉密人员范围： 无；
3. 保密期限： 无。
4. 泄密责任： 无。

乙方：

1. 保密内容（包括技术信息和经营信息）： 无。
2. 涉密人员范围： 无；
3. 保密期限： 无。
4. 泄密责任： 无。

第六条：本合同的变更必须由双方协商一致，并以书面形式确定。

但有下列情形之一的，一方可以向另一方提出变更合同权利与义务的请求，另一方应当在 7 日内予以答复；逾期未予答复的，视为同意：

1. 无

第七条：双方确定以下列标准和方式对乙方的技术服务工作成果进行验收：

1. 乙方完成技术服务工作的形式： 建立一套南方肉牛养殖模式和标准化饲养管理技术总结报告 1 份。
2. 技术服务工作成果的验收标准： 材料验收，双方认可。
3. 验收的时间和地点： 2022.10.31 于云浮市岭南肉牛科技有

限公司现场验收。

第八条：双方确定：

1. 在本合同有效期内，甲方利用乙方提交的技术服务工作成果所完成的新的技术成果，归双（甲、双）方所有。

2. 在本合同有效期内，乙方利用甲方提供的技术资料和工作条件所完成的新的技术成果，归双（乙、双）方所有。

第九条：双方确定，按以下约定承担各自的违约责任：

1. 无方违反本合同第无条约定，应当无（支付违约金或损失赔偿额的计算方法）。

第十条：双方确定，在本合同有效期内，甲方指定刘瑞华为甲方项目联系人，乙方指定柳广斌为乙方项目联系人。项目联系人承担以下责任：

1. 共同协助项目顺利完成。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十一条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力。

第十二条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第1种方式处理：

1. 提交广州仲裁委员会仲裁；

2. 依法向人民法院起诉。

第十三条：双方确定：本合同及相关附件中所涉及的有关名词和技术术语，其定义和解释如下：

1. _____ 无 _____

第十四条：与履行本合同有关的下列技术文件，经双方确认后，_____ 无 _____为本合同的组成部分：

1. 技术背景资料：_____ 无 _____；

2. 可行性论证报告：_____ 无 _____；

3. 技术评价报告：_____ 无 _____；

4. 技术标准和规范：_____ 无 _____；

5. 原始设计和工艺文件：_____ 无 _____；

6. 其他：_____ 无 _____；

第十五条：双方约定本合同其他相关事项为：_____ 无 _____。

第十六条：本合同一式 4 份，具有同等法律效力。

第十七条：本合同经双方签字盖章后生效。

甲方：_____云浮市岭南肉牛科技有限公司_____
(盖章)
法定代表人 / 委托代理人：_____
(签名)
2020年 10 月 29 日

乙方：_____华南农业大学_____
(盖章)
法定代表人 / 委托代理人：_____
(签名)
2020年 10 月 29 日

印花税票粘贴处：

(以下由技术合同登记机构填写)

合同登记编号:

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

1. 申请登记人: _____

2. 登记材料: (1) _____

(2) _____

(3) _____

3. 合同类型: _____

4. 合同交易额: _____

5. 技术交易额: _____

技术合同登记机构 (印章)

经办人:

年 月 日

合同编号:

技术服务合同

项目名称: 以牧草为主要原料的饲料配方设计及加工
技术服务

委 托 方: 海南金牧良品生态科技集团有限公司
(甲 方)

受 托 方: 华南农业大学
(乙 方)

签订时间: 2022 年 2 月 10 日

签订地点: 广州

有效期限: 三年

中华人民共和国科学技术部印制

填 写 说 明

一、本合同为中华人民共和国科学技术部印制的技术服务合同示范文本，各技术合同认定登记机构可推介技术合同当事人参照使用。

二、本合同书适用于一方当事人（受托方）以技术知识为另一方（委托方）解决特定技术问题所订立的合同。

三、签约一方为多个当事人的，可按各自在合同关系中的作用等，在“委托方”、“受托方”项下（增页）分别排列为共同委托人或共同受托人。

四、本合同书未尽事项，可由当事人附页另行约定，并作为本合同的组成部分。

五、当事人使用本合同书时约定无需填写的条款，应在该条款处注明“无”等字样。

技术服务合同

委托方（甲方）：海南金牧良品生态科技集团有限公司

住 所 地：海南省海口市龙华区金龙路金海广场 6 栋 1402 房

法定代表人：郝旭明

项目联系人：郝旭明

联系方式：18666622196

通讯地址：广州市海珠区琶洲街道保利叁悦广场 B 塔 1301 室

电 话： 传真：

电子信箱：haoxuming007@163.com

受托方（乙方）：华南农业大学

住 所 地：广州市天河区五山路 483 号

法定代表人：刘雅红

项目联系人：柳广斌

联系方式：020-85280275

通讯地址：广州市天河区五山路 483 号

电 话：13640835578 传真：020-85280740

电子信箱：dwliu@scau.edu.cn

本合同甲方委托乙方就以牧草为主要原料的反刍动物饲料配方设计及加工技术服务项目进行的专项技术服务，并支付相应的技术服务报酬。双方经过平等协商，在真实、充分地表达各自意愿的基础上，根据《中华人民共和国民法典》的规定，达成如下协议，并由

双方共同恪守。

第一条：甲方委托乙方进行技术服务的内容如下：

1. 技术服务的目标：制定反刍动物饲料的营养配方、标准化饲料加工工艺及产品检验标准，提高饲料生产的综合经济效益。

2. 技术服务的内容：(1) 为甲方制定以牧草为主要原料的反刍动物饲料的营养配方（青贮饲料、发酵饲料、颗粒饲料）、标准化饲料加工工艺及饲料检验标准提供技术支持；（2）为甲方提供相关技术信息咨询服务和现场生产指导；（3）对甲方申报项目进行指导。

3. 技术服务的方式：方案制订、现场指导和远程咨询。

第二条：乙方应按下列要求完成技术服务工作：

1. 技术服务地点：海南省定安县雷鸣镇山地村

2. 技术服务期限：三年

3. 技术服务进度：

2022.3.1-2022.9.30 对现有牧草品种及海南可利用的农作物进行评估，制定反刍动物的饲料配方及饲料生产工艺，并对饲料生产进行指导。

2022.10.1-2025.2.28 优化反刍动物饲料配方及饲料生产工艺。

4. 技术服务质量要求：根据甲方需要，乙方定期组织相关专家到甲方进行现场指导和技术培训，协助甲方完善饲料配方设计及标准化饲料加工工艺，提高饲料生产的综合效益。

5. 技术服务质量期限要求：2022.3.1-2025.2.28

第三条：为保证乙方有效进行技术服务工作，甲方应当向乙方提

供下列工作条件和协作事项：

1. 提供技术资料：

- (1) 牧草品种及种植情况；
- (2) 现有饲料配方及生产设备等相关资料；
- (3) 饲料厂发展规划及其他相关信息。

2. 提供工作条件：

- (1) 提供乙方到现场工作的基本交通和食宿条件；
- (2) 协助专家与相关技术管理人员的对接沟通工作，严格执行经双方确认的技术方案；
- (3) 协助开展相关实验研究；如提供实验场地、实验动物和生产人员等，具体试验需要双方另行商定。

3. 其他：无

4. 甲方提供上述工作条件和协作事项的时间及方式：双方协商。

第四条：甲方向乙方支付技术服务报酬及支付方式为：

- 1. 技术服务费总额为：叁万元整（¥30000 元）
- 2. 技术服务费由甲方一次性（一次或分期）支付乙方。

具体支付方式和时间如下：2022 年 4 月 1 日前由甲方一次性支付给乙方。

乙方开户银行名称、地址和帐号为：

开户银行：中国工商银行广州五山支行

地址：广州市天河区五山路 483 号

户名: 华南农业大学

帐号: 3602 0026 0900 0310 520

第五条: 双方确定因履行本合同应遵守的保密义务如下:

乙方的保密责任如下:

1. 保密内容(包括技术信息和经营信息): 共同制定的所有反刍动物饲料的营养配方及生产工艺。

2. 涉密人员范围: 项目组所有成员

3. 保密期限: 三年

4. 泄密责任: 承担泄密内容所造成的经济损失

第六条: 本合同的变更必须由双方协商一致, 并以书面形式确定。但有下列情形之一的, 一方可以向另一方提出变更合同权利与义务的请求, 另一方应当在 7 日内予以答复; 逾期未予答复的, 视为同意:

1. 无

2. 无

3. 无

4. 无

第七条: 双方确定以下列标准和方式对乙方的技术服务工作成果进行验收:

1. 乙方完成技术服务工作的形式: 建立一套完整的以牧草为主要原料的反刍动物饲料(青贮饲料、发酵饲料、颗粒饲料)的核心配方、生产工艺及产品检验标准的文件。

2. 技术服务工作成果的验收标准：饲料营养价值评估报告(料肉比、繁育率)的验收，双方认可。

3. 验收地点：海南省定安县雷鸣镇山地村现场验收。

第八条 侵权处理

1. 乙方应当保证，其依本合同为甲方提供技术服务过程中和/或其为甲方提供的服务成果不侵犯任何第三人的合法权益。如果有人提出法律或行政程序(合称“侵权指控”)，声称甲方侵犯了其知识产权等合法权益的，乙方应当负责解决，并赔偿甲方就此所承担的一切损失和费用，包括但不限于上述侵权指控中所产生的诉讼费用、合理的律师费用、调查费用、和解金额或生效法律文书中规定的赔偿金额。

2. 如果在侵权指控的审理过程中有关机关禁止甲方继续使用技术服务成果的部分或全部，乙方应当采取以下措施之一：

(1) 使甲方重新免费获得使用上述技术服务成果的权利。

(2) 免费更换或改造上述技术服务成果，使甲方不受上述禁令限制继续使用技术服务成果。

(3) 其它使甲方对技术服务成果拥有合法使用权，或其它弥补甲方受损利益、实现合同目的的合理方式。

乙方采取上述措施不能免除乙方就甲方因此遭受的损失进行赔偿的义务。

第九条：项目服务成果的权利归属

1. 在本合同有效期内，甲方利用乙方提交的服务成果所完成的新的技术成果，归双（甲、双）方所有。

2. 乙方利用甲方提供的技术资料和工作条件所完成的服务成果，归双（乙、双）方所有。

第十条：双方确定，在本合同有效期内，甲方指定郝旭明为甲方项目联系人，乙方指定柳广斌为乙方项目联系人。项目联系人承担以下责任：

1. 共同协助项目顺利完成。

一方变更项目联系人的，应当及时以书面形式通知另一方，未及时通知并影响本合同履行或造成损失的，应承担相应的责任。

第十一条：双方确定，出现下列情形，致使本合同的履行成为不必要或不可能的，可以解除本合同：

1. 发生不可抗力。

第十二条：双方因履行本合同而发生的争议，应协商、调解解决。协商、调解不成的，确定按以下第1种方式处理：

1. 提交广州市仲裁委员会仲裁；

2. 依法向人民法院起诉。

第十三条：双方确定：本合同及相关附件中所涉及的有关名词和技术术语，其定义和解释如下：无。

第十四条：双方约定本合同其他相关事项为：无。

第十五条：本合同一式4份，具有同等法律效力。

第十六条：本合同经双方签字盖章后生效。

甲方：海南金牧良品生态科技集团有限公司（盖章）

法定代表人 / 委托代理人：（签名）

2022年2月23日

乙方：华南农业大学（盖章）

法定代表人 / 委托代理人：（签名）

2022年2月23日

印花税票粘贴处:

(以下由技术合同登记机构填写)

合同登记编号:

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

1. 申请登记人: _____

2. 登记材料: (1) _____

(2) _____

(3) _____

3. 合同类型: _____

4. 合同交易额: _____

5. 技术交易额: _____

技术合同登记机构 (印章)

经办人:

年 月 日

合同编号：2024-XBH-00-002

2024年省级乡村振兴战略专项资金 种业振兴行动项目

合 同 书

项目名称：雷州山羊新种质资源引进与优质基因快速传递技术开发
 项目管理单位（甲方）：广东省农业农村厅
 项目牵头承担单位（乙方）：华南农业大学
 项目推荐（主管）单位（丙方）：
 项目负责人：刘德武 联系电话：13640835778
 项目联系人：柳广斌 联系电话：18802085530

广东省农业农村厅制

第一条 为保障 2024 年省级乡村振兴战略专项资金种业振兴行动项目顺利实施, 按时保质保量完成项目任务, 根据《中华人民共和国民法典》《广东省省级财政专项资金管理办法(修订)》(粤府〔2023〕34 号)、《广东省农业农村厅种业振兴行动专项资金管理办法(试行)》等文件有关规定, 经甲、乙、丙三方协商一致, 签署本合同书。

第二条 甲方的权利义务: 本合同履行过程中, 甲方有权对乙方项目的实施情况和资金到位、使用情况进行监督、检查, 提出改进要求。

第三条 乙方的权利义务:

1. 按财政资金管理规定, 对甲方核拨的资金做到专款专用, 单独列账, 并随时配合甲方进行监督检查。

2. 认真填写本合同书《项目任务书》, 《项目任务书》的内容应与乙方的《项目申报书》保持一致。

3. 严格按照本合同书及合同书《项目任务书》的要求及时完成项目建设内容, 项目实施完成后, 按照本合同验收报告模版要求提交验收报告。

4. 按照《广东省农业农村厅种业振兴行动专项资金管理办法(试行)》规定, 按期(每年 6 月 30 日、12 月 31 日)向甲方、丙方书面报告项目实施进展及资金使用情况等内容。

5. 乙方需保留与所有参与单位的合作实施协议和相关财务凭证, 并向甲方备案。

第四条 丙方的权利义务:

1. 为乙方项目实施提供必要的条件保障。

2. 负责对项目承担单位的实施条件、能力以及财务管理规范进行审查, 对推荐项目的实施场地、申报材料等进行真实性审核, 并监督项目实施、资金预算执行情况。

3.协助甲方完成对财政资金投资 500 万元（含）以上【科研项目财政资金在 200 万元（含）以上】项目验收等工作；负责完成对财政资金投资 500 万元以下（科研项目财政资金 200 万元以下）项目验收工作，并及时向甲方报告情况。

第五条 本项目资金不得用于以下方向：1.行政事业单位基本支出；2.各项奖金、津贴、福利补助、职工工资、奖励绩效等；3.企业担保金和弥补企业亏损；4.修缮楼堂馆所以及建造职工住宅；5.弥补单位预算支出缺口和偿还债务；6.购买交通工具及通讯设备；7.形成地方政府债务的支出；8.购买理财产品、发放借款及平衡预算等。

第六条 项目验收。项目验收及结果处理严格执行《广东省农业农村厅专项资金项目验收管理办法（试行）》（粤农农办〔2023〕73 号）的规定。乙方应在项目完成后 3 个月内，提出验收申请。申请验收除了规定材料外，还应该提交项目审计报告或者经费决算表，其中财政专项资金 50 万元以下的项目，需提交由项目承担单位财务部门出具的经费决算表，财政专项资金 50 万元（含）以上的项目，需提交由项目承担单位委托会计师事务所出具的审计报告。对财政资金投资 500 万元（含）以上【科研项目财政资金在 200 万元（含）以上】的项目，及乙方直接向甲方申报的项目，由甲方负责组织验收；对财政资金投资 500 万元以下（科研项目财政资金 200 万元以下）的项目，由项目推荐（主管）单位（丙方）负责验收，验收单位向甲方提交验收材料，甲方对验收材料进行审核确认。


第七条 在履行本合同的过程中，如出现相关法律法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。因非不可抗力因素导致的项目未履行或未履行完毕，或因乙方责任造成项目不能继续开展的，甲方有权终止项目合同，收回尚未使用和使用不符合规定的财政经费。

第八条 在履行本合同的过程中,乙方发现可能导致项目整体或部分失败的情形时,应及时通知甲方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

第九条 实施项目所获得的科技成果(项目成果)归属、成果转让和实施技术成果所产生的经济利益的分享,按照国家和广东省有关规定执行。项目研究成果应向省农业农村厅进行登记、备案,对外发布前应征求省农业农村厅的意见。

第十条 本合同在履行过程中发生的任何争议,由甲乙丙三方友好协商解决。

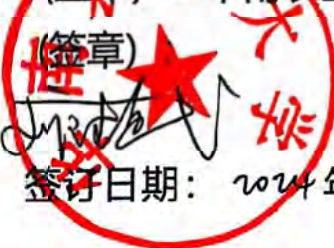
第十一条 本合同未尽事宜,各方同意按照《广东省省级财政专项资金管理办法(修订)》(粤府〔2023〕34号)、《广东省农业农村厅种业振兴行动专项资金管理办法(试行)》履行。

项目管理单位(甲方) (盖章):  广东省农业农村厅

法定代表人(或授权代表) (签章): 

签订日期: 2025年2月19日

项目牵头承担单位(乙方) (盖章):  华南农业大学

法定代表人(或授权代表) (签章): 

项目负责人(签章): 

签订日期: 2024年12月19日

乙方推荐(主管)单位(丙方) (盖章):

法定代表人(或授权代表) (签章):

签订日期: 年 月 日

项目任务书

填写说明

一、本项目任务书由乙方填写。

二、本项目任务书所列内容应实事求是填写，表达要明确、严谨。对填写不符合要求的，或填报内容出现虚报夸大、不切实际的，将退回项目承担单位修改。

三、项目任务书规定的项目考核指标、建设内容和绩效目标必须依据《项目申报书》填写，应遵循明确、量化、可考核的原则，其中技术指标应明确项目完成时达到的关键技术参数及预期可以形成的发明专利、标准、新技术、新产品、新装置、论文、专著等的数量。项目申报指南对项目技术、经济和成果等指标有明确要求的，应符合项目申报指南的要求，相关专项管理办法有特别规定的，应符合相关规定。

四、《项目申报书》及申报指南是本项目任务书填报的重要依据，项目任务书填报不得修改考核指标、绩效目标、资金预算等内容。《项目申报书》、申报指南和本项目任务书将共同作为项目过程管理、综合绩效评价（验收）和监督评估的重要依据。

五、省财政资金支出的预算计划应按照国家及省相关规定执行。

六、表格栏目不够可自行增加。

一、目的及意义

主要说明项目的建设目的、研究价值和意义。

雷州山羊是广东省独特的地方山羊品种，因其耐粗饲、耐高温高湿、抗病力强、性成熟早、肉用性能好等优点，已被列入《国家级畜禽遗传资源保护名录》。近年来，在广东省农业农村厅的支持下，虽然成立了两个省级雷州山羊保种场，并取得了一定的保种成果，但由于大量外来品种如川中黑山羊、努比亚黑山羊等的引入和杂交，导致保种场内雷州山羊的纯度受到严重影响，遗传结构复杂，纯种个体稀缺。现有保种群体受到了周边省份山羊的基因渗入，无法满足纯种保种和提纯复壮的需求。

这种状况对雷州山羊这一宝贵遗传资源的保护和利用提出了严峻挑战。为了有效地恢复和保持雷州山羊的纯种特性，亟需从国家家畜基因库引进保存的纯种雷州山羊冷冻精液和胚胎，利用先进的人工授精和胚胎移植技术，恢复纯种雷州山羊遗传资源，组建新的 F0 代基础群体。同时，开展分子遗传鉴定，确保新群体的遗传纯度。

此外，传统的繁育方式已无法满足快速提升种群质量的需求。开发冷冻精液、冷冻胚胎和体细胞克隆等优质基因快速传递技术，实现优良基因的高效利用和长期保存，是提升雷州山羊种群质量的关键途径。通过全基因组测序和表型关联分析，建立优良遗传特征识别与选择技术体系，能够为精准育种提供科学依据，进一步提升育种效率和种群质量。

本项目的实施，不仅有助于恢复和保护纯种雷州山羊的遗传资源，解决当前保种群体纯度不高的问题，还能促进雷州山羊产业的发展，提升其经济效益。通过技术创新和遗传改良，保持雷州山羊的优良特性，满足市场对高品质羊肉的需求，助力广东省畜牧业的可持续发展，具有重要的科学意义和

现实意义。

二、项目建设内容

详细说明项目建设内容（项目需求或项目建设任务，包含项目参与单位的建设内容）。

研究一：纯种雷州山羊遗传资源引进、恢复及 F0 群体组建

承担单位：广东海洋大学

任务负责人：赵志辉

鉴于雷州山羊纯种个体稀缺、遗传结构复杂，保种和提纯复壮工作面临严峻挑战，本研究拟从国家动物遗传资源基因库引进保存的纯种雷州山羊冷冻精液和胚胎，利用人工授精和胚胎移植技术实现其遗传物质的恢复。通过基因组测序技术，对恢复的群体及现有保种群体进行血缘鉴定，确保纯种群体的建立和遗传纯度的准确性。组建新的 F0 代纯种雷州山羊群体，扩大纯种资源的遗传基础。本研究将增强雷州山羊种群的遗传多样性，为后续的保种育种工作提供坚实的遗传资源保障，推动品种的可持续发展。

研究二：雷州山羊优质基因资源快速传递技术开发

承担单位：华南农业大学

任务负责人：柳广斌

传统的繁育方法已无法满足雷州山羊优质基因快速传递的需求，限制了种群改良的速度和效率。为此，本研究旨在开发和优化雷州山羊的冷冻精液、冷冻胚胎及体细胞克隆等先进繁育技术。通过改进冷冻保存方法，提高遗传物质的活性和保存期限；开展克隆胚胎生产和克隆个体培育研究，

建立完善的生物安全控制体系，确保克隆技术的安全应用。该技术体系的建立将实现优质基因资源的长期保存和高效利用，加速优良基因在种群中的传播，为雷州山羊产业的提升提供强有力的技术支持。

研究三：优良遗传特征识别与选择技术体系建立

承担单位：华南农业大学

任务负责人：刘德武

目前雷州山羊缺乏对其优良遗传特征的系统性认识，影响了精准育种工作的开展。本研究拟通过全基因组测序和表型关联分析，识别纯种雷州山羊的优良遗传特征，建立基因型特征库。利用高通量测序技术获取基因组数据，结合生产性能等表型数据，开展基因型与表型的关联分析，筛选出与重要性状相关的关键基因和遗传标记。开发精准的鉴定技术，建立标准化的遗传特征识别和选择体系。本研究将为雷州山羊的遗传评估和精准育种提供科学依据，提升保种场种群质量，推动品种改良和产业升级。

研究四：雷州山羊保种群体扩大及遗传改良

承担单位：雷州市状元黑山羊养殖有限公司

任务负责人：陈五拓

鉴于现有雷州山羊保种群体规模有限，制约了品种的保护和开发利用，本研究计划扩大核心保种群体数量，构建多个家系，优化种群结构。通过引进纯种雷州山羊遗传资源的引进与恢复，采用人工授精、胚胎移植等繁殖技术，提高核心保种群体的保种质量。同时，对当地及周边地区的雷州山羊进行遗传改良，推广优质种公羊，提升后代的生产性能。本研究将扩大良种的推广范围，提升区域内雷州山羊的生产性能和经济效益，促进产业的健康发展，助力乡村振兴和农民增收。

备注：项目建设内容（项目需求或项目建设任务）按《项目申报书》内容填写。

三、项目绩效目标

主要说明项目实施后，预期达到的目标和产生的效果，相关表述应量化。

（一）总体考核指标

1. 引进国家家畜基因库雷州山羊冷冻精液 300 剂，胚胎 30 枚；组建 F0 代纯种雷州山羊基础群体 100 只以上，扩大雷州山羊保种群 300 只以上；
2. 形成 DNA 血缘鉴定报告 1 份；
3. 形成雷州山羊胚胎移植及克隆技术体系各 1 套；
4. 建立优良遗传特征识别与选择技术体系 1 套；
5. 改良个体数量达到 2000 只以上；
6. 制作冷冻胚胎 100 枚和冷冻精液 800 支，部分返还国家基因库或广东省畜禽遗传资源库的保存。

（二）年度考核指标

项目实施期为两年，两年绩效目标分为：

第一年绩效目标

1. 引进国家家畜基因库雷州山羊冷冻精液 300 剂，胚胎 30 枚；组建 F0 代纯种雷州山羊基础群体 100 只以上；
2. 形成 DNA 血缘鉴定报告 1 份；
3. 形成雷州山羊胚胎移植技术体系 1 套；
4. 制作冷冻胚胎和冷冻精液，部分返还国家基因库或广东省畜禽遗传资源库的保存。

第二年绩效目标

1. 扩大雷州山羊保种群 300 只以上；
2. 形成雷州山羊克隆技术体系各 1 套；
3. 建立优良遗传特征识别与选择技术体系 1 套；
4. 改良个体数量达到 2000 只以上；
5. 制作冷冻胚胎 100 枚和冷冻精液 800 支，部分返还国家基因库或广东省畜禽遗传资源库的保存。

四、项目进度安排

详细说明各阶段的工作内容和时间安排情况。

项目实施期为两年，2024 年 12 月 29 日-2026 年 12 月 28 日，具体进度安排如下：

2024 年 12 月-2025 年 5 月：项目启动与准备，成立项目管理委员会和技术专家组，完善项目实施方案。开展纯种雷州山羊遗传资源的引进和恢复，实施人工授精和胚胎移植技术，组建 F0 代纯种群体。

2025 年 6 月-2025 年 11 月：组建 100 只以上的 F0 代纯种群体，完成 DNA 血缘鉴定报告。开发雷州山羊胚胎移植和克隆技术，优化技术参数。开展全基因组测序。

2025 年 12 月-2026 年 5 月：制作冷冻胚胎和冷冻精液，形成胚胎移植技术体系 1 套，优化克隆技术参数。开展优良性状基因型-表型关联分析。扩大保种核心群体规模。

2026 年 6 月-2026 年 12 月:形成克隆技术体系 1 套,制作冷冻胚胎 100 枚和冷冻精液 800 支。建立优良遗传特征识别与选择技术体系 1 套。保种群数量达到 300 只以上,推广优质种公羊,改良个体数量达到 2000 只以上。项目总结与验收,编写技术报告、专利申请和论文发表。

备注:项目绩效目标按《项目申报书》内容填写。

五、项目主要合作、参与单位(含牵头承担单位)					
单位名称		单位性质	统一社会信用代码	通讯地址	
华南农业大学		大专院校	124400004554165634	广东省广州市天河区五山路 483 号	
广东海洋大学		大专院校	1244000045625261X8	广东省湛江市麻章区海大路 1 号	
雷州市状元黑山羊养殖有限公司		企业	MA4UQ9UK-2	雷州市白沙镇黎郭村(邦企线公路北蒔甘岭)	
六、项目组主要成员(含项目负责人)					
姓名	性别	身份证号	单位	职称/职务	电话
刘德武	男	42010619660711563X	华南农业大学	正高级	13640835778
柳广斌	男	210103198204261211	华南农业大学	中级	18802085530
李耀坤	男	410423198607084036	华南农业大学	副高级	18620193682
卫恒习	男	130531198002062019	华南农业大学	副高级	15902067686
孙宝丽	女	411024198109241823	华南农业大学	正高级	13802405625
郭勇庆	男	13042119810616031X	华南农业大学	副高级	18122469798
邓铭	男	430726198601203910	华南农业大学	副高级	15915704462
赵志辉	男	220106196511039612	广东海洋大学	正高级	13578754340
甘尚权	男	340822197707162	广东海洋大学	正高级	13414887690

		837			
高蕊	女	659001198010133547	广东海洋大学	正高级	18999327691
尹福泉	男	152301197211091037	广东海洋大学	正高级	15913509615
于海滨	男	220106198802010210	广东海洋大学	副高级	18686609912
姜平	女	220182199206018620	广东海洋大学	副高级	15584169529
照那木拉	男	152323198110044613	广东海洋大学	中级	18813628301
林紫薇	女	440803199005101528	广东海洋大学	中级	13922077371
陈五拓	男	440824197905100638	雷州市状元黑山羊养殖有限公司	其他	13724707988
陈华明	男	440823199210083336	雷州市状元黑山羊养殖有限公司	其他	13692332147

七、资金使用预算

主要说明资金使用的范围或方向及资金使用进度安排(包含参与单位内容)。

(一) 资金使用的范围或方向

1. 本项目省级财政资金总经费 300.00 万元，各单位经费分配如下：

(1) 华南农业大学 150 万元，负责的研究任务包括：

1) 研究二：雷州山羊优质基因资源快速传递技术开发，70 万元，负责人：柳广斌；

2) 研究三：优良遗传特征识别与选择技术体系建立，80 万元，负责人：刘德武；

(2) 广东海洋大学 100 万元，负责的研究任务包括：

研究一：纯种雷州山羊遗传资源引进、恢复及 F0 群体组建，100 万元，负责人：赵志辉。

(3) 雷州市状元黑山羊养殖有限公司 50 万元，负责的研究任务包括：

研究四：雷州山羊保种群体扩大及遗传改良，50 万元，负责人：陈五拓。

2. 经费主要用途如下：

(1) 直接费用：285 万元。包括：

1) 材料费：162.75 万元，其中华南农业大学 61.14 万元，广东海洋大学 51.61 万元，雷州市状元黑山羊养殖有限公司 50.00 万元。主要用于采购项目实施所需的实验试剂耗材以及实验样品、饲料和药物等费用。

2) 测试化验加工费：55.60 万元，其中华南农业大学 41.00 万元，广东海洋大学 14.60 万元。主要用于基因组、转录组及微生物检测。

3) 出版/文献/信息传播/知识产权事务费: 18.55 万元, 其中华南农业大学 10.80 万元, 广东海洋大学 7.75 万元。用于论文出版、专利申请、资料打印、快递等相关费用。

4) 会议/差旅/国际合作交流费: 21.44 万元, 其中华南农业大学 14.10 万元, 广东海洋大学 7.34 万元。用于项目开展所需的差旅费。

5) 劳务费: 25.66 万元, 其中华南农业大学 12.96 万元, 广东海洋大学 12.70 万元。用于支付参与项目的研究生及临时聘用人员的劳务费。

6) 专家咨询费: 1.00 万元, 其中华南农业大学 1.00 万元。用于邀请高级职称专家进行技术指导的咨询费。

(2) 间接费用: 15.00 万元, 为项目承担单位华南农业大学与广东海洋大学的项目管理费, 按所分配经费的 6% 计算。

(二) 资金使用进度安排

2025 年 6 月前累计支出 75 万元, 占总经费 25%;

2025 年 12 月前累计支出 150 万元, 占总经费 50%;

2026 年 6 月前累计支出 225 万元, 占总经费 75%;

2026 年 12 月前完成经费全部支出。

八、保障措施

说明围绕完成项目任务、目标所要采取的具体措施。

1. 项目组织方式和机制

项目实施单位根据计划目标抓好财务管理和质量管理，加强项目组织管理，保证专款专用，任务落实到位。实施严格的岗位责任制，加强工作质量管理，提高管理水平和工作效率，保证项目开展合理科学，实施规范到位，保障项目建设顺利进行。做好计划管理，项目建设按照“统一计划，分步实施”的原则，根据批复的项目申报书等文件，确定本项目建设内容、物资采购、资金使用计划等，严格执行，不得随意变更。做好信息管理，做好文件、档案等资料的收集保管等。

2. 产学研结合机制

建立产学研合作的技术创新机制。通过项目建设，与相关企业单位建立创新联合体。各单位间通过人员互派、优势互补等有效措施，解决科研与应用相脱离的“两层皮”问题，打通技术研发与产业化应用的渠道，真正建立产学研合作的技术创新机制。

3. 创新人才队伍的凝聚和培养

项目承担单位华南农业大学及广东海洋大学是南方现代草牧业（羊）产业技术体系创新团队的重要成员。团队成员均长期从事草食动物生产技术研究，具有丰富一线实践经验。本项目为团队成员及参与项目的研究生提供良好的科学研究条件与合作交流机会，为人才培养提供有利条件。

（四）预算资金管理情况

统筹安排建设资金，严格按照有关财务制度及有关规定，科学、合理使用项目资金，确保资金使用效益。设立本项目资金专用账户，按照“统

—规划、专账核算、专款专用”的原则，确保项目建设进度和预期目标。另外，加强财务管理和预算管理，建立科学化、精细化经费预算管理机制，专款专用，独立核算；严格控制经费超支，确保经费使用规范、安全、有效。

联合申报项目协议书

甲方：华南农业大学

乙方：广东海洋大学

丙方：雷州市状元黑山羊养殖有限公司

甲方、乙方、丙方经友好协商决定联合申报 2024 年度 省级种业振兴行动和科技兴农专项资金 项目，项目名称：雷州山羊新种质资源引进与优质基因快速传递技术开发。并达成如下合作协议：

第一条：项目研究工作详细分工：

甲方(主持方)：负责项目的主持申报与管理；开发雷州山羊优质基因资源快速传递技术，形成雷州山羊胚胎移植及克隆技术体系；建立优良遗传特征识别与选择技术体系；制作冷冻胚胎；负责样品在广东省畜禽遗传资源库的保存。开展技术指导与培训工作，负责项目报告撰写及上传。

乙方(参与方)：协助甲方进行项目申报及实施工作；负责纯种雷州山羊遗传资源引进、恢复及 F0 群体组建；开展 DNA 血缘鉴定并形成报告；制作冷冻精液；开展技术指导与培训工作；协助甲方完成项目报告撰写。

丙方(参与方)：负责雷州山羊保种群及实验群的饲养管理以及表型测定，协助甲方和乙方开展技术开发及示范工作，提供必要的工作条件及实验羊群体；负责雷州山羊保种群体的扩大及遗传改良工作。

第二条：经费分配：

1、项目申报政府资助经费 300 万元，甲方、乙方、丙方同意此经费按甲方 150 万元、乙方 100 万元、丙方 50 万元 进行分配。如果政府下达的财政资助经费高于或低于 300 万元，则按相应的比例调增或调减。

2、甲方在收到 省财政资金 下达的资助经费后的 一个月 内将乙方、丙方所占经费支付给乙方、丙方指定帐户。

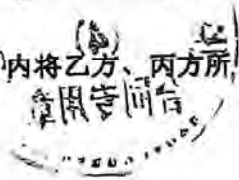
乙方账户信息：

账 户：广东海洋大学

帐 号：679557760592

开户行：中国银行股份有限公司湛江霞山支行

丙方账户信息：



账 户：雷州市状元黑山羊养殖有限公司

帐 号：2015021109200038565

开户行：中国工商银行股份有限公司雷州支行

第三条：企业配套经费比例： 无 。

第四条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有；各方共同完成的由各方共享，具体按照各方的贡献大小进行分配或各方另行商定。

②共同完成的项目成果的转让，须在各方同意的前提下进行，任何一方不得私自转让或许可实施。

2. 项目成果申报各级奖项，各方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第五条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第六条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第七条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第八条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第九条：本协议自各方签字盖章之日起生效，至项目完成之日起终止，若合作申请未获资助，本协议自动废止。



项目负责人（签字）：

[Signature]

2024年10月17日



项目负责人（签字）：

[Signature]

2024年10月17日



项目负责人（签字）：

[Signature]

2024年10月17日

凭证信息					
凭证内码:	25040110171	分录编号:	26	摘要:	恢复年底省财政收回零余额项目转基本户-转零B753
部门编号:	4300	部门名称:	动物科学学院		
项目编号:	F24103	项目名称:	转/零B753-种业振兴行动-雷州山羊新种质资源引进与优质基因快速传递技术开发		
科目编号:	80010301	科目名称:	财政直接支付		
经济分类科目编号:		经济分类科目名称:			
借金额:	0.00	贷金额:	3,000,000.00		
银行账号:		结算单号:		对方单位:	
支付状态:		打卡人:			

打印时间: 2025-07-17 13:26:49
打印单位: 华南农业大学

华南农业大学科研经费入账通知单（纵向）

L202500069

单号：
财务处：

单位：元

由我校 动科学院

刘德武 老师负责的纵向科研课题，现有经费到账

请按照下表的具体情况给予办理经费入账并开具相应的票据。

来款金额（元）	3000000	来款日期	20241230	经费卡号	F25103
项目名称	雷州山羊新种质资源引进与优质基因快速传递技术开发				
来款单位	广东省财政厅零余额				
到位金额	3000000	学校管理费（4%）	60000	性质备注	混合经费
外拨金额	1500000	学院管理费（2%）	30000		
可支配金额	1500000	课题组	0		
票据类型	开纵向新卡		办理日期	20250313	

注：经办人：李振梓 审核：李振梓

请依次到以下科室办理214

广东省林业科技创新项目

合 同 书

项目编号： 2023KJCX004

项目名称： 广东丘陵山地“林-草-羊”生态循环种养模式构建与示范

承担单位： 华南农业大学 (公章)

项目负责人： 贾小容 联系电话： 15918752817

项目联系人： 龙凤玲 联系电话： 17875571130

项目起止年限： 2023 年 1 月至 2025 年 12 月

广东省林业局制



填写说明

1. 本合同书为广东省林业科技创新项目专用，合同甲方为项目管理部门——广东省林业局，乙方为项目承担单位，丙方为项目承担单位的上级归口管理单位（市、县级林业行政主管部门，省或省林业局直属单位，中直驻粤单位）。
2. 合同书由项目承担单位填写，省林业局与项目承担单位及其上级归口管理单位共同签订。
3. 项目编号按省林业局下达的计划项目编号填写。
4. 项目申报时承诺配套或自筹资金的应按比例落实，否则不予签订合同书。
5. 合同书一律用仿宋体四号字体填写，A4 纸双面打印装订（有条件的可采用 A3 纸双面打印中缝装订）。
6. 本合同书 1 式 6 份，分别保存于项目管理部门、项目承担单位和上级归口管理单位。



项目信息表

项目编号		2023KJCX004									
项目名称		广东丘陵山地“林-草-羊”生态循环种养模式构建与示范									
项目承担单位	名称	华南农业大学									
	单位负责人	刘雅红	职务	校长	技术职称	教授					
	单位所在地	广东省广州市天河区									
	通讯地址	广州市天河区五山路 483 号						邮编	510642		
	单位类别	<input checked="" type="checkbox"/> 大专院校 <input type="checkbox"/> 科研院所 <input type="checkbox"/> 企业 <input type="checkbox"/> 其他									
	归口管理单位	广东省教育厅									
其他主要参加单位	序号	单位名称									
	1	广东态合堂实业有限公司									
	2										
项目负责人	姓名	贾小容	性别	<input type="checkbox"/> 男 <input checked="" type="checkbox"/> 女	出生年月	1978 年 8 月					
	学历	<input checked="" type="checkbox"/> 研究生 <input type="checkbox"/> 大学 <input type="checkbox"/> 大专 <input type="checkbox"/> 中专 <input type="checkbox"/> 其他									
	学位	<input type="checkbox"/> 博士 <input checked="" type="checkbox"/> 硕士 <input type="checkbox"/> 学士									
	职称	<input type="checkbox"/> 正高级 <input checked="" type="checkbox"/> 副高级 <input type="checkbox"/> 中级 <input type="checkbox"/> 初级 <input type="checkbox"/> 其他									
	移动电话	15918752817			E-mail	xiaorongj@scau.edu.cn					
项目组人数	26 人	高级	5 人	中级	6 人	初级	5 人	其他	10 人		
起始时间	2023 年 1 月				终止时间			2025 年 12 月			
项目类型	<input checked="" type="checkbox"/> 应用基础研究 <input type="checkbox"/> 应用开发 <input type="checkbox"/> 产业化开发 <input type="checkbox"/> 成果转化 <input type="checkbox"/> 其它										
创新类型	<input type="checkbox"/> 原始创新 <input checked="" type="checkbox"/> 集成创新 <input type="checkbox"/> 引进消化吸收再创新										



主要研究内容 (100 字以内)		项目拟在广东省丘陵山地开展林草优化配置和不同林草组合下的山羊生态养殖技术研究, 阐明“林-草-羊”种养模式与森林环境的耦合机制, 构建符合广东省林下经济发展实际的生态优先、绿色发展的林下种养生态循环模式。				
预期成果		<input checked="" type="checkbox"/> 专利 <input checked="" type="checkbox"/> 技术标准 <input type="checkbox"/> 新产品 (或林木新品种) <input type="checkbox"/> 新工艺 <input type="checkbox"/> 新材料 <input type="checkbox"/> 计算机软件 <input checked="" type="checkbox"/> 论文论著 <input checked="" type="checkbox"/> 研究报告 <input type="checkbox"/> 其他				
预期知识产权		获得国家发明专利____项, 其他专利 2 项; 植物新品种权____项。				
预期技术标准制定		<input type="checkbox"/> 国际标准 <input type="checkbox"/> 国家标准 <input type="checkbox"/> 行业标准 <input type="checkbox"/> 地方标准 <input checked="" type="checkbox"/> 团体标准 <input checked="" type="checkbox"/> 企业标准				
产学研联合		<input checked="" type="checkbox"/> 是 <input type="checkbox"/> 否				
经费 预算	总预算	150 万元, 其中省财政资金 150 万元, 地方财政 0 万元, 其他 0 万元。				
	专项	2023 年	2024 年	2025 年	____年	____年
	预算	150 万元	____万元	____万元	____万元	____万元
一、项目目标与任务						
1、 创新与示范目标 (1) 阐明“林-草-羊”种养模式与森林环境的耦合机制: 监测林地生物多样性和小气候特征, 评价林地土壤肥力变化, 揭示“林-草-羊”种养模式与森林环境的相互关系。 (2) 筛选兼具生态效益和经济效益的林草优化配置模式: 开展林草优化配置实验, 比较不同模式下牧草的产量、营养成分和饲用价值, 结合森林环境指标, 筛选出兼具生态和经济效益的林草优化配置组合 2-3 种。						



(3) 确定合理的林下饲养模式: 设置在林草优化组合下半放牧半舍饲饲养、天然林草下半放牧半舍饲饲养和全舍饲饲养等三种种养模式, 分析比较不同模式下山羊的生理生化指标、生长性能、屠宰性能及肉品质等, 确定较优的林下饲养模式, 明确林草优化模式对生态养羊的影响机理。

(4) 构建广东丘陵山地“林-草-羊”生态循环种养模式: 综合林地生态效益和经济效益, 基于筛选出的林草优化配置模式、合理的林下饲养模式, 凝练并集成广东丘陵山地林草优化配置与生态养羊的关键技术。

2、 创新与示范内容

(1) “林-草-羊”种养模式在提高生物多样性、保持水土和改善小气候等方面的效益变化机制研究

通过监测林下环境、林草生长、土壤性状及养分、土壤生物多样性, 评价固土保肥效益、生物多样性效益、微气候效益, 分析系统碳储量变化规律等探索“林-草-羊”种养模式与森林环境的耦合机制。

(2) 林草优化配置技术研究

研究不同林草配置模式下牧草鲜草及干草产量、营养成分(粗蛋白、粗纤维、粗脂肪和粗灰分等)的动态变化特征, 开展产量及营养成分的综合评价和适应性分析, 筛选适应该地区气候和立地条件的林草优化配置组合。

(3) 生态养羊技术研究

首先探索天然林草放牧模式下的生态承载力, 初步确定合理的山羊养殖密度和轮牧方式; 之后, 通过对比天然林草、林草优化配置组合和舍饲模式下, 基于山羊的生理生化指标、生长性能、屠宰性能及肉品质等, 评价不同



林草组合及饲养模式对山羊林下放牧养殖效果的影响。

(4) “林-草-羊”生态循环种养模式构建和示范

合理配置“林-草-羊”系统中资源、环境和生产要素，集成“林-草-羊”系统结构优化技术，构建“林-草-羊”生态循环种养模式，开展林下种养生态效益、经济效益和社会效益的评估，进行示范。

3、 要解决的主要技术难点和问题

要解决的主要技术难点：筛选适应该地区气候、立地条件和山羊营养需求的林草优化配置组合，确定合理的山羊载畜量和放牧方式是本项目的主要技术难点。

要解决的主要技术问题：揭示“林-草-羊”种养模式在提高生物多样性、保持水土和改善小气候等方面的效益变化机理；筛选出兼具生态效益和经济效益的林草优化组合；阐明天然林草和林下人工套种牧草 2 种模式下的生态养羊技术；构建“林-草-羊”生态循环种养模式。

4、 技术路线

通过资料收集、野外调查与监测、试验分析、综合分析等技术措施开展林草优化配置与生态养羊技术研究（技术路线见图 1）。

(1) 动态监测林地效益。利用样方调查法调查林地群落物种组成和数量特征，测定土壤理化性质、土壤碳储量，分析土壤微生物群落组成、土壤酶活性等指标，评价土壤肥力和林地生物多样性，同时监测林地小气候特征，揭示“林-草-羊”种养模式在提高生物多样性、保持水土和改善小气候等方面的效益变化机理；



(2) 优化林草配置模式。选取 1 种林分和 2 种豆科牧草及 2 种禾本科牧草等为主要试验对象, 设置不同林草组合, 以天然林草地为对照, 分析比较不同组合下牧草产量、营养成分和饲用价值, 结合森林环境指标分析, 筛选出兼具生态效益和经济效益的林草优化配置组合;

(3) 探索生态养羊关键技术。设置林草优化组合的林下进行半放牧半舍饲饲养、天然林草下半放牧半舍饲饲养和全舍饲饲养等三种种养模式, 分析比较不同模式下山羊的生理生化指标、生长性能、屠宰性能及肉品质等, 确定合理的饲养模式;

(4) 建设示范基地。综合林地生态效益和经济效益评价, 基于筛选出的林草优化配置组合、合理的林下饲养模式, 集成广东丘陵山地林草优化配置与生态养羊的关键技术, 构建广东丘陵山地“林-草-羊”生态循环种养模式, 开展示范。

5、创新点

理论创新:结合林学、生态学、草业科学、动物科学、土壤学、植物学、生物化学、生理学、营养学等学科知识, 在林地开展生态循环种养模式探索, 研究当前广东丘陵山地“林-草-羊”生态循环种养模式的机理、机制、技术和示范, 为我省丘陵山地林下种养提供系统的、合理的、科学的理论依据, 形成一套我省丘陵山地适宜的林下种养技术体系。本项目研究成果不仅为类似地区林下种养的发展提供借鉴和参考, 也为后续深入研究全产业链生态循环发展模式奠定理论基础。

实践创新: (1) 通过林草的优化配置来提高植被盖度达到改善小生境、



二、预期成果及考核指标

1、 主要技术经济指标 (如形成的知识产权、技术标准、新技术、新产品、新装置、论文专著等数量、指标及其水平等)

阐明“林-草-羊”种养模式与森林环境的耦合机制;筛选出兼具生态效益和经济效益的林草优化配置组合;获得天然林草和林下人工套种牧草2种模式下的生态养羊技术;构建“林-草-羊”生态循环种养模式。

- (1) 建立林下种养生态循环示范基地 100 亩;
- (2) 制订团体标准/企业标准 (草案) 1 项;
- (3) 申请专利 2 项;
- (4) 发表论文 3-5 篇;
- (5) 筛选林草优化配置组合 2-3 个;
- (6) 构建“林-草-羊”生态循环种养模式 1 个;
- (7) 编写“林-草-羊”生态循环种养关键技术研究报告 1 份。

2、 项目实施中形成的示范基地、中试线、生产线及其规模

联合国家林业重点龙头企业—广东态合堂实业有限公司在其基地建设 100 亩的林下种养生态循环模式示范基地 1 个。

3、 人才队伍建设

通过本项目的实施,培养研究生和本科生及林业相关专业技术人才 6 名以上。

4、 其它应考核的指标

无



三、预期主要经济、社会、生态效益（如技术及产品应用产业化前景，在项目
实施期内能够形成的市场规模与效益，对保障国家安全、促进社会可持续发展及提升相
关产业竞争力的作用等）

经济效益：一方面可以有效提升林地利用效率，拓宽林地利用方式，降
低单位面积成本，增加经济收入；另一方面降低饲养成本，提升山羊品质的
同时提高其市场价格，可显著提高养殖户的经济收入。

社会效益：生态种养模式的推广，可提高林地集约利用水平，改善农村
人居环境。同时，由于产业链条延伸，解决农村富余劳动力就业问题，为实
现山区林农增收致富拓宽路径，助推乡村振兴。

生态效益：羊粪还林不仅可以提高土壤养分含量，改善土壤结构，提升
土壤肥力，促进林木生长，同时减少化肥施用，实现污染减排。此外，草本植
物适应能力强，草地植被覆盖率大、生长速度快，能快速减少水土流失。



四、年度计划安排及目标	
项目实施时间	2023 年 1 月至 2025 年 12 月
年度	年度计划及年度目标
2023 年	拟定详细的实施方案；开展示范地天然林草地现状调查；进行山羊天然林草下放牧实验；布置林下套种牧草优化配置实验；提交项目实施方案；撰写年度总结报告。
2024 年	按照实验设计安排牧草采样及土壤理化性质取样、测定，获取数据进行初步分析；持续进行山羊天然林草下放牧实验；初步形成基于林草生长指标、牧草产量品质的林草优化配置技术和天然林草山羊放牧技术；发表论文 1 篇以上，培养研究生和本科生及相关技术人员 2 名以上；申请专利 1 项；撰写年度总结报告。
2025 年	开展基于林草优化配置模式的山羊林下放牧试验；集成林草优化配置技术和林下生态养羊技术，构建林-草-羊生态循环种养模式，开展示范；发表论文 2 篇以上；培养研究生和本科生及林业相关专业技术人才 4 名以上；制订团体标准或企业标准（草案）1 项；申请专利 1 项；完成项目总结报告。



五、经费来源与支出预算					
单位：万元					
序号	预算科目名称	合计	专项经费	配套或自筹经费	
1	一、直接费用	116	116		
2	1.设备费	0	0		
3	2.业务费	71	71		
4	3.直接人力资源成本费	45	45		
5	4.直接费用其他支出	0	0		
6	二、间接费用	34	34		
7	间接费用	34	34		
8	三、经费来源				
9	(一) 申请从省级专项资金获得的补助	150	150		
10	(二) 配套或自筹经费来源				
11	1.地级以上市财政配套				
12	2.县及县以下财政配套				
13	3.单位自有配套资金				
14	4.其他资金				
15	(三) 已获得省级专项资金补助				
16	(四) 财政专项经费拨付进度申请	第1年	第2年	第3年	第4年
17	年度资金预算	150			
18	年度资金比例 (%)	100%			
其他需说明的情况：无					

制表人：李永强

财务部门负责人(签字)：吴晗

单位财务专用章：2022年12月

注：①设备费：是指与科研项目直接相关的计算类仪器设备、软件工具，及仪器设备的租赁、现有仪器设备的升级改造等支出。项目经费要严格控制设备购置总支出。②业务费：为完成科研项目任务目标而产生的材料费、测试化验加工费、燃料动力费、差旅费/会议费/国际合作与交流费、专家咨询费、出版/文献/信息传播/知识产权事务费等。③直接人力资源成本费：在项目实施过程中，参与项目研究的科研人员和科研教辅人员的劳务支出以及临时聘请专家的咨询费用等。项目所有参与单位的编制内人员不得在项目中编列劳务费。④间接经费：间接费用不超过直接费用扣除设备费后的30%核定。



六、实施机制

1.运行机制

(1) 组织管理：项目由承担单位华南农业大学和参加单位共同负责，承担单位全面负责项目组织与实施，参与单位负责示范基地的建设、运行和管理并配合外业调查。项目参与人员明确分工、责权利清楚。

(2) 合作机制：项目采取分工负责制，申报单位及参与单位分别设立项目研究小组开展研究，合作双方分别指定项目联系人，制定有效的沟通机制，定期就项目进度、经费开支、技术问题等进行交流，确保项目如期按质完成。

(3) 档案管理：主要由华南农业大学负责，专人专柜，分类管理，保存纸质版档案的同时，备份电子版档案。示范基地管理技术档案由参与单位负责。

2.实施措施

(1) 项目实施采用课题分工专人负责制，任务和责任分配到人，并由项目负责人定期跟踪任务进度，确保各项指标按时完成。

(2) 牧草种植和管护、羊舍等基础设施的建设、羊只管护：由华南农业大学负责指导并由参与企业负责实施。具体由企业指定专人负责，并按有关管理制度执行。

3.项目参与单位的任务分工

项目单位	任务分工
华南农业大学	负责项目方案的制定、实施和总结
广东恣合堂实业有限公司	负责示范基地的建设、管理和运行，协助科研调查



4.项目参与单位的经费分配（单位：万元）

项目单位	专项经费	设备费	业务费	直接人力资 源成本费	间接费用
华南农业大学	110	0	52	33	25
广东态合堂实业有限公司	40	0	19	12	9

5.知识产权与成果管理及权益分配

项目研究成果及其形成的知识产权，除涉及国家安全、国家利益和重大社会公共利益的以外，省林业局授予科研项目承担单位。项目承担单位可以依法自主决定实施、许可他人实施、转让、作价入股等，并取得相应的收益。



七、项目承担单位、参加单位及项目负责人、主要参加人员

项目承担单位（签章）：



主要参加单位（签章）：



项目负责人

姓 名	性 别	年 龄	职务/职称	从事专业	任务分工	所在单位	签名
贾小容	女	44	教研室主任/ 副教授	林学	项目主持	华南农业大学 林学与风景园林学院	贾小容
柳广斌	男	40	讲师	动物科学	林下养殖	华南农业大学 动物科学学院	柳广斌

主要参加人员

李峰	男	41	技术总工/林 业工程师	生态学	示范基地建 设	广东态合堂实业有限 公司	李峰
陈东明	男	55	国家林草 乡土专家	林业	林下种植 技术顾问	广东态合堂实业有限 公司	陈东明
刘德武	男	55	教授	动物科学	林下养殖 技术顾问	华南农业大学 动物科学学院	刘德武
王晓亚	女	38	讲师	草业科学	牧草管理	华南农业大学 林学与风景园林学院	王晓亚
赵倩	女	30	讲师	土壤学	土壤监测	华南农业大学 林学与风景园林学院	赵倩
林福宇	男	35	科研经理	林业	示范基地运 行	广东态合堂实业有限 公司	林福宇
陈一安	女	27	申报主管	林业	示范基地管 理	广东态合堂实业有限 公司	陈一安



孙宝丽	女	41	副教授	动物科学	林下养殖	华南农业大学 动物科学学院	孙宝丽
邓铭	男	34	实验师	动物科学	林下养殖	华南农业大学 动物科学学院	邓铭
郑明轩	男	30	实验师	植物学	林木监测	华南农业大学 林学与风景园林学院	郑明轩
汤学志	男	39	项目经理	林业	示范基地建设	广东态合堂实业有限公司	汤学志
刘文晓	女	28	科研专员	林业	示范基地建设	广东态合堂实业有限公司	刘文晓
周庆	男	51	副教授	林学	林下种植	华南农业大学 林学与风景园林学院	周庆
龙凤玲	女	32	实验师	森林生态	植物调查、测定	华南农业大学 林学与风景园林学院	龙凤玲
张成龙	男	25	在读硕士	林业	土壤取样	华南农业大学 林学与风景园林学院	张成龙
晏媛	女	23	在读硕士	林业	牧草监测	华南农业大学 林学与风景园林学院	晏媛
李梦仙	女	22	在读硕士	林业	林木监测	华南农业大学 林学与风景园林学院	李梦仙
黄飞	男	22	在读硕士	林业	牧草测定	华南农业大学 林学与风景园林学院	黄飞
吕建达	男	25	在读硕士	动物科学	羊只样品采集、测定	华南农业大学 动物科学学院	吕建达
胡长胜	男	25	在读硕士	动物科学	羊只样品采集、测定	华南农业大学 动物科学学院	胡长胜
贺梓晴	女	24	在读硕士	林业	土壤微生物测定	华南农业大学 林学与风景园林学院	贺梓晴
胡雪花	女	22	在读硕士	林业	林木监测	华南农业大学 林学与风景园林学院	胡雪花
王艺颖	女	24	在读硕士	林业	土壤测定	华南农业大学 林学与风景园林学院	王艺颖
刘悦	男	23	在读硕士	林业	土壤测定	华南农业大学 林学与风景园林学院	刘悦



八、共同条款

根据《广东省林业局科技项目管理办法》及广东省财政专项资金管理的有关要求,为顺利完成 2023 年度广东省林业科技创新项目计划任务,经协商一致,特订立本合同,各方共同遵守。

1. 甲方负责按合同要求做好经费核拨和工作协调,对项目实施、经费使用进行检查、监督和管理,并按科技项目管理的有关要求组织项目验收鉴定。

2. 乙方负责按合同要求组织项目实施,对专项资金进行单独列账,按开支范围实行专款专用;并于每年 12 月 31 日前向甲方提交本年度项目实施情况、经费决算的书面报告;项目完成后的 1 个月内向甲方提交项目实施情况总结报告;项目完成后的 3 个月内向甲方申请进行验收或鉴定,验收时论文、专著等研究成果以第一标注为准;项目验收后的 1 个月内向甲方提供完整的验收或鉴定材料。

3. 丙方负责按合同要求督促项目的执行并负责监督经费的使用。

4. 在履行合同过程中,一方发现可能导致项目失败或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

5. 合同执行过程中,乙方如需调整任务,应根据有关规定,经丙方审核同意并签署意见后,向甲方提出变更内容及其理由的申请报告,经甲方审批同意后方可执行。

6. 乙方因某种原因致使计划无法执行,而要求中止合同,应视不同情况,部分、全部退还所拨经费;如乙方没有提出中止合同的要求,甲方可根据调查核实情况有权做出停拨、缓拨项目经费或中止合同、追回所拨经费的



决定。

7. 丙方在督促项目的执行和监督经费使用的过程中, 如发现乙方有违规行为, 应及时向甲方汇报并负责提出调整意见。必要时, 甲方有权依据乙方违规事实直接调整专项经费或撤销专项任务。

8. 乙方违反规定而造成项目工作停滞、延误或失败, 在延期一年后仍不能通过验收的, 应解除合同, 乙方退还财政已核拨的经费, 并自行承担由此引起的损失。

9. 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享, 除本合同约定外, 按国家和省的有关法规执行。

10. 根据项目具体情况, 经各方协商订立的技术保密条款、附加条款等作为合同文本的一部分。

11. 本合同的争议应由各方本着协商一致的原则解决, 当合同需要更改或解除时, 双方应订立变更条款或协议, 需要仲裁和审理应在甲方所在地进行。

12. 本合同正式文本一式六份, 甲方、乙方、丙方各二份, 自签字之日起生效, 有效期至项目验收后一年内。各方均应负合同规定的法律责任, 不应受机构、人事变动的影响。



项目绩效目标申报表

项目名称	广东丘陵山地“林-草-羊”生态循环种养模式构建与示范		
项目类型	2023 年度自然资源事务专项资金-生态林业建设（省级组织实施）		
项目等级	二级项目		
主管部门	广东省林业局	用款单位	华南农业大学
实施期限	起始年度	2023	终止年度
			2025
预算金额（万元）	立项总金额	150	当年度金额
			150
设立依据	《广东省林业局关于做好 2023 年有关林业科技类项目入库申报的通知》		
项目概述	<p>为充分利用森林空间发展林下经济，践行习近平总书记提出的“绿水青山就是金山银山”和“大食物观”，华南农业大学联合广东合盛实业有限公司，针对示范区内林草生物生产力不高、林草配置组合方式欠佳、林地资源利用率低等问题，选取典型人工林分，开展林草优化配置和林地山羊放牧实验，监测林地生态效益、牧草产量和营养成分的变化规律，及山羊的生长规律和养殖效果，以广东丘陵山地林草优化配置与生态养羊的关系为立足点，揭示林草优化配置对生态养羊的影响机理，探讨林草优化配置、林下养羊与森林环境效益的耦合机制，凝练林草优化配置及生态养羊的关键技术，构建广东丘陵山地“林-草-羊”生态循环种养模式，提高广东省林下种养专业化、标准化水平，助推乡村振兴。</p>		
总体绩效目标	实施周期总目标 (跨年度项目需填写)	年度绩效目标	
	<p>揭示林草优化配置、林下养羊与森林环境效益的耦合机制；筛选林草优化配置组合 2-3 个；获得天然林草和林下人工套种牧草 2 种模式下的生态养羊技术；构建“林-草-羊”生态循环种养模式 1 个；营建 100 亩林下种养生态循环示范基地 1 个；制订团体标准/企业标准（草案）1 项；申请专利 2 项；发表论文 3-5 篇；培养学生及相关专业技术人才 6 名以上。</p>	<p>2023 年 拟定详细的实施方案；开展示范地天然林草地现状调查；进行山羊天然林草下放牧实验；布置林下套种牧草优化配置实验。①提交项目实施方案；②撰写年度总结报告。</p> <p>2024 年 按照实验设计安排牧草采样及土壤理化性质取样、测定，获取数据进行初步分析；持续进行山羊天然林草下放牧实验；初步形成基于林草生长指标、牧草产量品质的林草优化配置技术和天然林草山羊放牧技术。①获得林草优化配置组合 2-3 个；②发表论文 1 篇以上；③申请专利 1 项；④撰写年度总结报告。</p> <p>2025 年 开展基于林草优化配置模式的山羊林下放牧试验；集成林草优化配置技术和林下生态养羊技术，构建林-草-羊生态循环种养模式，开展示范。①构建林-草-羊生态循环种养模式 1 个；②制订团体标准或企业标准（草案）1 项；③完成项目总结报告；④发表论文 2 篇以上；⑤申请专利 1 项。</p>	



绩效 指标	一级指标	二级指标	三级指标	实施周期指标值	年度指标值
	产出指标	数量指标	建立示范地 (亩)	100	2023 (0) 2024 (30) 2025 (70)
			制订团体标准 或企业标准 (草案) (项)	1	2023 (0) 2024 (0) 2025 (1)
			申请专利 (项)	2	2023 (0) 2024 (1) 2025 (1)
			发表论文 (篇)	3-5	2023 (0) 2024 (1) 2025 (2)
			提交关键技术 研究报告 (份)	1	2023 (0) 2024 (0) 2025 (1)
			构建生态循环 种养模式 (个)	1	2023 (0) 2024 (0) 2025 (1)
			筛选林草优化 配置组合 (个)	2-3	2023 (0) 2024 (2) 2025 (0)
			培养人才 (人)	6	2023 (0) 2024 (0) 2025 (6)
		质量指标	山羊生产性能	符合品种标准	
		时效指标	项目进度计划 完成率 (%)	100	2023 (30) 2024 (30) 2025 (40)
		成本指标	减少草地管理 成本 (%)	10	
	效益指标	社会效益指标	所取得成果的 推广应用前景	良好	
	满意度指标	服务对象满意 度指标	技术服务对象 的满意度 (%)	90	



九、合同签约各方

(一) 项目管理部门 (甲方) : 广东省林业局(公章)

单位负责人 (签章) :

永郑光

联系人及电话: 谢步丹 020-81702338

2023年3月13日



(二) 项目承担单位 (乙方) : 华南农业大学 (单位名称及公章)

单位负责人 (签章) :

农业

联系人及电话:

2022年12月15日



(三) 项目归口管理部门: (单位名称及公章)

县级林业主管部门:

地级以上市林业主管部门 (或省及省
林业局所属单位、中央驻粤单位) :

单位负责人 (签章) :

单位负责人 (签章) :

联系人及电话:

联系人及电话:

年 月 日

年 月 日



合同编号: 2022-XPK-00-009

2022年省级乡村振兴战略专项资金 种业振兴项目

合 同 书



项目名称: 雷州山羊繁育体系建立及快大高繁新品系培育

项目管理单位(甲方): 广东省农业农村厅

项目牵头承担单位(乙方): 华南农业大学

项目推荐(主管)单位(丙方): 华南农业大学

项目负责人: 刘德武 联系电话: 13640835778

项目联系人: 柳广斌 联系电话: 18802085530

广东省农业农村厅制

第一条 为保障 2022 年省级乡村振兴战略专项种业振兴项目顺利实施，按时保质保量完成项目任务，根据《中华人民共和国民法典》、《广东省省级财政专项资金管理办法（试行）》、《广东省财政厅关于安排 2022 年省级乡村振兴战略专项资金种业振兴项目资金的通知》（粤财农[2022]184 号）、《广东省农业农村厅财政专项资金管理办法》等文件有关规定，经甲、乙、丙三方协商一致，签署本合同书。

第二条 甲方的权利义务：本合同履行过程中，甲方有权对乙方项目的实施情况和资金到位、使用情况进行监督、检查，提出改进要求。

第三条 乙方的权利义务：

1. 按财政资金管理规定，对甲方核拨的资金做到专款专用，单独列账，并随时配合甲方进行监督检查；

2. 认真填写本合同书附件 1《项目任务书》，《项目任务书》的内容应与乙方的《项目申报书》保持一致；

3. 严格按照本合同书及合同书附件 1《项目任务书》的要求及时完成项目建设内容，项目实施完成后，按照本合同附件 2 的要求提交验收报告；

4. 按照《广东省农业农村厅财政专项资金管理办法》规定，按季度向甲方、丙方报告项目实施情况、财政资金开支进度等内容；

5. 乙方需保留与所有参与单位的合作实施协议和相关财务凭证，并向甲方备案。

第四条 丙方的权利义务：

1. 为乙方项目实施提供必要的条件保障；

2. 负责对项目承担单位的实施条件、能力以及财务管理规范进行审查,对推荐项目的实施场地、申报资料等进行真实性审核,并监督项目实施、资金预算执行情况;

3. 受甲方委托或协助甲方完成项目验收等工作,并及时向甲方报告情况。

第五条 本项目资金不得用于以下方向:1. 行政事业单位基本支出;2. 各项奖金、津贴和福利补助;3. 企业担保金和弥补企业亏损;4. 修缮楼堂馆所以及建造职工住宅;5. 弥补单位预算支出缺口和偿还债务;6. 购买交通工具及通讯设备;7. 形成地方政府债务的支出;8. 购买理财产品、发放借款及平衡预算等。

第六条 项目验收。对财政资金投资 500 万元(含)以上(科研项目财政资金在 200 万元(含)以上)的项目,及乙方直接向甲方申报的项目,由甲方负责组织验收;对财政资金投资 500 万元以下(科研项目财政资金 200 万元以下)的项目,甲方自主或委托推荐(主管)单位负责验收,由验收单位向甲方提交验收材料,甲方对验收材料进行审核确认。

第七条 在履行本合同的过程中,如出现相关法律法规重大改变等不可抗力情况,甲方有权对所核拨经费的数量和时间进行相应调整。因非不可抗力因素导致的项目未履行或未履行完毕,或因乙方责任造成项目不能继续开展的,甲方有权终止项目合同,收回尚未使用和使用不符合规定的财政经费。

第八条 在履行本合同的过程中,当事人一方发现可能导致项目整体或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,

没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

第九条 实施项目所获得的科技成果(项目成果)归属、成果转让和实施技术成果所产生的经济利益的分享,按照国家和广东省有关规定执行。项目研究成果应向省农业农村厅进行登记、备案,对外发布前应征求省农业农村厅的意见。

第十条 本合同在履行过程中发生的任何争议,由甲乙丙三方友好协商解决。

项目管理单位(甲方) (盖章): 广东省农业农村厅

法定代表人(或授权代表) (签章):

刘中国

项目牵头承担单位(乙方) (盖章): 华南农业大学

法定代表人(或授权代表) (签章):

刘雅红

项目负责人(签章):

刘雅红

签订日期: 2023年1月19日

乙方推荐(主管)单位(丙方) (盖章): 华南农业大学

法定代表人(或授权代表) (签章):

签订日期: 年 月 日

附件 1

项目任务书

填写说明

一、本项目任务书由乙方填写。

二、本项目任务书所列内容应实事求是填写，表达要明确、严谨。对填写不符合要求的，或填报内容出现虚报夸大、不切实际的，将退回项目承担单位修改。

三、项目任务书规定的项目考核指标、建设内容和绩效目标必须依据《项目申报书》填写，应遵循明确、量化、可考核的原则，其中技术指标应明确项目完成时达到的关键技术参数及预期可以形成的发明专利、标准、新技术、新产品、新装置、论文、专著等的数量。项目申报指南对项目技术、经济和成果等指标有明确要求的，应符合项目申报指南的要求，相关专项管理办法有特别规定的，应符合相关规定。

四、《项目申报书》及申报指南是本项目任务书填报的重要依据，项目任务书填报不得修改考核指标、绩效目标、资金预算等内容。《项目申报书》、申报指南和本项目任务书将共同作为项目过程管理、综合绩效评价（验收）和监督评估的重要依据。

五、省财政资金支出的预算计划应按照国家及省相关规定执行。

六、表格栏目不够可自行增加。

一、目的及意义

主要说明项目的建设目的、研究价值和意义。

广东省经济发达，人口众多，羊肉需求量较大，但广东省肉羊产业发展严重滞后，羊肉产量无法满足市场需求，外省引进羊肉往往质量差，无法满足人们对高质量肉质的要求。广东有着优良的地方山羊品种—雷州山羊，其能很好地适应广东高温、高湿的生态条件，具有性成熟早、耐粗饲特点，更由于其肉质细嫩，味道鲜美而受到广东市场青睐。但由于长期缺乏系统的选育，近亲繁殖现象严重，从而出现体型小、生长慢、繁殖力低等生产性能下降或者产弱羔、畸形羔等现象，优良性状正逐渐消失，丧失市场竞争力。近年来虽引进优良种羊进行杂交改良，可由于缺乏系统的繁育体系和保种措施，不但没有起到杂交改良的应有效果，反而导致本地优秀品种资源遭到严重破坏。畜禽良种是现代牧业发展的核心基础，因此本项目针对我省本地山羊品种开发利用存在的问题，系统性的开展雷州山羊遗传结构研究，挖掘特色优异种质性状和优异基因，建立雷州山羊数字化育种体系，应用山羊现代化创新育种及繁殖技术，培育高大、快繁的雷州山羊新品系并进行示范推广，对我省肉羊产业以及畜牧业的发展具有重要的意义。

二、项目建设内容

详细说明项目建设内容（项目需求或项目建设任务）。

（一）项目（课题）牵头承担单位

1. 单位名称：华南农业大学

项目（课题）负责人：刘德武

2. 研究开发内容：

研究一：雷州山羊育种体系建立及新品系培育

利用基因组测序技术研究雷州山羊的遗传结构特征，深度发掘特色优异种质性状和优异基因，研究其遗传规律，开展是优良肉用山羊种质资源表型与基因型精准分析，构建重要经济性状表型-基因型数据库，建立雷州山羊数字化育种体系。制定育种方案，利用现代分子育种技术培育生长速度快、繁殖力高的肉用山羊新品系。

研究二：雷州山羊良种快速扩繁关键技术建立

研究适用于雷州山羊人工受精、早期妊娠诊断、同期发情、超数排卵、胚胎移植等快速扩繁的技术流程，建立雷州山羊良种高效繁殖体系，提高雷州山羊核心育种群的培育效率。

（二）项目（课题）参与单位

1. 单位名称：广东海洋大学

项目（课题）负责人：周光现

2. 研究开发内容:

研究三：雷州山羊良种评估及高效生产关键技术建立

开展性能测定、良种登记评估工作。研究不同生理阶段肉羊的生长发育规律，确定优质肉羊生产的影响因素，建立优质肉羊高效生产及胴体质量评价技术。建设雷州山羊饲料营养、饲养管理、环境控制和疫病防治等诸方面规范化、标准化生产技术，形成雷州山羊优质高效健康养殖的技术体系，提高种羊生产性能。

3. 单位名称：雷州市状元黑山羊养殖有限公司

项目（课题）负责人：陈五拓

4. 研究开发内容:

研究四：雷州山羊育种群体建立与示范

进行雷州山羊的养殖，协助开展性能测定工作，开展生产记录与系谱登记工作，按育种方案组建雷州山羊育种群体，并进行提纯复壮。运用山羊高效生产关键技术提高生产效率，形成标准化羊场运作体系并进行示范。

备注：项目建设内容（项目需求或项目建设任务）按《项目申报书》内容填写。

三、项目绩效目标

主要说明项目实施后，预期达到的目标和产生的效果，相关表述应量化。

1. 三年总体目标：

建成母羊 300 头、公羊 30 头以上的雷州山羊育种群，建立雷州山羊数字化育种体系 1 套，培育快大高繁的雷州山羊新种群 1 个。六月龄体重达到公羊 18.0 千克以上，母羊 14.4 千克以上；经产母羊产羔率达到 195.5%以上。

2. 当年绩效目标

(1) 建成母羊 300 头、公羊 30 头以上的雷州山羊育种群。

(2) 建立雷州山羊数字化育种体系 1 套。

(3) 六月龄体重达到公羊 16.0 千克以上，母羊 12.8 千克以上；经产母羊产羔率达到 171.2%以上。

四、项目进度安排

详细说明各阶段的工作内容和时间安排情况。

2023 年 1 月-2023 年 4 月：开展雷州山羊性能测定及良种登记评估工作；制定育种方案，并初步组建雷州山羊育种群体，开展生产记录与系谱登记工作。

2023 年 5 月-2023 年 8 月：根据育种方案进一步对育种群体进行选育，并建立数字化育种体系；开展血液及肉品质检测研究；采集雷州山羊的耳样，提取基因组 DNA，并进行基因组重测序；

2023 年 9 月-2023 年 12 月：通过数据分析挖掘与雷州山羊优良性状相关的分子标记；对育种群体生产性能进行测定评估，撰写育种报告。

备注：项目绩效目标按《项目申报书》内容填写。

五、项目主要合作、参与单位(含牵头承担单位)

单位名称	单位性质	统一社会信用代码	通讯地址
华南农业大学	大专院校	124400004554165634	广东省广州市天河区五山路483号
广东海洋大学	大专院校	45625261X	广东省湛江市麻章区海大路1号
雷州市状元黑山羊养殖有限公司	企业	MA4UQ9UK-2	雷州市白沙镇黎郭村(邦企线公路北蒨甘岭)

六、项目组主要成员(含项目负责人)

姓名	性别	身份证号	单位	职称/职务	电话
刘德武	男	42010619660711563X	华南农业大学	正高级	13640835778
柳广斌	男	210103198204261211	华南农业大学	中级	18802085530
孙宝丽	女	411024198109241823	华南农业大学	副高级	13802405625
郭勇庆	男	13042119810616031X	华南农业大学	中级	18122469798
贾坤	男	372330198111274212	华南农业大学	中级	13501516198
邓铭	男	430726198601203910	华南农业大学	中级	15915704462
周光现	男	500224198603045475	广东海洋大学	中级	15768597638
姜平	女	220182199206018620	广东海洋大学	副高级	15143059097
于海滨	男	220106198802010210	广东海洋大学	中级	18686609912
康丹菊	女	61270119850425532X	广东海洋大学	中级	15768679622
陈五拓	男	440824197905100638	雷州市状元黑山羊养殖有限公司	总经理	13724707988
陈华明	男	440823199210083336	雷州市状元黑山羊养殖有限公司	场长	13692332147

七、资金使用预算

主要说明资金使用的范围或方向及资金使用进度安排。

（一）资金使用的范围

本项目 2022 年省级财政资金预算 100.00 万元，总经费按华南农业大学 50%，广东海洋大学 30%，雷州市状元黑山羊养殖有限公司 20%分配。

主要用途如下：

1. 直接费用：94.90 万元。包括：

（1）材料费：29.80 万元，用于饲料及保育种实验的试剂耗材购买。

（2）测试化验加工费：43.80 万元，用于基因测序、血液生理生化指标、病原抗体及肉品质检测等。

（3）出版/文献/信息传播/知识产权事务费：6.00 万元，用于论文出版、专利和软件著作权申请等相关费用。

（4）会议/差旅/国际合作交流费：8.82 万元，用于差旅费。

（5）劳务费：6.48 万元，用于支付参与项目的研究生劳务费。

2. 间接费用：5.10 万元，为项目承担单位的项目管理费。

（二）经费使用进度安排

2023 年 4 月前累计支出 23 万元以上，占总经费 23%；

2023 年 8 月前累计支出 83 万元以上，占总经费 83%；

2023 年 12 前完成经费全部支出。

八、保障措施

说明围绕完成项目任务、目标所要采取的具体措施。

（一）项目组织方式和机制

项目实施单位根据计划目标抓好财务管理和质量管理，加强项目组织管理，保证专款专用，任务落实到位。实施严格的岗位责任制，加强工作质量管理，提高管理水平和工作效率，保证项目开展合理科学，实施规范到位，保障项目建设顺利进行。做好计划管理，项目建设按照“统一计划，分步实施”的原则，根据批复的项目申报书等文件，确定本项目建设内容、物资采购、资金使用计划等，严格执行，不得随意变更。做好物资管理，按照广东省相关文件要求，项目建设所需的设备、仪器和其他物资公开向社会招标；要求设备仪器厂商负责安装调试；指定专人对其进行管理。做好信息管理，做好文件、档案等资料的收集保管等。

（二）产学研结合机制

建立产学研合作的技术创新机制。通过项目建设，与相关企业单位建立创新联合体。各单位间通过人员互派、优势互补等有效措施，解决科研与应用相脱离的“两层皮”问题，打通技术研发与产业化应用的渠道，真正建立产学研合作的技术创新机制。

（三）创新人才队伍的凝聚和培养

项目承担单位华南农业大学及广东海洋大学是南方现代草牧业（羊）产业技术体系创新团队的重要成员，包含肉羊体系首席专家（本项目负责人）及岗位科学家。团队成员均长期从事草食动物生产技术研究，具有丰富一线实践经验。本项目可为团队成员及参与项目的研究生提供良好的科学研究条件与合作交流机会，为人才培养提供有利条件。

（四）预算资金管理情况

统筹安排建设资金，严格按照有关财务制度及有关规定，科学、合理使用项目资金，确保资金使用效益。设立本项目资金专用账户，按照“统一规划、专账核算、专款专用”的原则，确保项目建设进度和预期目标。另外，加强财务管理和预算管理，建立科学化、精细化经费预算管理机制，专款专用，独立核算；严格控制经费超支，确保经费使用规范、安全、有效。

联合申报项目协议书

甲方：华南农业大学

乙方：广东海洋大学

丙方：雷州市状元黑山羊养殖有限公司

甲方、乙方、丙方经友好协商决定联合申报 2022-2023 年度 乡村振兴战略专项省级种业振兴行动项目入库申报 项目，项目名称：雷州山羊繁育体系建立及快大高繁新品系培育。并达成如下合作协议：

第一条：项目研究工作详细分工：

甲方(主持方)：负责项目的主持申报与管理，育种体系建立方案的制定，生产性能测定及检测，数据分析，技术指导与培训，项目报告撰写及上传。

乙方(参与方)：协助甲方开展种羊生产性能测定及检测工作，协助实施选育方案，开展现场繁育技术指导与培训。

丙方(参与方)：种羊饲养管理，繁育方案具体实施，协助甲乙双方开展种羊生产性能测定及检测工作，并提供必要的工作条件。

第二条：经费分配：

1、如果本申报项目获批立项，按政府下达的资助经费，甲方、乙方、丙方同意此经费分别按政府资助经费的甲方：50%、乙方：30%、丙方：20%进行分配。

2、甲方在收到 省财政资金 下达的资助经费后的一个月内将乙方、丙方所占经费支付给乙方、丙方指定帐户。

乙方账户信息：

账 户：广东海洋大学

帐 号：679557760592

开户行：中国银行股份有限公司湛江霞山支行

丙方账户信息：

账 户：雷州市状元黑山羊养殖有限公司

帐 号：2015021109200038565

开户行：中国工商银行股份有限公司雷州支行

第三条：企业配套经费比例：无。

第四条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有；各方共同完成的由各方共享，具体按照各方的贡献大小进行分配或各方另行商定。

②共同完成的项目成果的转让，须在各方同意的前提下进行，任何一方不得擅自转让或许可实施。

2. 项目成果申报各级奖项，各方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第五条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第六条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第七条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第八条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第九条：本协议自各方签字盖章之日起生效，至项目完成之日起终止；若合作申请未获资助，本协议自动废止。



项目负责人(签字):

[Signature]

2022年8月23日



项目负责人(签字):

[Signature]

2022年8月24日



项目负责人(签字):

[Signature]

2022年8月24日

受理编号: c1730550100150

项目编号: 2017B020201014

文件编号: 粤科规财字[2017]50号

广东省省级科技计划项目

合同书

项目名称: 广东雷州黑山羊快大高繁新品系培育

专项资金类别: 公益研究与能力建设

计划类别: 农村科技领域

项目起止时间: 2017-01-01 至 2019-12-31

管理单位(甲方): 广东省科学技术厅

承担单位(乙方): 华南农业大学

乙方主管部门(丙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 刘德武

联系电话: 020-85284816

项目联系人: 刘德武

联系电话: 13640835778



(广东科技微信公众号)

广东省科学技术厅
二〇一七年制

(受理纸质材料二维码)



一、研发内容和关键技术

1. 研究内容

(1) 利用川中黑山羊及努比亚黑山羊父本对雷州黑山羊母本进行三元杂交, 改善雷州黑山羊的肉用体型及繁殖能力, 并对杂交子代进行横交固定。通过BLUP育种值估计、分子标记辅助选择等方法选育提纯, 形成雷州黑山羊体大及高繁新品系。

(2) 在选育过程中, 逐步制定出雷州黑山羊体大高繁新品系相关的品种标准。

2. 技术路线

(1) 利用川中黑山羊公羊与雷州黑山羊母羊杂交改善雷州黑山羊的肉用体型和繁殖能力。选择300只健康, 1岁-2岁之间的雷州黑山羊母羊做为杂交母本。10只健康, 2岁-3岁之间的川中黑山羊公羊做为杂交父本。母羊分娩后测定杂交子代(川雷杂交子代)生产性能, 育成母羊8月龄时进行育成鉴定。达到育成标准的母羊定为三元杂交母本。

(2) 利用努比亚黑山羊公羊与川雷杂交子代母羊进行杂交进一步改善雷州黑山羊的肉用体型并稳固高繁殖能力。选择第一步育成川雷杂交子代母羊300只作为杂交母本, 以10只健康, 年龄在2岁-3岁之间的努比亚黑山羊作为杂交父本, 进行第三元杂交。母羊分娩后, 对产羔数进行统计, 并对子代的生产性能进行测定。通过BLUP育种值估计, 对后备公羊进行选育, 并最终生产出用于横交固定的种公羊。育成母羊测定及培育方法同上。

(3) 利用BLUP育种值估计、分子标记辅助选择方法对杂交后代进行横交固定。利用上一步培育的三元杂交子代的公羊与母羊进行交配。并对母羊的产羔数, 以及子代的生长性能进行测定, 方法同上。结合系谱信息及相关测定结果, 利用BLUP方法计算子代个体育种值, 及分子标记辅助选择技术组建快大及高繁两个品系培育群体。之后各品系内进行纯繁培育, 进一步对体大高生长性能及高繁殖力的相关基因进行纯化, 最终形成稳定品系。

3. 解决的关键问题

雷州黑山羊是广东地区优秀的地方黑山羊品种, 具有肉质细嫩, 本地适应性强的优点, 但体型小, 繁殖力低, 不满足当前高效生产的市场要求。本项目通过引入川中黑山羊及努比亚黑山羊血缘, 改善雷州黑山羊的肉用体型, 并提高其繁殖力。培育雷州黑山羊高生产性能新品系, 解决广东地区缺乏可推广的本地高生产性能肉羊品种的问题。

4. 创新点

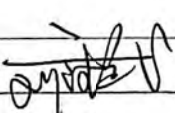
通过育成杂交的方法, 在保留雷州黑山羊肉质及本地适应性的优点基础上, 向其导入快大及高繁基因。利用BLUP育种值估计、分子标记辅助选择等现代化育种技术, 提高育种效率, 培育适合广东本地饲养的雷州黑山羊快大高繁新品系。



二、项目考核指标

1. 项目完成后提供的研究开发成果及形式 (须明确产品、专利、版权、标准等成果的类型及数量)					
成果形式		成果数量	成果形式		成果数量
发明专利	申请		引进人才(人)		
	授权		培养人才(人)		2
实用新型专利	申请		科技人才奖励(人)		
	授权		技术标准制定	牵头(个)	
外观设计专利	申请			参与(个)	
	授权		科技报告(篇)		
国外专利	PCT受理		软件著作权(项)		
	授权		论文论著(篇)		2
获得国家级奖项(项)			其中: 被收录论文数(篇)	SCI	1
获得省级奖项(项)				EI	
新服务(项)				ISTP	
新产品(或新材料、新装备、新品种(系))		1	新工艺(或新方法、新模式、新技术)		
创新载体项目必填		技术服务数量(项)			
		服务企业数量(家)			
科技金融项目必填		开展培训宣讲活动场次(次)			
		服务企业数量(家)			
		帮助企业融资(万元)			
		引进专业机构(家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告(篇)			
		研究总报告(篇)			
		研究中后期报告(篇)			
		研究分报告(篇)			
		调研报告(篇)			
		专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		核心期刊论文(篇)[以第一作者发表, 须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		培养人才(人)			



	获国家级奖项(项)	
	获省级奖项(项)	
	其他	
2. 其他成果及形式说明:		
3. 主要技术经济指标及社会效益		
累计新增销售收入(万元)		
累计新增利税(万元)		
4. 其他主要技术经济指标及社会效益说明:		
1. 培育成雷州黑山羊新品系1-2个。 2. 雷州黑山羊新品系品种标准1个。 3. 发表学术论文1-2篇。 4. 培养硕士研究生2名。		
项目负责人(签章):  2017年6月25日		



三、项目进度和阶段目标

开始日期	结束日期	主要工作内容
2017-01-01	2017-12-31	川中黑山羊公羊与雷州黑山羊母羊杂交，并产出川雷二元杂交子代。二元杂交子代母羊育成。
2018-01-01	2018-12-31	川雷二元杂交子代母羊与努比亚黑山羊公羊杂交，并产出努川雷三元杂交子代。三元杂交子代母羊及公羊选育。
2019-01-01	2019-12-31	选择优秀的三元杂交子代母羊及公羊进行横交，产生子代并进行生产性能测定及分子标记检测。根据生产性能及分子标记特点特点组建体大及高繁品系群体，并对群体进行持续的提纯选育。



四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
华南农业大学	负责育种方案的制定, 个体生产性能测定, 数据分析, BLUP育种值估计, 分子标记检测及分析, 品系建立方案的制定, 新品系标准制定工作。	30.00	30.00
广东温氏食品集团股份有限公司	负责提供实验羊只及饲养场地, 提供饲养员并对实验羊只进行饲养管理, 配合主持单位完成羊只个体生产性能测定, 以及执行选配育种方案。	20.00	20.00
合计		50.00	50.00



五、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额: (大写) 伍拾万圆整; (小写) 50万元;						
2. 省科技厅经费拨付方式: 一次性拨款						
3. 省科技厅经费年度下达计划: (大写) 伍拾万圆整; (小写) 50万元;						
分期				经费(万元)		
第1期				50		
4. 总经费开支预算计划:						
经费筹集情况:						(单位: 万元)
总投入经费: 50.00						
	省科技厅经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费:						
新增经费:	50.00					50.00
政府部门、境外资金及其他资金投入情况说明: <div style="text-align: center; font-size: 2em; opacity: 0.5; transform: rotate(-15deg);">2017B0202010</div>						




新增经费预算:			(单位: 万元)	
	新增经费总额		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:				
1、直接费用:	47.50		47.50	
(1) 设备费:				
(2) 材料费:	30.54	1) 屠宰实验羊; 2) 饲料费; 3) 实验试剂及耗材	30.54	1) 屠宰实验羊; 2) 饲料费; 3) 实验试剂及耗材
(3) 测试化验加工外协费:	6.40	1) 肉质检测; 2) 引物合成	6.40	1) 肉质检测; 2) 引物合成
(4) 燃料动力费:				
(5) 差旅费/会议费/国际合作与交流费:	7.20	用于实验室与羊场之间的往返车费。	7.20	用于实验室与羊场之间的往返车费。
(6) 出版/文献/信息传播/知识产权事务费:	1.20	1) 文献资料查新; 2) 论文版面费	1.20	1) 文献资料查新; 2) 论文版面费
(7) 劳务费:				
(8) 人员费:	2.16	用于在读研究生的补助	2.16	用于在读研究生的补助
(9) 专家咨询费:				
(10) 直接费用其他支出:				
(11) 科技金融服务体系其他费用:				
①信用评级补贴:				
②大赛场租:				
③特派员奖励与补贴:				
2、间接费用:	2.50		2.50	
(1) 间接成本:				
(2) 管理成本:				
(3) 绩效支出:				
合计:	50.00		50.00	

特别提醒: 2017年3月份, 广东省《关于进一步完善省级财政科研项目资金管理等政策的实施意见(试行)》(粤委办〔2017〕13号)出台, 对间接费用比例、劳务费开支范围、人员费用安排等进行了调整优化。为及时拨付2017年度科研经费, 平台直接提取申报书相关信息生成合同书并进行了预签订, 但各项目负责人、承担单位、主管部门须认真领会相关文件精神, 在合同书签订完成后2个月内通过平台提请项目经费变更或确认, 对相关经费开支进行细化完善, 否则, 将影响其科研信用评级或申报新的省级科技项目。



六、人员信息

项目负责人情况

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
刘德武	51	男	教授	系主任	博士研究生	项目主持	华南农业大学	

主要研究开发人员

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
柳广斌	34	男	讲师	无	博士研究生	设计实验及项目指导	华南农业大学	
李耀坤	30	男	讲师	无	博士研究生	生产数据收集、整理及实验指导	华南农业大学	
孙宝丽	35	女	副教授	无	博士研究生	数据分析	华南农业大学	
陈水龙	44	男	未取得	副总畜牧师	硕士研究生	羊病防治	广东温氏食品集团股份有限公司	
孙建宽	38	男	未取得	副总经理	中专	项目实施管理	广东温氏食品集团股份有限公司	
李和平	38	男	未取得	副经理	硕士研究生	羊场管理	广东温氏食品集团股份有限公司	
林金荣	37	男	未取得	主任畜牧师	本科	项目实施管理	广东温氏食品集团股份有限公司	
廖其升	41	男	未取得	副主任畜牧师	本科	项目实施管理	广东温氏食品集团股份有限公司	



苏敏	31	男	未取得	厂长	本科	项目实施管理	广东温氏食品集团股份有限公司	苏敏
崔振亮	30	男	未取得	羊的营养管理	博士研究生	羊的饲养配方制定	广东温氏食品集团股份有限公司	崔振亮
周多恩	26	男	未取得	管理类	硕士研究生	羊的饲养监督	广东温氏食品集团股份有限公司	周多恩
代童童	25	女	未取得	研究生	本科	SNP检测	华南农业大学	代童童
何玉强	23	男	未取得	研究生	本科	生产数据测定	华南农业大学	何玉强



七、承担、参与单位合作

联合申报项目协议书

甲方：华南农业大学

乙方：广东温氏食品集团股份有限公司

甲方与乙方经友好协商决定联合申报 2017年度广东省公益研究与能力建设专项项目，项目名称：广东雷州黑山羊快大高繁新品系培育。并达成如下合作协议：

第一条：项目研究工作分工：

甲方(主持方)：负责项目管理和实施。

乙方(参与方)：配合甲方做好项目各项管理和协调工作。

第二条：经费分配：

1、如果本申报项目获批立项，按政府下达的资助经费，甲方、乙方同意此经费分别按政府资助经费的甲方：60%、乙方：40%进行分配。

2、甲方在收到政府下达的资助经费后的一个月内将乙方所占经费支付给乙方指定帐户。

帐户：广东温氏食品集团股份有限公司

帐号：670457744671

开户行：中国银行新兴支行

第三条：知识产权归属：

1. 项目实施过程中所产生的知识产权，优先执行任务下达单位的知识产权管理政策，在此前提下，作如下规定：

①各方独立完成的所有权归各自所有，对方有使用权；双方共同完成的由双方共享，具体按照双方的贡献大小进行分配或双方另行商定。

②项目成果的转让，须在双方同意的前提下进行，任何一方不得私自转让或许可实施，独立完成的除外。

2. 项目成果申报各级奖项，双方单位排名根据具体情况另行商定，人员排名原则上按贡献大小先后排名。

第四条：合作项目各方应严格遵守共同签订的合作协议书，除因不可抗拒的客观原因，不得中途撤消或中止合同。在合同期内，某方要求修改合同条款，须各方协商，确认后方能生效。

第五条：如合作方因各种原因无法履行合同条款时，由项目负责人报项目主管部门同意后，另寻合作者。

第六条：经批准中途退出合作的一方，应视具体情况将所余经费退回项目主持方，已用经费由项目负责人提出审查报告，报项目主管部门审批。

第七条：合作一方在工作进行中有问题不及时报告，影响项目整体的年度进展者，项目负责人有权缓拨或停拨下一年度经费，并通报项目主管部门。如影响项目整体无法完成者，将承担相关责任，并报主管部门。

第八条：本协议自双方签字盖章之日生效，若合作申请未获资助，本协议自动废止。



八、合同条款

第一条	甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定,为顺利完成(2017)年广东雷州黑山羊快大高繁新品系培育专项项目(项目编号: 2017B020201014)经协商一致,特订立本合同,作为甲乙双方在项目实施管理过程中共同遵守的依据。
第二条	甲方的权利义务: 1. 按合同书规定进行经费核拨的有关工作协调。 2. 根据甲方需要,在不影响乙方工作的前提下,定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。 3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。
第三条	乙方的权利义务: 1. 确保落实自筹经费及有关保障条件。 2. 按合同书规定,对甲方核拨的经费实行专款专用,单独列账,并随时配合甲方进行监督检查。 3. 使用财政资金采购设备、原材料等,按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定,符合招标条件的须进行招标。 4. 项目实施完成或实施到一定程度,须按照《广东省省级科技计划项目结题管理的实施细则(试行)》提出验收或终止结题的申请,并按甲方要求做好项目结题工作。 5. 在每年1月向甲方如实提交上年度工作情况报告,报告内容包含上年度项目进展情况、经费决算和取得的效果等。 6. 按照国家和省有关规定,每年须提交年度科技报告;项目验收时,须提交验收科技报告。
第四条	在履行本合同的过程中,如出现广东省相关法律法规重大改变等不可抗力情况,甲方有权对所核拨经费的数量和时间进行相应调整。
第五条	在履行本合同过程中,需要对项目起止时间、项目经费使用(包括自筹经费、经费分配及经费支出预算等)、项目内容(包括研发内容、技术指标、经济指标及成果指标等)、项目名称、项目承担单位(包括承担单位更名、承担单位替换)、参与单位、项目负责人和成员等进行变更的,甲乙双方按照《广东省省级科技计划项目合同书管理的实施细则(试行)》有关规定执行。
第六条	在履行本合同的过程中,当事人一方发现可能导致项目整体或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。
第七条	本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享,除双方另有约定外,按国家和广东省有关法规执行。



第八条	属技术保密的项目,甲乙双方应另行订立技术保密条款,作为本合同正式内容的一部分,与本合同具有同等效力。
第九条	根据项目具体情况,经双方另行协商订立的附加条款,作为本合同正式内容的一部分,与本合同具有同等效力。
第十条	本合同的争议应由双方本着协商一致的原则解决,如双方协商不成的,则应向甲方所在地法院提起诉讼。
第十一条	保密条款: 1. 本合同保密内容范围为: / 2. 本合同保密期限为: / 3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。 4. 各方应建立技术秘密保护制度。 5. 属技术保密的项目必须经省负责技术保密部门审查后,确定可否发表或用于国际合作和交流。
第十二条	甲方可根据具体情况决定乙方是否需要单位担保,若需要保证单位,应订立担保条款,作为本合同正式内容一部分。当乙方不履行或不完全履行本合同,以及没有或没有完全承担违约责任时,乙方的保证单位承担连带保证责任。
第十三条	本合同一式六份,各份具有同等效力。甲方存三份,乙方存二份,丙方存一份,本合同自签字之日起生效,有效期至项目结题后一年内。各方均应负合同的法律责任,不应受机构、人事变动的影响。
说明:本合同书中,凡是当事人约定无需填写的内容,应在空白处划(/)。	



九、本合同签约各方

管理单位（甲方）： 广东省科学技术厅 （盖章） 单位地址： 连新路171号 法定代表人（或授权代表）： 黄宁生 （签章） 立项责任人： 叶毓峰 叶毓峰 （签章）		2017-07-26 年 月 日
承担单位（乙方）： 华南农业大学 （盖章） 二级部门： 华南农业大学动物科学学院 单位地址： 五山路483号 法定代表人（或法人代理）： 陈晓阳 （签章） 联系人（项目主管）姓名： 石睿 （签章） Email: kjcgxk@scau.edu.cn 电话： 020-85285390 / 13430350923 开户单位名称： 华南农业大学 开户银行及帐号： 广东广州工行五山支行 3602002609000310520		2017年 7月 2日 年 月 日
乙方主管部门（丙方）： 华南农业大学 （盖章） 单位地址： 五山路483号 法定代表人（或法人代理）： 陈晓阳 （签章）		2017年 7月 2日 年 月 日



受理编号: c19140500000024

项目编号: 2019B1515210017

文件编号: 粤基金字〔2020〕6号

广东省基础与应用基础研究基金项目 合同书

项目名称: 南方荷斯坦奶牛重要经济性状全基因组选择

项目类别: 企业(温氏)联合基金-重点项目

项目起止时间: 2020-01-01 至 2022-12-31

管理单位(甲方): 广东省基础与应用基础研究基金委员会

依托单位(乙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-85283435

项目负责人: 李耀坤

联系电话: 18620193682



(广东科技微信公众号)

广东省基础与应用基础研究
基金委员会
二〇二〇年制



(受理纸质材料二维码)

一、主要研究内容和要达到的目标

一、研究内容：

基于高密度芯片杂交、基因测序和搜集本地化物种表型等技术和手段，得到表型关联的位点信息，挖掘调控奶牛产奶量、奶品质等重要经济性状的相关位点。再结合生物信息学分析，探究关键基因的作用机理，揭示相关“表型-基因型”的分子作用机制，筛选影响荷斯坦奶牛重要经济性状的分子遗传标记。在南方高温高湿条件下，对奶牛重要经济性状全基因组选择信号进行综合评估，开发适合南方荷斯坦奶牛选育的分子育种模型和全基因组选择方案，为育种奠定基础。

1、高精度分子遗传标记的开发和鉴定

1) 荷斯坦奶牛多世代群体的表型持续搜集

中国南方荷斯坦奶牛生活、繁衍在中国南方特有的生态环境下，故一般认为拥有与世界其他地区的该物种不同的表型，以及基因组水平上不同的变异和转录组水平上不同的基因表达量。持续搜集的表型数据将用于位点的关联和验证。

2) 基因芯片杂交定位

当前已有的荷斯坦奶牛基因芯片主要针对国外或中国北方的奶牛，所以需要首先使用基因芯片对中国南方荷斯坦奶牛的表型关联位点进行杂交定位和位点验证，并将杂交的结果与该芯片已有的公开数据（比如其他地区的荷斯坦奶牛芯片杂交结果）进行对比。

3) 生物信息学分析进一步开发及验证位点

在单纯芯片得到位点信息无法满足需求的情况下，使用生物信息学方法：如GWAS分析、家系连锁分析以及QTL定位的方法，再结合搜集到的表型数据进行关联，得到更精准的关联变异位点或者QTL区间。

另外针对基因组定位到的结果，再采用转录组的测序进一步的筛选和验证位点信息。最终已有结果进行多重对比和验证，筛选并整合转录组测序分析结果、基因组位点关联结果以及基因芯片杂交结果，最终得到精准的中国南方荷斯坦奶牛分子遗传标记。

2、分子育种模型的开发

1) 已有分子育种算法的计算结果比较

针对得到的分子遗传标记，采用现有的分子育种算法进行计算，比较不同算法模型的计算结果，估算各模型对于中国南方荷斯坦奶牛这个物种的适用程度。

2) 新型分子育种算法的建模和对比验证

根据不同算法模型的评估结果以及已有的新表型、新位点信息，的对各模型进行调整和优化，并据此开发出一套最适合中国南方荷斯坦奶牛的新型分子育种模型。

二、研究目标：

- 1、筛选出中国南方荷斯坦奶牛优良经济性状相关，并可用于早期选育种的分子遗传标记23 个；
- 2、结合全基因组扫描芯片数据、RNA-Seq、奶牛DHI数据对现有育种模型分析结果进行验证及优化；
- 3、建立适用于南方奶牛选育种的全基因组选择技术方案1 套。

二、研究成果及形式

论文及专著情况	国家统计源刊物以上刊物 发表论文（篇）		2		科技报告（篇）		1	
	被SCI/EI/ISTP收录论文 数（篇）		1		培养人才（人）		1	
	专著（册）		0		引进人才（人）		0	
专利情况(项)	发明专利		实用新型专利		外观设计专利		国外专利	
	申请	授权	申请	授权	申请	授权	申请	授权
	2	0	0	0	0	0	0	0
其他								

三、项目进度和阶段目标

(一) 项目起止时间： 2020-01-01 至 2022-12-31		
(二) 项目实施进度及阶段主要目标：		
开始日期	结束日期	主要工作内容
2020-01-01	2020-12-31	奶牛样品采集及生产数据跟踪测定；样品核酸提取；芯片杂交及转录组测序。
2021-01-01	2021-12-31	芯片数据分析及验证；转录组数据分析；QTL挖掘及验证。
2022-01-01	2022-12-31	育种模型验证与参数优化；撰写论文与研究报告。

四、项目总经费及省基金委经费预算

(一) 省基金委经费下达总额: (大写) 伍拾万圆整; (小写) 50万元;					
(二) 省基金委经费年度下达计划:					
年度	2019 年	年	年	年	年
经费(万元)	50.00				
(三) 总经费及省基金委经费开支预算计划:					
经费筹集情况:					(单位: 万元)
省基金委经费	自筹资金				合计
	自有资金	贷款	地方政府投入	其它	
50.00	0	0	0	0	50.00
政府部门、境外资金及其他资金投入情况说明:	<p>本项目总经费投入50万元, 均来自省基金委, 包括直播经费47.5万元, 间接经费2.5万元。</p> <p>直接经费中, 9万元用于基因检测与核酸提取所用的试剂耗材等内容, 22万元用于奶牛性状检测及测序费用等, 6万元用于调研、往返奶牛养殖基地及参加有关学术会议等, 3.5万元用于文献发表、论文检索及专利发表费用等, 7万元用于参与项目学生劳务及临时雇用人员等方向。</p> <p>间接费用为2.5万元, 单位按总经费的5%收取项目管理费。</p>				

经费预算			(单位: 万元)	
	总投入经费		省基金委经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:				
1、直接费用:	47.50		47.50	
(1) 设备费:	0	无	0	无
(2) 材料费:	9.00	基因检测与核酸提取所用的试剂耗材等	9.00	基因检测与核酸提取所用的试剂耗材等
(3) 测试化验加工外协费:	22.00	奶牛性状检测及测序费用等	22.00	奶牛性状检测及测序费用等
(4) 燃料动力费:	0	无	0	无
(5) 差旅费/会议费/国际合作与交流费:	6.00	调研、往返奶牛养殖基地及参加有关学术会议等	6.00	调研、往返奶牛养殖基地及参加有关学术会议等
(6) 出版/文献/信息传播/知识产权事务费:	3.50	文献发表、论文检索及专利发表费用等	3.50	文献发表、论文检索及专利发表费用等
(7) 劳务费:	7.00	参与项目学生劳务及临时雇用人员等	7.00	参与项目学生劳务及临时雇用人员等
(8) 人员费:	0	无	0	无
(9) 专家咨询费:	0	无	0	无
(10) 直接费用其他支出:	0	无	0	无
(11) 科技金融服务体系其他费用:	0.00		0.00	
①信用评级补贴:	0	无	0	无
②大赛场租:	0	无	0	无
③特派员奖励与补贴:	0	无	0	无
2、间接费用:	2.50		2.50	
(1) 间接成本:	0	无	0	无
(2) 管理成本:	2.50	按总经费的5%收取项目管理费	2.50	按总经费的5%收取项目管理费
(3) 绩效支出:	0	无	0	无
合计:	50.00	无	50.00	无

五、人员信息

项目负责人								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
李耀坤	410423198607084036	34	男	副教授	博士研究生	项目负责人	华南农业大学	

项目组主要成员								
姓名	证件号码	年龄	性别	职称	学历	在项目中承担的任务	所在单位	签名
柳广斌	210103198204261211	38	男	讲师	博士研究生	论文发表及专利申请	华南农业大学	
毛宜军	41010519791206285X	41	男	讲师	博士研究生	负责奶牛基因组芯片检测	华南农业大学	
刘德武	42010619660711563X	54	男	教授	博士研究生	样品采集统筹协调	华南农业大学	
戚晓鸿	441228198111192217	39	男	管理员	硕士研究生	奶牛系谱信息收集	肇庆市鼎湖温氏畜牧有限公司	
魏建生	522224198106150018	39	男	管理员	硕士研究生	负责奶牛DHI测定及数据收集	肇庆市鼎湖温氏畜牧有限公司	
肖帆	41132319830905001X	37	男	技术员	大专	协助实验奶牛筛选及样品采集	肇庆市鼎湖温氏畜牧有限公司	
陈墩金	445221198303154599	37	男	技术员	硕士研究生	育种模型优化	广州明领基因科技有限公司	
曾千里	430502199003301011	30	男	技术员	硕士研究生	RNA-Seq数据分析	广州明领基因科技有限公司	
谢岑瑶	440583199511090767	25	女	技术员	本科	SNP芯片测序数据分析	广州明领基因科技有限公司	

六、依托单位与合作单位的合作协议

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省基金委经费分配 (万元)
华南农业大学	育种方案制定；奶牛基因组芯片检测；RNA-Seq测序；结题报告撰写、论文发表、专利申请。	25.00	25.00
肇庆市鼎湖温氏畜牧有限公司	提供实验奶牛及样品；提供奶牛系谱信息；奶牛DHI测定。	15.00	15.00
广州明领基因科技有限公司	QTL数据分析；RNA-Seq数据分析；育种模型优化。	10.00	10.00
	合计	50.00	50.00

七、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定，为顺利完成（2019）年南方荷斯坦奶牛重要经济性状全基因组选择 专项项目（文件编号：粤基金字〔2020〕6号）经协商一致，特订立本合同，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 乙方是项目资金管理的责任主体，应当建立健全科研项目资金管理制度，严格按照省科技经费使用范围和有关规定管好用好财政资金；应当按合同书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 项目负责人是项目资金使用的直接责任人，对资金使用的合规性、合理性、真实性和相关性承担法律责任。
4. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
5. 项目合同任务完成后，或合同书规定的任务、指标及经费投入等提前完成的，乙方可按照《广东省省级科技计划项目结题管理实施细则（试行）》提出验收结题申请，并按甲方要求做好项目验收结题工作。
6. 若项目发生需要终止结题的情况，乙方须按照《广东省省级科技计划项目结题管理的实施细则（试行）》提出终止结题申请，并按甲方要求做好项目终止结题工作。
7. 在每年规定时间内向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的成果等。
8. 按照国家和省有关规定，提交科技报告及其他材料。
9. 利用甲方的经费获得的研究成果，项目负责人和参与者应当注明获得“广东省基础与应用基础研究基金（英文：Guangdong Basic and Applied Basic Research Foundation）（项目编号）”资助或作有关说明。
10. 乙方要恪守科学道德准则，遵守科研活动规范，践行科研诚信要求，不得抄袭、剽窃他人科研成果或者伪造、篡改研究数据、研究结论；不得购买、代写、代投论文，虚构同行评议专家及评议意见；不得违反论文署名规范，擅自标注或虚假标注获得科技计划（专项、基金等）等资助；不得弄虚作假，骗取科技计划（专项、基金等）项目、科研经费以及奖励、荣誉等；不得有其他违背科研诚信要求的行为。
11. 确保本项目开展的研究工作符合我国科研伦理管理相关规定。

第四条 在履行本合同的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本合同的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第六条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第七条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第八条 本合同一式三份，各份具有同等效力。甲、乙方及项目负责人各执一份，三方签字、盖章后即生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

第九条 乙方必须接受甲方聘请的本项目合同监理单位的监督和管理。监理单位按照甲方赋予的权利对本项目合同的履行进行审核、进度调查，对项目合同变更、经费使用情况进行监督管理及组织项目验收。

说明：1. 本合同书中，凡是当事人约定无需填写的条款，应在该条款的空白处划（/）。

2. 委托代理人签订本合同书的，应出具合法、有效的委托书。

八、本合同签约各方

管理单位（甲方）：广东省基础与应用基础研究基金委员会（盖章）

法定代表人（或法人代理）：_____（签章）

年 月 日

依托单位（乙方）：华南农业大学（盖章）

法定代表人（或法人代理）：刘雅红_____（签章）

联系人（项目主管）姓名：郑鹏_____（签章）

Email: kjcgxk@scau.edu.cn

电话：020-85283435 / 13560344902

开户单位名称：华南农业大学

开户银行名称：广东广州工行五山支行

开户银行帐号：3602002609000310520

年 月 日

联系人（项目负责人）姓名：李耀坤（签名）

Email: ykli@scau.edu.cn

电话：18620193682

年 月 日

受理编号: c1630550100239

项目编号: 2016A020210083

文件编号: 粤科规财字(2016)47号



广东省省级科技计划项目

合同书

项目名称: 辣木对荷斯坦奶牛生产性能和牛奶品质的影响研究

计划类别: 农村科技领域

项目起止时间: 2016-01-01 至 2017-12-31

管理单位(甲方): 广东省科学技术厅

承担单位(乙方): 华南农业大学

乙方主管部门(丙方): 华南农业大学

通讯地址: 广东省广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 孙宝丽

联系电话: 020-85280283

项目联系人: 孙宝丽

联系电话: 020-85280283

广东省科学技术厅
二〇一四年制

一、项目实施内容

主要研究内容：

- (1) 采集新鲜辣木细枝叶烘干制粉，测定其总能、粗蛋白、粗脂肪、无氮浸出物、粗纤维、灰分、钙、磷、NDF、ADF、木质素、单宁、Lys和Met等指标。
- (2) 将辣木枝叶在实验室青贮罐发酵后进行指标的测定，研究辣木枝叶的青贮工艺和参数，同时在刈割的辣木枝叶中添加乳酸菌和葡萄糖进行发酵，研究乳酸菌和葡萄糖对发酵品质的调控特点及青贮发酵的动态变化规律，选择最佳的辣木青贮处理模式。
- (3) 添加不同比例青贮辣木枝叶替代苜蓿干草对泌乳期奶牛泌乳性能、免疫性能、抗氧化能力及牛奶品质的影响，确定最佳的青贮辣木添加比例。
- (4) 在内容(3)的基础上选择合适的青贮辣木枝叶添加量替代苜蓿干草，研究辣木对奶牛繁殖性能的影响。
- (5) 选择辣木最佳的处理方式和最佳饲用量，在风行集团种养基地进行应用和示范。

二、项目考核指标

1. 项目完成后提供的研究开发成果及形式(须明确产品、专利、版权、标准等成果的类型及数量)


成果形式		成果数量	成果形式		成果数量
发明专利	申请		引进人才(人)		
	授权		培养人才(人)		2
实用新型专利	申请		科技人才奖励(人)		
	授权		技术标准制定	牵头(个)	
外观设计专利	申请			参与(个)	
	授权		科技报告(篇)		
国外专利	PCT受理		软件著作权(项)		
	授权		论文论著(篇)		2
获得国家级奖项(项)			其中:被收录论文数(篇)	SCI	
获得省级奖项(项)				EI	
新服务(项)				ISTP	
新产品(或新材料、新装备、新品种(系))			新工艺(或新方法、新模式、新技术)		
创新载体项目必填		技术服务数量(项)			
		服务企业数量(家)			
科技金融项目必填		开展培训宣讲活动场次(次)			
		服务企业数量(家)			
		帮助企业融资(万元)			
		引进专业机构(家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告(篇)(至少1篇)			
		研究总报告(篇)(至少1篇)			
		研究中期报告(篇)			
		研究分报告(篇)			
		调研报告(篇)			
		专著(篇)[须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		核心期刊论文(篇)[以第一作者发表,须注明“广东省软科学研究计划项目(项目编号:)资助”]			
		培养人才(人)			

	获国家级奖项(项)	
	获省级奖项(项)	
	其他(具体形式: 用户填)	
其他成果及形式说明:		
2. 主要技术经济指标及社会效益		
累计新增销售收入(万元)		
累计新增利税(万元)		
其他主要技术经济指标及社会效益说明:		
项目负责人(签章): 孙金明 2016 年 8 月 24 日		

三、项目进度和阶段目标

开始日期	结束日期	主要工作内容
2016-01-01	2016-03-31	从种植基地采集青绿生长期的辣木枝叶，进行三种不同的处理，分别测定营养成分，配制饲料。
2016-04-01	2016-10-31	开展晾干、青贮、微贮辣木枝叶与常规饲料组合对比的饲养实验和青贮辣木叶替代优质苜蓿干草饲养实验。
2016-11-01	2017-06-30	对泌乳期奶牛饲养试验的生产数据进行统计分析，同时测定奶样和血清的各项指标。同时开展辣木对奶牛繁殖性能影响的实验，并对一系列繁殖指标进行测定和统计分析。
2017-07-01	2017-08-31	对所有实验数据进行整理和统计分析，撰写论文。
2017-09-01	2017-12-31	确定辣木的最佳处理方式和青贮辣木叶的最佳添加量，开展辣木种植和饲用一体化的应用和示范。撰写研究报告，申请验收。

四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
 华南农业大学	本项目由华南农业大学独立承担，在华南农业大学与广州风行奶牛有限公司长期稳定合作的基础上开展工作，建立不同学科间协同创新、研究和示范同步、科研和应用一体化的组织方式。遵循华南农业大学标准的项目管理体系，项目负责人负责本项目所有资源的运用、人员的管理等，保证项目的顺利开展和完成。项目组成员根据各自的分工开展工作，其中1名项目成员长期在企业全职挂职工作，为项目的顺利开展尤其是应用示范和推广奠定了基础。基地在场地、人员、资金、时间等各方面提供全面的保障。	15.00	15.00
	合计	15.00	15.00

五、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额：（大写）壹拾伍万圆整；（小写）15.00万元；

2. 省科技厅经费拨付方式：一次性拨款

3. 省科技厅经费年度下达计划：总额15.00(万元)

分期	经费(万元)
第1期	15

4. 总经费开支预算计划：

经费筹集情况：（单位：万元）

总投入经费：15.00

	省科技厅经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费：						
新增经费：	15.00					15.00

政府部门、境外资金及其他资金投入情况说明：

新增经费预算:			(单位: 万元)	
	新增经费总额		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明
基建费:				
1、直接费用:	14.25		14.25	
(1) 设备费:				
(2) 材料费:	7.25	实验饲料、动物、试剂、耗材等	7.25	实验饲料、动物、试剂、耗材等
(3) 测试化验加工外协费:	4.00	饲料营养成分分析, 发酵指标测定等	4.00	饲料营养成分分析, 发酵指标测定等
(4) 燃料动力费:				
(5) 差旅费:	2.00	基地往返、调研、推广、国内学术交流等	2.00	基地往返、调研、推广、国内学术交流等
(6) 会议费:				
(7) 国际合作与交流费:				
(8) 出版/文献/信息传播/知识产权事务费:	1.00	论文版面费、文献检索、文献打印复印费、网络费等	1.00	论文版面费、文献检索、文献打印复印等
(9) 租赁费:				
(10) 人员费:				
(11) 专家咨询费:				
(12) 直接费用其他支出:				
(13) 科技金融服务体系其他费用:	0.00		0.00	
①信用评级补贴:				
②大赛场租:				
③特派员奖励与补贴:				
2、间接费用:	0.75		0.75	
科研管理费用:	0.75	科研管理费用: $5\% \times 15 = 0.75$ 万元	0.75	科研管理费用: $5\% \times 15 = 0.75$ 万元
合计:	15.00		15.00	

六、人员信息

项目负责人情况

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
孙宝丽	35	女	副教授	教师	博士研究生	项目总负责人，负责本项目所有资源的运用、人员的管理等，保证项目的顺利开展和完成。	华南农业大学	孙宝丽

主要研究开发人员

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
柳广斌	33	男	讲师	无	博士研究生	负责种养基地的整体管理、辣木种养工艺的建立及后续的培训推广工作。	华南农业大学	柳广斌
刘德武	49	男	教授	动物生产系主任	博士研究生	辣木对荷斯坦奶牛乳品质的影响	华南农业大学	刘德武
张永亮	49	男	教授	教务处处长	博士研究生	血清的采集和抗氧化、生化等指标的测定与分析	华南农业大学	张永亮
马静云	41	女	副教授	教师	博士研究生	不同组合日粮配方的制定、疾病防控和抗体等免疫指标的测定	华南农业大学	马静云
丁美美	47	女	副教授	教师	博士研究生	辣木的种植、刈割和处理	华南农业大学	丁美美
汪胜楠	24	男	未取得	学生	硕士研究生	开展饲养实验，样品的采集和处理	华南农业大学	汪胜楠
朱潇	23	女	未取得	学生	本科	奶牛生产、繁殖性能相关指标的测定	华南农业大学	朱潇

七、承担、参与单位合作协议（须与申报书中合作协议或意向书相一致）

/

八、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定，为顺利完成（2016）年**辣木对荷斯坦奶牛生产性能和牛奶品质的影响研究**专项项目（项目编号：2016A020210083）经协商一致，特订立本合同，作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务：

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要，在不影响乙方工作的前提下，定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务：

1. 确保落实自筹经费及有关保障条件。
2. 按合同书规定，对甲方核拨的经费实行专款专用，单独列账，并随时配合甲方进行监督检查。
3. 使用财政资金采购设备、原材料等，按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定，符合招标条件的须进行招标。
4. 项目实施完成或实施到一定程度，须按照《广东省省级科技计划项目结题管理的实施细则（试行）》提出验收或终止结题的申请，并按甲方要求做好项目结题工作。
5. 在每年1月向甲方如实提交上年度工作情况报告，报告内容包含上年度项目进展情况、经费决算和取得的效果等。
6. 按照国家和省有关规定，每年须提交年度科技报告；项目验收时，须提交验收科技报告。

第四条 在履行本合同的过程中，如出现广东省相关政策法规重大改变等不可抗力情况，甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本合同过程中，需要对项目起止时间、项目经费使用（包括自筹经费、经费分配及经费支出预算等）、项目内容（包括研发内容、技术指标、经济指标及成果指标等）、项目名称、项目承担单位（包括承担单位更名、承担单位替换）、参与单位、项目负责人和成员等进行变更的，甲乙双方按照《广东省省级科技计划项目合同书管理的实施细则（试行）》有关规定执行。

第六条 在履行本合同的过程中，当事人一方发现可能导致项目整体或部分失败的情形时，应及时通知另一方，并采取适当措施减少损失，没有及时通知并采取适当措施，致使损失扩大的，应当就扩大的损失承担责任。

第七条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享，除双方另有约定外，按国家和广东省有关法规执行。

第八条 属技术保密的项目，甲乙双方应另行订立技术保密条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第九条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第十条 本合同的争议应由双方本着协商一致的原则解决，如双方协商不成的，则应向甲方所在地法院提起诉讼。

第十一条 保密条款：

1. 本合同保密内容范围为：

/

2. 本合同保密期限为：

/

3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。

4. 各方应建立技术秘密保护制度。

5. 属技术保密的项目必须经省负责技术保密部门审查后，确定可否发表或用于国际合作和交流。

第十二条 甲方可根据具体情况决定乙方是否需要单位担保，若需要保证单位，应订立担保条款，作为本合同正式内容一部分。当乙方不履行或不完全履行本合同，以及没有或没有完全承担违约责任时，乙方的保证单位承担连带保证责任。

第十三条 本合同一式六份，各份具有同等效力。甲方存三份，乙方存二份，丙方存一份，本合同自签字之日起生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

说明：本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

九、本合同签约各方

管理单位（甲方）： 广东省科学技术厅 （盖章）

单位地址： 广东省广州市连新路171号

法定代表人（或授权代表）： 黄宁生

联系人（经办人）姓名： 刘世伟

Email: liusw@gdstc.gov.cn

电话： 020-83163909



2016年 9月 1日

承担单位（乙方）： 华南农业大学 （盖章）

二级部门： 华南农业大学动物科学学院

单位地址： 广东省广州市天河区五山路483号

法定代表人（或法人代理）： 陈晓阳 （盖章）

联系人（项目主管）姓名： 石睿 （盖章）

Email: 77909213@qq.com

电话： 020-85283435

开户单位名称： 华南农业大学

开户银行及帐号： 广东广州工行五山支行 3602002609000310520

2016年 9月 1日

乙方主管部门（丙方）： 华南农业大学 （盖章）

单位地址： 广东省广州市天河区五山路483号

法定代表人（或法人代理）： 陈晓阳 （盖章）

联系人（项目主管）姓名： 石睿 （盖章）

Email: 77909213@qq.com

电话： 020-85283435

开户单位名称： 华南农业大学

开户银行及帐号： 广东广州工行五山支行 3602002609000310520

2016年 9月 1日

受理编号: c1430550100075

项目编号: 2014A020208104

文件编号: 粤科规财字[2015]72号



2014A020208104

广东省省级科技计划项目

合同书

项目名称: 黄梁木对乐至黑山羊饲用效果和肉品质的影响研究与应用示范

计划类别: 农村科技领域

项目起止时间: 2015-01-01 至 2016-12-31

管理单位(甲方): 广东省科学技术厅

承担单位(乙方): 华南农业大学

乙方主管部门(丙方): 华南农业大学

通讯地址: 广东省广州市天河区广州市天河区五山路483号

邮政编码: 510642

单位电话: 020-38632819

项目负责人: 孙宝丽

联系电话: 020-85280283

项目联系人: 孙宝丽

联系电话: 020-85280283

广东省科学技术厅
二〇一四年制

一、项目实施内容

主要研究内容：

(1) 将黄梁木叶经过晾干、青贮、微贮处理后与常规饲料组合进行对比实验，研究其对生长期黑山羊养分消化率、生长性能、抗氧化能力及胴体品质的影响，选择最佳的黄梁木处理方式。

(2) 添加不同比例晾干黄梁木叶替代羊草对生长期黑山羊养分消化率、生长性能、抗氧化能力及胴体品质的影响，确定最佳的晾干黄梁木添加比例。

(3) 选择(1)和(2)肉质最佳组合，研究不同MyHC亚型mRNA在黑山羊不同部位肌肉的表达量，确定黑山羊肉中被表达的MyHC亚型，分析不同亚型的表达差异与胴体肉质性状的关系。

(4) 选择黄梁木最佳的处理方式和最佳饲用量，在合作建立的广东温氏食品集团种养基地进行应用示范，为广东地区乃至全国相关企业提供示范、技术培训和推广工作。

拟解决的关键问题：

(1) 从生长屠宰性能、抗氧化能力、肉质等方面研究黄梁木替代常规饲料的饲用效果。

(2) 确定黄梁木叶的最佳处理方式和晾干黄梁木叶的最佳添加量。

(3) 确定山羊肉中被表达的MyHC亚型，分析MyHC不同亚型的表达差异与胴体肉质性状的关系。

(4) 建立多学科的协同创新研究模式，以广东省温氏食品集团为基地，开展黄梁木种植和饲用一体化的应用、示范和推广。

技术路线见可行性报告。

创新点：

在南方丘陵地区养殖草食动物，开发新型的青绿优质饲料是解决草料匮乏的主要途径。黄梁木作为一种优质蛋白来源的绿色木本植物，在畜禽养殖应用上未有报道。本项目组前期的研究基础发现，饲用黄梁木不仅可以提高家兔的生产性能，还可以改善抗氧化性、免疫、屠宰性能和肉质。本项目的创新点主要体现在：

(1) 首次研究黄梁木叶饲喂反刍动物的效果及其对肉质的影响，从木本饲料角度开发适合南方地区利用的优质青粗饲料。

(2) 以广东省温氏食品集团为基地，开展黄梁木种植和饲用一体化的应用、示范和推广。

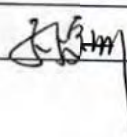
(3) 高校和企业紧密合作，建立不同学科间协同创新、研究和示范同步、科研和应用一体化的组织模式。

(4) 确定山羊肉中被表达的MyHC亚型，分析MyHC不同亚型的表达差异与胴体肉质性状的关系，初步探索黄梁木影响羊肉品质的作用机理。

二、项目考核指标

1. 项目完成后提供的研究开发成果及形式（须明确产品、专利、版权、标准等成果的类型及数量）

成果形式		成果数量	成果形式		成果数量
发明专利	申请	1	引进人才(人)		
	授权		培养人才(人)		2
实用新型专利	申请		科技人才奖励(人)		
	授权		技术标准制定	牵头(个)	
外观设计专利	申请			参与(个)	
	授权		科技报告(篇)		
国外专利	PCT受理		软件著作权(项)		
	授权		论文论著(篇)		3
获得国家级奖项(项)			其中：被收录论文数(篇)	SCI	1
获得省级奖项(项)				EI	
新服务(项)				ISTP	
新产品（或新材料、新装备、新品种（系））			新工艺（或新方法、新模式、新技术）		
创新载体项目必填		技术服务数量（项）			
		服务企业数量（家）			
科技金融项目必填		开展培训宣讲活动场次(次)			
		服务企业数量(家)			
		帮助企业融资(万元)			
		引进专业机构(家)			
院士工作站项目必填		引进院士及其团队科技成果转化数量			
		院士开展的战略咨询和技术指导次数			
		院士年进站次数			
		院士及院士团队年进站时间			
软科学项目必填		决策咨询报告(篇)(至少1篇)			
		研究总报告(篇)(至少1篇)			
		研究中期报告(篇)			
		研究分报告(篇)			
		调研报告(篇)			
		专著(篇)[须注明“广东省软科学研究计划项目(项目编号：)资助”]			
		核心期刊论文(篇)[以第一作者发表，须注明“广东省软科学研究计划项目(项目编号：)资助”]			
		培养人才(人)			


	获国家级奖项(项)	
	获省级奖项(项)	
	其他(具体形式: 用户填)	
其他成果及形式说明:		
2. 主要技术经济指标及社会效益		
累计新增销售收入(万元)		
累计新增利税(万元)		
其他主要技术经济指标及社会效益说明:		
项目负责人(签章):  2015年 9 月 11 日		

2014A02020810A

三、项目进度和阶段目标

开始日期	结束日期	主要工作内容
2015-01-01	2015-04-30	从种植基地采集青绿生长期的黄梁木叶，进行三种不同的处理：晾干、青贮和微贮，分别测定经不同处理后的黄梁木叶的营养成分，根据NY/T816-2004配制不同实验不同处理组的饲料。
2015-05-01	2015-10-31	开展晾干、青贮、微贮黄梁木叶与常规饲料组合对比的饲养实验和晾干黄梁木叶替代优质羊草干草饲养实验。
2015-11-01	2016-01-31	对饲养试验的生产数据进行统计分析，同时开展屠宰实验，测定屠宰羊只的胴体和肉品质性状，采集血、肝脏、背最长肌、半腱肌和冈上肌，测定血清、肝脏、肌肉抗氧化、免疫和生化等相关指标。选择肉质最佳组合，选取2只羊进行屠宰，取背最长肌、半腱肌和冈上肌抽取总RNA备用。
2016-02-01	2016-05-31	确定山羊肉中被表达的MyHC亚型，分析MyHC不同亚型的表达差异与胴体肉质性状的关系。同时对所有实验数据进行整理和统计分析，撰写论文和专利。
2016-06-01	2016-11-30	确定黄梁木叶的最佳处理方式和晾干黄梁木叶的最佳添加量，在广东省温氏食品集团开展黄梁木种植和饲用一体化的应用、示范和推广。同时撰写详细的研究报告。
2016-12-01	2016-12-31	对整个项目进行整理和归纳，申请验收。

四、承担、参与单位工作分工及经费分配情况

承担/参与单位名称 (盖章)	工作分工	总经费分摊 (万元)	省科技厅经费分配 (万元)
 华南农业大学	本项目由华南农业大学独立承担，在华南农业大学与广东温氏食品集团长期稳定合作的基础上开展工作，建立不同学科间协同创新、研究和示范同步、科研和应用一体化的组织方式。遵循华南农业大学标准的项目管理体系，项目负责人负责本项目所有资源的运用、人员的管理等，保证项目的顺利开展和完成。项目组成员根据各自的分工开展工作，其中1名项目成员在基地全职挂职工作，为项目的顺利开展尤其是应用示范和推广奠定了基础。基地在场地、人员、资金、时间等各方面提供全面的保障。	20.00	20.00
	合计	20.00	20.00

五、项目总经费及省科技厅经费预算

1. 省科技厅经费下达总额：（大写）贰拾万圆整；（小写）20.00万元；						
2. 省科技厅经费年度下达计划：（第一期）20.00万元；（余额）0.00万元						
3. 总经费开支预算计划：						
经费筹集情况：						（单位：万元）
总投入经费：20.00						
	省科技厅经费	自筹资金				合计
		自有资金	贷款	地方政府投入	其它	
已投入经费：						
新增经费：	20.00					20.00
政府部门、境外资金及其他资金投入情况说明：	<div style="text-align: center; font-size: 2em; opacity: 0.3; transform: rotate(-30deg);">2014A020208104</div>					

新增经费预算:				(单位: 万元)	
		新增经费总额		省科技厅经费	
支出经费	经费额	用途说明	经费额	用途说明	
基建费:					
1、直接费用:	19.00		19.00		
(1)设备费:					
(2)材料费:	14.00	实验饲料、动物、试剂、耗材等	14.00	实验饲料、动物、试剂、耗材等	
(3)测试化验加工外协费:	3.00	组织抗氧化、生化等指标的测定等	3.00	组织抗氧化、生化等指标的测定等	
(4)燃料动力费:					
(5)差旅费:	1.00	基地往返、调研、推广、参加学术交流等交通住宿费用	1.00	基地往返、调研、推广、参加学术交流	
(6)会议费:					
(7)国际合作与交流费:					
(8)出版/文献/信息传播/知识产权事务费:	1.00	文章版面费、专利申请费等	1.00	文章版面费、专利申请费等	
(9)租赁费:					
(10)人员费:					
(11)专家咨询费:					
(12)直接费用其他支出:					
(13)科技金融服务体系其他费用:	0.00		0.00		
①信用评级补贴:					
②大赛场租:					
③特派员奖励与补贴:					
2、间接费用:	1.00		1.00		
科研管理费:	1.00	科研管理费用	1.00	科研管理费	
合计:	20.00		20.00		

六、人员信息

项目负责人情况								
姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
孙宝丽	34	女	副教授	教师	博士	项目总负责人，负责本项目所有资源的运用、人员的管理等，保证项目的顺利开展和完成。	华南农业大学	

2014A020208104

主要研究开发人员

姓名	年龄	性别	职称	职务	学历	在项目中承担的任务	所在单位	签名
柳广斌	32	男	讲师	教师	博士	因在基地全职挂职，主要负责种养基地的整体管理、黄梁木示范工艺的建立及后续的培训推广工作。	华南农业大学	柳广斌
刘德武	48	男	教授	教师	博士	黄梁木对山羊肉品质的影响和机理的初步探讨	华南农业大学	刘德武
张永亮	48	男	教授	院长	博士	血清、肝脏、肌肉等组织的采集和抗氧化、生化等指标的测定与分析	华南农业大学	张永亮
马静云	40	女	副教授	教师	博士	肉山羊不同组合日粮配方的制定、疾病防控和抗体等免疫指标的测定	华南农业大学	马静云
丁美美	46	女	副教授	教师	博士	黄梁木的种植、刈割和处理	华南农业大学	丁美美
李果	23	男	未取得	无	硕士	肉羊生产、屠宰性能的测定等	华南农业大学	李果
廖海辉	21	女	未取得	无	本科	开展饲养实验，所有样品的采集和处理	华南农业大学	廖海辉

七、承担、参与单位合作协议（须与申报书中合作协议或意向书相一致）

2014A020208104

八、合同条款

第一条 甲方与乙方根据《中华人民共和国合同法》及国家有关法规和规定,为顺利完成(2014)年黄梁木对乐至黑山羊饲用效果和肉品质的影响研究与应用示范专项项目(项目编号: 2014A020208104)经协商一致,特订立本合同,作为甲乙双方在项目实施管理过程中共同遵守的依据。

第二条 甲方的权利义务:

1. 按合同书规定进行经费核拨的有关工作协调。
2. 根据甲方需要,在不影响乙方工作的前提下,定期或不定期对乙方项目的实施情况和经费使用情况进行检查或抽查。
3. 根据《广东省科技计划项目信用管理办法(试行)》对乙方进行科技计划信用管理。

第三条 乙方的权利义务:

1. 确保落实自筹经费及有关保障条件。
2. 按合同书规定,对甲方核拨的经费实行专款专用,单独列账,并随时配合甲方进行监督检查。
3. 使用财政资金采购设备、原材料等,按照《广东省实施〈中华人民共和国招标投标法〉办法》有关规定,符合招标条件的须进行招标。
4. 项目实施完成或实施到一定程度,须按照《广东省省级科技计划项目结题管理的实施细则(试行)》提出验收或终止结题的申请,并按甲方要求做好项目结题工作。
5. 在每年1月向甲方如实提交上年度工作情况报告,报告内容包含上年度项目进展情况、经费决算和取得的效果等。
6. 按照国家和省有关规定,每年须提交年度科技报告;项目验收时,须提交验收科技报告。

第四条 在履行本合同的过程中,如出现广东省相关政策法规重大改变等不可抗力情况,甲方有权对所核拨经费的数量和时间进行相应调整。

第五条 在履行本合同过程中,需要对项目起止时间、项目经费使用(包括自筹经费、经费分配及经费支出预算等)、项目内容(包括研发内容、技术指标、经济指标及成果指标等)、项目名称、项目承担单位(包括承担单位更名、承担单位替换)、参与单位、项目负责人和成员等进行变更的,甲乙双方按照《广东省省级科技计划项目合同书管理的实施细则(试行)》有关规定执行。

第六条 在履行本合同的过程中,当事人一方发现可能导致项目整体或部分失败的情形时,应及时通知另一方,并采取适当措施减少损失,没有及时通知并采取适当措施,致使损失扩大的,应当就扩大的损失承担责任。

第七条 本项目技术成果的归属、转让和实施技术成果所产生的经济利益的分享,除双方另有约定外,按国家和广东省有关法规执行。

第八条 属技术保密的项目，甲乙双方应另行订立技术保密条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第九条 根据项目具体情况，经双方另行协商订立的附加条款，作为本合同正式内容的一部分，与本合同具有同等效力。

第十条 本合同的争议应由双方本着协商一致的原则解决，如双方协商不成的，则应向甲方所在地法院提起诉讼。

第十一条 保密条款：

1. 本合同保密内容范围为：

/

2. 本合同保密期限为：

/

3. 乙方应与可能知悉保密内容的人员签订技术秘密保护协议。

4. 各方应建立技术秘密保护制度。

5. 属技术保密的项目必须经省负责技术保密部门审查后，确定可否发表或用于国际合作和交流。

第十二条 甲方可根据具体情况决定乙方是否需要单位担保，若需要保证单位，应订立担保条款，作为本合同正式内容一部分。当乙方不履行或不完全履行本合同，以及没有或没有完全承担违约责任时，乙方的保证单位承担连带保证责任。

第十三条 本合同一式六份，各份具有同等效力。甲方存三份，乙方存二份，丙方存一份，本合同自签字之日起生效，有效期至项目结题后一年内。各方均应负合同的法律责任，不应受机构、人事变动的影响。

说明：本合同书中，凡是当事人约定无需填写的内容，应在空白处划（/）。

九、本合同签约各方

管理单位（甲方）：广东省科学技术厅（盖章）

单位地址：广州市连新路171号大院信息大楼

法定代表人（或授权代表）：黄宁生（签章）

联系人（经办人）姓名：林振亮（签章）

Email: linzl@gdstc.gov.cn

电话：020-83163905



年 月 日

承担单位（乙方）：华南农业大学（盖章）

二级部门：华南农业大学动物科学学院

单位地址：广东省广州市天河区广州市天河区五山路483号

法定代表人（或法人代理）：陈晓阳（签章）

联系人（项目主管）姓名：夏斌（签章）

Email: kjcgxk@scau.edu.cn

电话：020-85283435

开户单位名称：华南农业大学

开户银行及帐号：广东广州工行五山支行 3602002609000310520

年 月 日

乙方主管部门（丙方）：华南农业大学（盖章）

单位地址：广东省广州市天河区广州市天河区五山路483号

法定代表人（或法人代理）：陈晓阳（签章）

联系人（项目主管）姓名：夏斌（签章）

Email: kjcgxk@scau.edu.cn

电话：020-85283435

开户单位名称：华南农业大学

开户银行及帐号：广东广州工行五山支行 3602002609000310520

年 月 日

检索证明

根据委托人提供的论文材料，委托人华南农业大学动物科学学院 柳广斌 17 篇论文收录情况如下表。

序号	论文名称	发表刊物及发表的年月卷期/页码等	作者排名	论文等级	作者工作单位	收录情况	影响因子	中科院大分区
1	Effects of chronic heat stress on mRNA and miRNA expressions in dairy cows	GENE 出版年: 2020 出版日期: JUN 5 卷期: 742 页码: - 文献号: 144550 文献类型: Article	第一作者	B 类	华南农业大学 动物科学学院	SCI	IF2-year=3.688 IF5-year=3.329 (2020)	生物学 3 区 Top 期刊: 否 (2020)
2	Effects of moringa polysaccharides on growth performance, immune function, rumen morphology, and microbial community structure in early-weaned goat kids	FRONTIERS IN VETERINARY SCIENCE 出版年: 2024 出版日期: NOV 6 卷期: 11 页码: - 文献号: 1461391 文献类型: Article	共同通讯作者 (倒数第一)		华南农业大学 动物科学学院	SCI	IF2-year=2.9 IF5-year=3.3 (2024)	农林科学 2 区 Top 期刊: 否 (2025)
3	Transcriptome analysis reveals mRNAs and long non-coding RNAs associated with fecundity in the hypothalamus of high-and low-fecundity goat	FRONTIERS IN VETERINARY SCIENCE 出版年: 2023 出版日期: MAR 28 卷期: 10 页码: -	共同通讯作者 (倒数第一)	A 类	华南农业大学 动物科学学院	SCI	IF2-year=2.6 IF5-year=3.1 (2023)	农林科学 2 区 Top 期刊: 是 (2023)

	文献号: 1145594 文献类型: Article		共同通讯作者 (倒数第一)				SCI	IF2-year=4.9 IF5-year=5.6 (2023)	生物学 2 区 Top 期刊: 否 (2023)
4	Integrating Analysis to Identify Differential circRNAs Involved in Goat Endometrial Receptivity	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 出版年: 2023 出版日期: JAN 卷期: 24 2 页码: - 文献号: 1531 文献类型: Article					华南农业大学 动物科学学院		
5	雄激素受体抑制剂恩杂鲁胺对山羊卵泡颗粒细胞增殖凋亡的影响	畜牧兽医学报 出版年: 2024 卷期: 55 3 页码: - 文献号: 1531 文献类型: Article	通讯作者	A类			华南农业大学 动物科学学院	无	无
6	Screening of SNP Loci Related to Leg Length Trait in Leizhou Goats Based on Whole-Genome Resequencing	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 出版年: 2024 出版日期: NOV 卷期: 25 22 页码: - 文献号: 12450 文献类型: Article	共同通讯作者 (倒数第一)	B类			华南农业大学 动物科学学院	IF2-year=4.9 IF5-year=5.7 (2024)	生物学 3 区 Top 期刊: 否 (2025)

7	Selection and Regulatory Network Analysis of Differential CircRNAs in the Hypothalamus of Goats with High and Low Reproductive Capacity	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 出版年: 2024 出版日期: OCT 卷期: 25 19 页码: - 文献号: 10479 文献类型: Article	共同通讯作者 (倒数第一)	B类	华南农业大学 动物科学学院	SCI	IF2-year=4.9 IF5-year=5.7 (2024)	生物学 3区 Top 期刊: 否 (2025)
8	miR-128-3p Regulates Follicular Granulosa Cell Proliferation and Apoptosis by Targeting the Growth Hormone Secretagogue Receptor	INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 出版年: 2024 出版日期: MAR 卷期: 25 5 页码: - 文献号: 2320 文献类型: Article	共同通讯作者 (倒数第一)	B类	华南农业大学 动物科学学院	SCI	IF2-year=4.9 IF5-year=5.7 (2024)	生物学 3区 Top 期刊: 否 (2025)
9	Expression profile and bioinformatics analysis of circRNA and its associated ceRNA networks in longissimus dorsi from Lufeng cattle and Leiqiong cattle	BMC GENOMICS 出版年: 2023 出版日期: AUG 29 卷期: 24 1 页码: - 文献类型: Article	共同通讯作者	A类	华南农业大学 动物科学学院	SCI	IF2-year=3.5 IF5-year=4.1 (2023)	生物学 2区 Top 期刊: 是 (2023)
10	Transcriptome analysis reveals pituitary lncRNA, circRNA and mRNA affecting fertility in high- and low-yielding goats	FRONTIERS IN GENETICS 出版年: 2023 出版日期: DEC 12 卷期: 14 页码: -	共同通讯作者 (倒数第一)	B类	华南农业大学 动物科学学院	SCI	IF2-year=2.8 IF5-year=3.3 (2023)	生物学 3区 Top 期刊: 否 (2023)

	文献号: 1303031 文献类型: Article	通讯作者 B类						
11	Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats GENES 出版年: 2023 出版日期: FEB 卷期: 14 2 页码: - 文献号: 444 文献类型: Article	共同通讯作者 (倒数第一)	华南农业大学 动物科学学院	SCI	IF2-year=2.8 IF5-year=3.3 (2023)	生物学 3 区 Top 期刊: 否 (2023)		
12	Identification and Comparative Analysis of Long Non-coding RNAs in High- and Low-Fecundity Goat Ovaries During Estrus FRONTIERS IN GENETICS 出版年: 2021 出版日期: JUN 23 卷期: 12 页码: - 文献号: 648158 文献类型: Article	共同通讯作者 (倒数第一)	华南农业大学 动物科学学院	SCI	IF2-year=4.772 IF5-year=4.933 (2021)	生物学 3 区 Top 期刊: 否 (2021)		
13	湖羊和川中黑山羊在华南地区适应性研究 华南农业大学学报 出版年: 2016 卷期: 37 5 页码: - 文献号: 文献类型:	通讯作者 B类	华南农业大学 动物科学学院	北大核心	无	无		
14	华南地区舍饲川中黑山羊与雷州山羊的生殖繁殖性能及死亡状况研究 中国畜牧杂志 出版年: 2021 卷期: 57 02 页码: -	通讯作者 C类	华南农业大学 动物科学学院	北大核心	无	无		

		文献号: 文献类型:							
15	牛血清蛋白及大豆卵磷脂对山羊精子保存 效果的影响	中国畜牧杂志 出版年: 2020 卷期: 56 05 页码: - 文献号: 文献类型:	通讯作者	C类	华南农业大学 动物科学学院	北大核心	无	无	
16	湖羊和川中黑山羊 GDF9、BMPR-IB、 GnRHR 基因多态性及其与产羔数的关联分 析	黑龙江畜牧兽医 出版年: 2018 卷期: 15 页码: 文献号: 文献类型:	通讯作者	C类	华南农业大学 动物科学学院	北大核心	无	无	
17	华南地区不同耐热性荷斯坦奶牛血液生化 指标比较	黑龙江畜牧兽医 出版年: 2018 卷期: 03 页码: - 文献号: 文献类型:	通讯作者	C类	华南农业大学 动物科学学院	北大核心	无	无	

说明: 论文等级和中科院大类分区按《华南农业大学学位论文评价方案(试行)》划分。

报告免责声明: 如未盖章, 报告无效



ISSN 0378-1119

Volume 742

5 June 2020



GENE



CONTENTS

(Abstracted / Indexed in: BIOSIS, Chemical Abstracts, Current Contents / Life Sciences, EMBASE, EMBiology, MEDLINE®, Pascal et Francis (INST-CNRS), Reference Update. Also covered in the abstract and citation database Scopus®. Full text available on ScienceDirect®)

Review papers

Epigenetic modifications in gastric cancer: Focus on DNA methylation

Ebrahimi, V., Soleimanian, A., Ebrahimi, T., Azargun, R., Yazdani, P., Eyvazi, S. and Tarhriz, V.

Research Papers

Sophoridine inhibits lung cancer cell growth and enhances cisplatin sensitivity through activation of the p53 and Hippo signaling pathways

Zhu, L., Huang, S., Li, J., Chen, J., Yao, Y., Li, L., Guo, H., Xiang, X., Deng, J. and Xiong, J.

Let-7b-5p promotes electroacupuncture tolerance by downregulating *Penk1* gene in CFA-induced inflammatory nociception rats

Zhang, Q., Liu, A., Abouelfetouh, M.M., Ma, N., Li, M., Chen, S., Ding, M. and Ding, Y.

Association of 5'UTR polymorphism of secretory phospholipase A2 group IIA (PLA2G2A) gene with prostate cancer metastasis

Ozturk, K., Onal, M.S., Efiloglu, O., Nikerel, E., Yildirim, A. and Telci, D.

New age-related hearing loss candidate genes in humans: an ongoing challenge

Di Stazio, M., Morgan, A., Brumat, M., Bassani, S., Dell'Orco, D., Marino, V., Garagnani, P., Giuliani, C., Gasparini, P. and Giroto, G.

DDC expression is not regulated by NFAT5 (TonEBP) in dopaminergic neural cell lines

Pineda-Cirera, L., Cabana-Domínguez, J., Benetó, N., Díez, H., Arenas, C., Cormand, B. and Fernández-Castillo, N.

PNPLA3 rs738409 is not associated with the risk of hepatocellular carcinoma and persistent infection of hepatitis B virus (HBV) in HBV-related subjects: A case-control study and meta-analysis on Asians

Wang, P., Wu, C., Li, Y., Gong, Y. and Shen, N.

Identification and expression profiling of sugar transporter genes during sugar accumulation at different stages of fruit development in apricot

Iqbal, S., Ni, X., Bilal, M.S., Shi, T., Khalil-ur-Rehman, M., Zhenpeng, P., Jie, G., Usman, M. and Gao, Z.

144577 Codon usage bias in radioresistant bacteria

Dilucca, M., Pavlopoulou, A., Georgakilas, A.G. and Giansanti, A.

144584

Involvement of tumor necrosis factor- α , interferon gamma- γ , and interleukins 1 β , 6, and 10 in immunosuppression due to long-term exposure to five common food preservatives in rats

144556

Abd-Elhakim, Y.M., Hashem, M.M.M., Abo-EL-Sooud, K., Ali, H.A., Anwar, A., El-Metwally, A.E., Mahmoud, E.A. and Moustafa, G.G.

144590

Transcriptome profiling of lncRNA related to fat tissues of Qinchuan cattle

144583

Jiang, R., Li, H., Huang, Y., Lan, X., Lei, C. and Chen, H.

144587

Overexpressing *Jatropha curcas* CBF2 in *Nicotiana benthamiana* improved plant tolerance to drought stress

144589

Wang, L., Wu, Y., Tian, Y., Dai, T., Xie, G., Xu, Y. and Chen, F.

144588

Effects of chronic heat stress on mRNA and miRNA expressions in dairy cows

144561

Liu, G., Liao, Y., Sun, B., Guo, Y., Deng, M., Li, Y. and Liu, D.

144550

Spatial and temporal expression pattern of sex-related genes in ovo-testis of the self-fertilizing mangrove killifish (*Kryptolebias marmoratus*)

144569

Qu, M., Ding, S., Scharf, M. and Adolphi, M.C.

144581

Bioinformatics analysis reveals novel core genes associated with nonalcoholic fatty liver disease and nonalcoholic steatohepatitis

144585

Feng, G., Li, X.-P., Niu, C.-Y., Liu, M.-L., Yan, Q.-q., Fan, L.-P., Li, Y., Zhang, K.-L., Gao, J., Qian, M.-R., He, N. and Mi, M.

144549

contents continued on inside back cover



ELSEVIER



0378-1119(20200605)742:C;1-6

Available online at www.sciencedirect.com

ScienceDirect

Genetic compensation of γ CaMKII, an evolutionarily conserved gene Rothschild, S.C., Ingram, S.R., Lu, F.-I., Thisse, B., Thisse, C., Parkerson, J.A. and Tombes, R.M.	144567	Short Communications	
The neuroprotective effect of NeuroAid on morphine-induced amnesia with respect to the expression of TFAM, PGC-1 α , Δ fosB and CART genes in the hippocampus of male Wistar rats Malboosi, N., Nasehi, M., Hashemi, M., Vaseghi, S. and Zarrindast, M.-R.	144601	Genome sequence of the fungus <i>Pycnoporus sanguineus</i> , which produces cinnabarinic acid and pH- and thermo- stable laccases Lin, W., Jia, G., Sun, H., Sun, T. and Hou, D.	144586
Novel Nile tilapia Neu1 sialidases: Molecular cloning and biochemical characterization of the sialidases Neu1a and Neu1b Honda, A., Chigwechokha, P.K., Takase, R., Hayasaka, O., Fujimura, K., Kotani, T., Komatsu, M. and Shiozaki, K.	144538	A novel pathogenic variant in <i>MYO18B</i> associating early-onset muscular hypotonia, and characteristic dysmorphic features, delineation of the phenotypic spectrum of <i>MYO18B</i> -related conditions Brunet, T., Westphal, D.S., Weber, S., Juenger, H., Vlaho, S., Hoefele, J., Meitinger, T., Ricger-Fackeldecy, E. and Wagner, M.	144542
Transcriptomic response to cold of thermophilous medicinal plant <i>Marsdenia tenacissima</i> Long, G., Zhao, C., Zhao, P., Zhou, C., Ntirenganya, E. and Zhou, Y.	144602	Errata	
Genomic landscape of transcriptionally active histone arginine methylation marks, H3R2me2s and H4R3me2a, relative to nucleosome depleted regions Beacon, T.H., Xu, W. and Davie, J.R.	144593	Erratum to "Genome-wide identification and expression analysis of the superoxide dismutase (<i>SOD</i>) gene family in <i>Salvia miltiorrhiza</i> " [Gene 742C (2020) 144603] Han, L.-m., Hua, W.-p., Cao, X.-y., Yan, J.-a., Chen, C. and Wang, Z.-z.	144639
Genome-wide identification and expression analysis of the superoxide dismutase (<i>SOD</i>) gene family in <i>Salvia miltiorrhiza</i> Han, L.-m., Hua, W.-p., Cao, X.-y., Yan, J.-a., Chen, C. and Wang, Z.-z.	144603		



Research paper

Effects of chronic heat stress on mRNA and miRNA expressions in dairy cows

Guangbin Liu^{a,1}, Yingxin Liao^{a,b,1}, Baoli Sun^a, Yongqing Guo^a, Ming Deng^a, Yaokun Li^{a,*}, Dewu Liu^{a,*}^a College of Animal Science, South China Agricultural University, Guangzhou 510642, China^b Guangdong Maoming Agriculture & Forestry Technical College, Maoming 525000, China

ARTICLE INFO

Keywords:

Heat stress
Expression profiles
Holstein
Blood
High-throughput sequencing

ABSTRACT

Heat stress has a negative impact on dairy cow productivity. In order to reveal the mechanisms of heat-stress response, the mRNA and miRNA expression profiles of five cows under chronic heat-stress and thermoneutral conditions were assayed in blood by high-throughput sequencing technology. A total of 540 mRNAs and 9 miRNAs were expressed differently under heat-stress and thermoneutral conditions. Functional analyses revealed that MAPK signaling pathway, cellular senescence, circadian entrainment, aldosterone synthesis and secretion, and pathways in cancer were enriched for differently expressed mRNAs; meanwhile cGMP-PKG signaling pathway, thermogenesis, and protein digestion and absorption were enriched for differently expressed miRNAs. In addition, *GADD45G*, *TGFB2*, and *GNG11* may play roles in the heat stress, and bta-miR-423-5p might be one of the regulators of heat-stress response in cows as potential mediators of chronic heat-stress response. In conclusion, the present study described the mRNA and miRNA expression patterns in blood extracted from cows during the transition from heat-stress to thermoneutral conditions. The results provide new data that could help in identifying mechanisms that mediate cows' response to chronic heat stress.

1. Introduction

Elevated environmental temperatures lead to heat stress, a condition that is triggered when the animal body temperature exceeds the upper threshold of the thermoneutral zone (Belhadj Slimen et al., 2016). Heat stress has a huge impact on many aspects of animal biology. It not only reduces productivity but also influences physiological and molecular processes. Thus, heat stress has become a major concern because it causes large financial losses to dairy enterprises (Bernabucci et al., 2010; Polsky and von Keyserlingk, 2017; Tao et al., 2018; Schüller et al., 2014).

Cows exhibit different responses to acute and chronic heat stress, such as increased protein catabolism and reduced protein synthesis; meanwhile, chronic heat stress decreases protein catabolism and synthesis (Tabiri et al., 2000; Temim et al., 2000). Moreover, acute and chronic heat stress exert different influences on carbohydrate metabolism and heat-shock protein (HSP) generation (Bharati et al., 2017; Deb et al., 2014; O'Brien et al., 2010). Research over last the few years has

identified some of the physiological, metabolic, cellular, and molecular responses to heat stress. Recently, many studies have reported changes in genes when cows undergo heat stress (Sengar et al., 2018; Li et al., 2018; Min et al., 2015; Srikanth et al., 2017), but few reports have focused on the molecular differences between chronic heat-stress and thermoneutral conditions. Comparing the gene expression profiles of cows during chronic heat-stress and thermoneutral conditions might lead to a better understanding of mechanisms regulating the heat-stress response.

The present study was conducted in Guangdong Province, China, located at 23° N and 113° E, and characterized by a long period of moderate heat stress during the summer. Specifically, the average ambient temperature (AT) exceeded 30 °C and the temperature–humidity index (THI) exceeded 80 for approximately 4 months (De, 2016). In the present study, we investigated the mRNA and miRNA expression profiles in blood samples extracted from cows, with the goal of identifying genes involved in chronic heat-stress regulation.

Abbreviations: THI, Temperature humidity index; TMR, Total mixed rations; RT, Rectal temperature; RPM, Reads per million mapped reads; FPKM, Fragments per kilobase of transcript per million mapped reads; BP, Biological process ontology; CC, Cellular component ontology; MF, Molecular function ontology

* Corresponding authors.

E-mail addresses: gblu@scau.edu.cn (G. Liu), yingxinliao1992@outlook.com (Y. Liao), baolisun@scau.edu.cn (B. Sun), yongqing@scau.edu.cn (Y. Guo), dengming@scau.edu.cn (M. Deng), ykli@scau.edu.cn (Y. Li), dwliu@scau.edu.cn (D. Liu).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.gene.2020.144550>

Received 2 September 2019; Received in revised form 20 January 2020; Accepted 8 March 2020

Available online 09 March 2020

0378-1119/ © 2020 Published by Elsevier B.V.

2. Materials and methods

2.1. Ethical statement

The study was approved by the Ethics Committees of the Laboratory Animal Center of South China Agricultural University (Permit Number: SYXK-2014-0136). All experiments were performed in accordance with the South China Agricultural University guidelines.

2.2. Animals and sample collection

Five Holstein cows (female, healthy) kept in a commercial farm (Wens Foodstuff Group Incorporated Company, Guangdong, China) were selected. They were matched for age (4 years old), parity number (3), and calving time (April). The selected cows were fed with total mixed rations (TMR) throughout the year. The TMR of dairy cows included whole corn silage (22.8%), alfalfa hay (19.9%), steam-flaked corn (6.3%), whole cotton seed (10.6%), beet meal (2.2%), wet brewer's grains (5%), sugarcane molasses (1.6%), rumen protected fat (1.44%), and commercial concentrate supplement (30.1%) (DM basis); the nutritional composition was as follows: CP 18.22%, NE 6.99 MJ/kg, NDF 35.56%, ADF 23.38%, Ca 0.92%, and P 0.43%. Clean water was available *ad libitum*. All the five cows calved in April. The milk yield data were recorded each day. AT (°C), relative humidity (RH, %), and cow rectal temperature (RT, °C) were recorded in the summer (August) and winter (December). The environmental temperature humidity index (THI) is calculated as follows: $(1.8 \times AT + 32) \times (0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)$, where AT is the ambient temperature (°C) and RH is the relative humidity (%) (Oceanic and Administration, 1976). Blood samples were obtained from each selected cow at the same moment by coccygeal venipuncture in the summer (August) and winter (December), following the RT recording on the same day. Each blood sample was divided into two tubes. The first tube was centrifuged at $5000 \times g$ for 10 min to prepare the serum and analyzed for T3, T4, and Cortisol using the RIA kit (Beijing North Institute of Biological Technology, Beijing, China) and HSP70 using the ELISA kit (CUSABIO, Wuhan, Hubei, China). The significant tests were conducted with paired T-test. The second tube was mixed with RNALock blood RNA protection reagent (TIANGEN, Beijing, China), followed by the isolation of total RNAs using HiPure Blood RNA Mini Kit (Magen, Guangzhou, Guangdong, China).

2.3. Illumina sequencing and annotation

The mRNA and miRNA libraries were constructed for each sample (in total, 10 mRNA and 10 miRNA libraries) as suggested by Illumina (San Diego, California, USA) and sequenced on a HiSeq2000 platform by the BGI Company (Shenzhen, Guangdong, China). The raw reads were filtered to obtain clean reads following this procedure: (1) remove reads with adaptors; (2) remove reads in which unknown bases exceeded 10%; and (3) remove low quality reads whose percentage of low quality bases (sequencing quality lower than 5) exceeds 50%. After that, for mRNA, the clean reads were mapped to the reference gene and genome base on the NCBI database version UMD3.1 (Center for Bioinformatics and Computational Biology, University of Maryland, Bos taurus assembly Database version: 3.1) (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bos_taurus/) using Bowtie2 (Langmead and Salzberg, 2012) and HISAT (Kim et al., 2015) software. The alignment parameters for Bowtie2 were as follows: -q -phred64 -sensitive -dpad 0 -gbar 99999999 -mp 1,1 -np 1 -score-min L,0,-0.1 -p 16 -k 200; and for HISAT were: -p 8 -phred64 -sensitive -l 1 -X 1000. Then, the quantification of mRNA was performed using RSEM tool (Li and Dewey, 2011) with default parameters. The Fragments Per Kilobase of transcript per Million mapped reads (FPKM) method was used in calculating the mRNA expression level. For miRNAs, the clean reads were mapped to the reference genome (UMD3.1) using the SOAP2 software (Li et al.,

2009) with default parameters. Then, the mapped reads were aligned to the miRbase version 21 (Kozomara and Griffiths-Jones, 2014) (<http://www.mirbase.org/>) to identify the known miRNAs and Rfam database version 11 (<http://rfam.janelia.org/>) and to remove the rRNA, scRNA, snoRNA, snRNA, and tRNA associated reads. Finally, the miRNA expression levels were normalized by following the RPM (Reads Per Million mapped reads) method.

2.4. Differential expression and functional analyses

For differential expression gene screening, the quasi-likelihood F-test was performed using edgeR (McCarthy et al., 2012; Robinson et al., 2010) R package with criteria foldchange ≥ 2 and P -value < 0.01 (5 samples vs. 5 samples). The gene ontology (GO) analyses were performed by DAVID 6.8 (<https://david.ncifcrf.gov/>) with the following parameters: count = 2, EASE = 0.1 (Huang da et al., 2009; Huang da et al., 2009). The KEGG pathways analyses were performed by Cytoscape software (Shannon et al., 2003) with the ClueGO tool (Bindea et al., 2009) (Kappa Score = 0.4). RNAhybrid (Rehmsmeier et al., 2004); miRanda (Enright et al., 2003) and Targetscan (Agarwal and Bell, 2015) were used with default parameters to predict the miRNA target gene and target genes common to all three algorithms were selected for functional analysis.

For QPCR (qRT-PCR), the stem-loop method (Chen et al., 2005) was performed for the miRNA reverse transcription with PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, Liaoning, China). The QPCR assays were performed by SYBR Select Master Mix (ThermoFisher, Waltham, Massachusetts, USA) using 50 ng cDNA for each reaction. U6 small nuclear RNAs were used as the reference miRNAs. The information on primers can be found in Table S6. The annealing temperature of 60 °C was used for amplification. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level (Livak and Schmittgen, 2001).

3. Results

3.1. Cows featured distinct mRNA and miRNA expression patterns between summer and winter

3.1.1. Blood variable detection

The RT of 5 cows were recorded under the heat-stress condition in the summer (mid-August) and under thermoneutral condition in the winter (mid-December). Blood samples were collected following RT recording under both conditions. The AT and THI at the time of RT recording were 32.5 °C and 87.3 during the summer, and 11.0 °C and 52.3 during the winter, respectively (Fig. 1). The milk yields were 24.9 ± 6.2 kg/day in August and 22.3 ± 2.5 kg/day in December. There was no significant difference in milk yield between August and December ($P > 0.05$). However, there was a significant drop in the milk yield from July to August ($P < 0.05$). The RT and selected blood variables are reported in Table 1. As can be seen, RT was significantly increased in all cows in the summer ($P < 0.01$). Blood cortisol concentrations were significantly increased in all cows in the summer ($P < 0.01$), whereas Triiodothyronine (T3) and Thyroxine (T4) were decreased ($P < 0.05$). Blood HSP70 concentrations did not increase in the summer.

3.1.2. Identification of differentially expressed mRNAs and miRNAs by sequencing

Total RNAs were isolated from blood samples collected under thermal conditions and subjected to Illumina sequencing. After filtration, mapping, and annotation (Table 2), a total of 9019 mRNAs and 135 miRNAs were detected in blood (Table S1). To identify the mRNAs and miRNAs, which might play important roles in heat-stress response, mRNA and miRNA expression were compared between the summer (heat-stress) and winter (thermoneutral) months. This analysis revealed

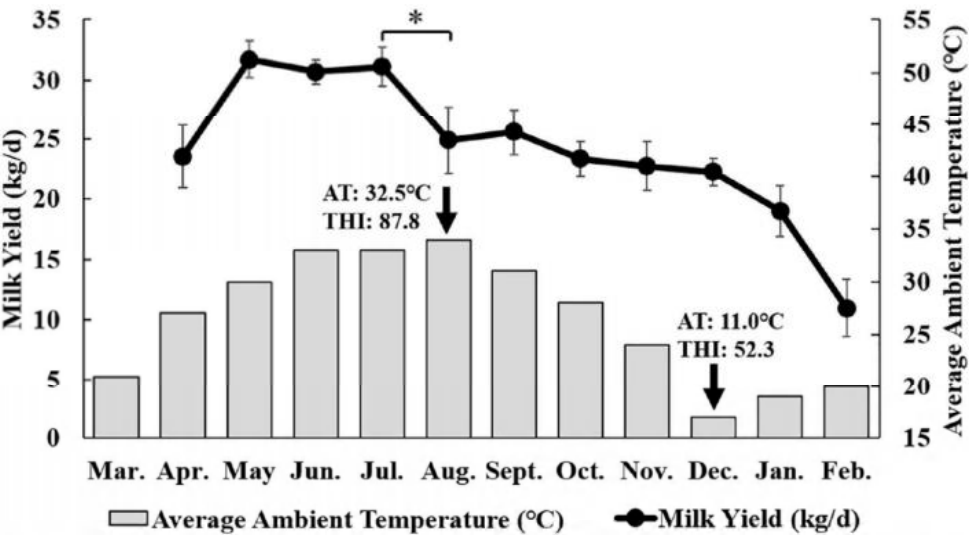


Fig. 1. Ambient temperature (AT) and milk yield of dairy cows. The monthly AT data are from the China Meteorological Administration. Arrows indicate the AT and THI of samples collected in the summer and winter. **P* < 0.05.

Table 1
Cow rectal temperature and blood parameters during the summer and winter months.

Items	Summer	Winter	P-values
Rectal temperature (°C)	39.54 ± 0.14	38.22 ± 0.10	< 0.01
T3 (ng/mL)	1.38 ± 0.12	2.66 ± 0.20	< 0.01
T4 (ng/mL)	71.35 ± 16.51	126.66 ± 9.33	0.02
Cortisol (ng/mL)	11.53 ± 0.74	3.98 ± 0.91	< 0.01
HSP70 (ng/mL)	1.40 ± 0.14	2.02 ± 0.03	0.02

T3, T4, Cortisol, and HSP70 were assayed in serum. Data came from n = 5 and presented as mean ± SE. The *P*-values were assessed by paired T-test.

differential expressions for 540 mRNAs (15 up-regulated and 525 down-regulated in summer) (Table 3 and Table S2) and 9 miRNAs (2 upregulated and 7 down-regulated) (Table 4). The expression levels of bta-miR-423-5p and bta-miR-451, which were up-regulated and down-regulated during the summer, respectively, were verified by QPCR and their expression patterns were consistent with the sequencing data (Fig. 2).

3.2. Functional analyses of differentially expressed mRNAs and miRNAs

3.2.1. Gene ontology and KEGG pathway analyses of differentially expressed mRNAs

To understand the basis for the heat-stress response, we subjected differentially expressed mRNAs to GO and KEGG pathway analyses. The most highly enriched GO terms were related to processes associated with transcription. Other highly enriched terms were “negative protein ubiquitination,” “regulation of cell proliferation,” “peptidyl-serine

Table 3
Differentially expressed mRNAs between the summer and winter months.

No.	GeneBank ID	Gene Symbol	mRNA Expression Level (RPKM)		Log2Ratio (Summer/ Winter)	P-values
			Summer	Winter		
Top 10 up-regulated mRNA in summer						
1	NM_205786	LOC404103*	838	408	1.04	9.65E-03
2	NM_001024523	GNG11	340	121	1.49	8.06E-04
3	NM_001075143	GNLY	290	144	1.01	3.56E-03
4	NM_001098930	MZB1	59	28	1.08	7.13E-03
5	NM_001077856	LY6G6C	33	16	1.04	4.61E-03
6	NM_001045901	GADD45G	24	11	1.13	8.22E-04
7	NM_001046098	LTC4S	18	9	1.00	6.21E-03
8	NM_001101268	FAM167B	14	7	1.00	2.41E-05
9	NM_001046535	BPMS	8	4	1.00	4.88E-03
10	NM_001046032	ISYNA1	4	2	1.00	6.86E-04
Top 10 down-regulated mRNA in summer						
1	NM_001077900	STAT1	64	136	-1.09	6.30E-04
2	NM_001192994	SF3B1	50	106	-1.08	1.89E-04
3	NM_001205381	CD1D	46	98	-1.09	4.41E-04
4	NM_001099106	ETS1	44	98	-1.16	2.57E-04
5	NM_001034650	CCNL2	44	89	-1.02	1.20E-04
6	NM_001102271	CLK1	41	84	-1.03	2.58E-03
7	NM_001098070	OGT	27	77	-1.51	7.14E-06
8	NM_001075621	SRSF11	27	74	-1.45	4.47E-05
9	NM_001193017	ZAP70	27	65	-1.27	8.34E-07
10	NM_001242585	PNN	29	62	-1.10	2.23E-04

*LOC404103 is *Bos taurus* spleen trypsin inhibitor; Data are from cow blood and n = 5; RPKM means Reads Per Kilobase of transcript per Million mapped reads. The *P*-values were assessed by quasi-likelihood F-test.

Table 2
The percentages of mapped and unmapped reads to the genome.

Cows	Summer			Winter		
	Total Reads	Total Mapped Reads (%)	Total Unmapped Reads (%)	Total Reads	Total Mapped Reads (%)	Total Unmapped Reads (%)
1	22479022	94.0	6.0	24015519	93.6	6.4
2	23928057	93.7	6.3	23912321	93.6	6.5
3	23896568	93.1	6.9	23837277	92.4	7.6
4	23965409	93.6	6.4	23917988	93.5	6.5
5	23893576	89.5	10.5	23810001	93.0	7.0

Table 4
Differentially expressed miRNAs between the summer and winter months.

No.	miRNA Name	miRNA Expression Level (RPM)		Log2Ratio (Summer/Winter)	P-values
		Summer	Winter		
Up-regulated miRNA in summer					
1	bta-miR-423-5p	3727	729	2.35	0.00E + 00
2	bta-miR-6529a	152	66	1.19	2.80E-03
3	bta-miR-320a	113	53	1.08	1.60E-03
4	bta-let-7b	97	27	1.87	1.00E-04
5	bta-miR-296-3p	78	14	2.48	0.00E + 00
6	bta-miR-744	10	4	1.24	8.50E-03
7	bta-miR-877	8	2	1.99	1.00E-04
Down-regulated miRNA in summer					
1	bta-miR-451	73	212	-1.54	2.40E-03
2	bta-miR-199b	0	3	-2.60	2.00E-04

Data were from cow blood and n = 5; RPM means Reads Per Million mapped reads. The P-values were assessed by quasi-likelihood F-test.

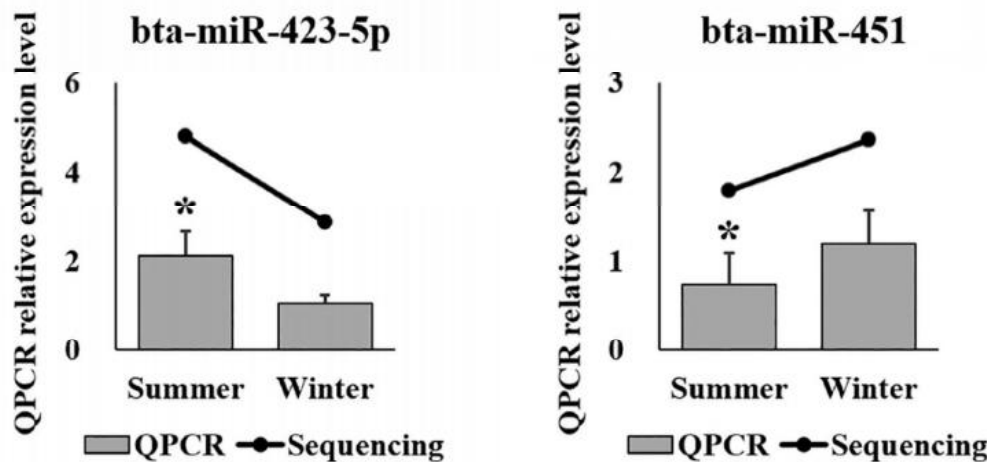


Fig. 2. Verification of miRNA expression patterns by QPCR. QPCR results are presented as columns, and sequencing data are presented as linear graphs. * $P < 0.05$.

phosphorylation,” and “regulation of apoptotic process” (Fig. 3 and Table S3). KEGG analyses showed that the “MAPK signaling pathway,” “cellular senescence,” “circadian entrainment,” “aldosterone synthesis and secretion,” and “pathways in cancer” were enriched (Fig. 4).

3.2.2. Prediction of miRNA target genes and functional analyses

The functional significance of differentially regulated miRNAs was assessed as follows: candidate mRNA targets were identified for each differentially expressed miRNA by bioinformatics, followed by their evaluation by GO and KEGG analyses. A total of 1266 potential target mRNAs was obtained (Table S4). The GO terms relating to “regulation of transcription” and “cell adhesion” and “cell proliferation” were highly enriched (Table S5). Notably, “extracellular space” was the most highly enriched term for this miRNA subset when using cellular component as the classification component, whereas it was “nucleus” for differentially expressed mRNAs (Table S3). Three pathways were identified by KEGG analyses, including the “cGMP-PKG signaling pathway,” “thermogenesis,” and “protein digestion and absorption” pathways (Fig. 5).

3.2.3. Co-analyses of differently expressed mRNAs and miRNAs

Finally, we performed co-analyses of differently expressed mRNAs and miRNAs to identify the interactions between them in the present data. Four differentially expressed miRNAs were found to be potential targets to eight mRNAs, and their change directions of their expression levels from summer to winter were the opposite (Table 5).

4. Discussion

THI is an important environment parameter for assessing heat-stress condition for dairy cows. The thermal comfort zone occurs at THI < 72.

Cows experiencing mild (THI = 72–79) to moderate heat stress (THI = 80–89) suffer from reduced milk production, whereas exposure to severe heat stress (THI > 90) has deleterious health effects and can even cause death (Hagiya et al., 2017; Armstrong, 1994; De Rensis et al., 2015). In the present work, blood samples were taken in summer when cows were under moderate heat-stress (THI = 87.8) and in winter when cows were under thermoneutral condition (THI = 52.3). This is supported by the elevated RT (39.54 °C) and declined milk yield (Fig. 1) in the summer. We also observed a decline in concentrations of plasma triiodothyronine (T3) and thyroxine (T4) in the summer and an increase in the plasma cortisol level (Table 1). Overall, these data indicate that cows suffer from heat stress in summer but not in the winter (Weitzel et al., 2017; Rees and Fischer-Tenhagen, 2016; Hao et al., 2014). In contrast, we did not find increased expression or serum levels for the heat shock protein HSP70 in the summer. HSPs are heat-shock proteins that play important roles in cellular stress resistance and damage repair. An increased HSP70 level can be observed in the early stage of acute heat-stress and are presumed to protect animals and cells from heat-induced injuries (Min et al., 2015; Hu et al., 2016). Nevertheless, the expression of HSP70 level has been shown to vary dynamically during chronic heat stress in Tharparkar cattle (Bharail et al., 2017), suggesting that the HSP70 expression patterns varied between acute and chronic heat-stress conditions. Another study reported increased HSP70 level was recovery within 6 h of heat stress in cattle peripheral blood mononuclear cells (PBMCs) (Bhanuprakash et al., 2016), indicating that HSP70 may not be elevated during the chronic heat stress. Consistent with this, the cows in our study were exposed to heat stress from May (THI > 72) to August (Sample collection, THI = 87.9) and serum HSP70 levels did not increase during this long heat-stress period.

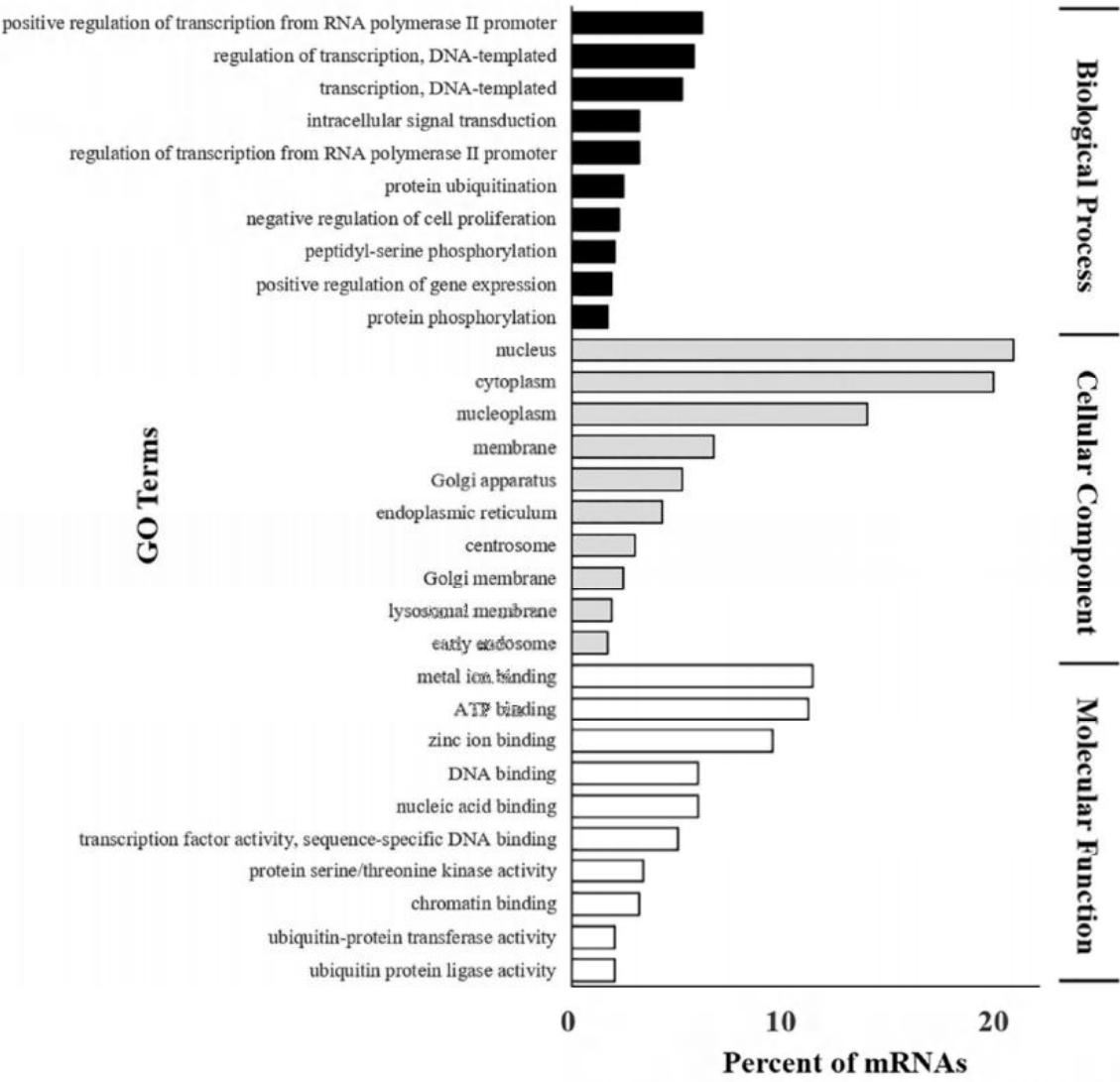


Fig. 3. Gene ontology analyses of differentially expressed mRNAs between summer and winter. Classifications include biological process ontology, cellular component ontology and molecular function ontology.

Heat stress has a huge impact on metabolic processes (Lu et al., 2007; Ain Baziz et al., 1996; Geraert et al., 1996) and can also cause cell damage. It induces oxidative stress in animals by enhancing the production of reactive oxygen species (ROS), resulting in oxidative damage and cell apoptosis (Li et al., 2018). In the present study, 540 mRNAs were expressed differently under heat-stress and thermoneutral conditions. The KEGG analyses results showed that the following pathways: MAPK signaling pathway, cellular senescence, circadian entrainment, aldosterone synthesis and secretion, and pathways in cancer were enriched for differently expressed mRNAs (Fig. 4). The functions of these pathways were related to cell survival, apoptosis, and stress response. Of these, the MAPK signaling pathway is believed to play a critical role in the response to stress stimuli (Sui et al., 2014; Nlaudet et al., 2017), including mediation of heat stress-induced cell death (Liu et al., 2016). A report showed that the inhibition of the p38-MAPK signaling pathway decreased heat stress-induced ROS generation and cell apoptosis (Li et al., 2018). In the present study, we found that the expression levels of *GADD45G*, *KRAS*, *PPP3CA*, *TGFB1*, *TGFB2*, *RASGRP1*, *RPS6KA5*, and *PRKACB*, which belong to the MAPK signaling pathway and intersect with other pathways, were significantly changed during heat-stress and thermoneutral conditions, indicating that these genes and pathways are involved in the regulation of cell heat-stress response. Notably, *GADD45G* and *TGFB2* were found to be upregulated in heat stress condition.

It has been reported that *GADD45G* served as a stress-response gene

and that it inhibited cell growth and induced apoptosis in stress shock (Ying et al., 2005). Meanwhile, *TGFB2* served as a key factor in the cold-inducible RNA-binding protein (CIRBP) regulation of heat shock-induced testicular injury. In the present study, the upregulated expression levels of these two genes in heat stress suggest that they may also play important roles in heat-stress regulation in cows. Moreover, there were some other important genes that intersected multiple signaling pathways (cellular senescence, circadian entrainment, aldosterone synthesis and secretion, and pathways in cancer), including *GNG11*, *MDM2*, *ETS1*, *GNB4*, *CAMK2D*, and *ADCY7* (Fig. 4). They also showed differential expression levels during heat-stress and thermoneutral conditions (Table S2). Among these, *GNG11* has been shown to increase ROS generation and suppressed growth in human SUSM-1 cells in a previous study (Takauji et al., 2017). ROS has also been reported to cause oxidative damage and cell apoptosis, which could be introduced by heat stress. In the present study, the expression level of *GNG11* was upregulated in heat stress, indicating that *GNG11* was involved in heat-stress response in cows.

In addition, we also found that nine miRNAs were expressed differently during heat-stress and thermoneutral conditions in the present study (Table 4). The KEGG analyses results showed that the cGMP-PKG signaling pathway, thermogenesis, and protein digestion and absorption pathways were enriched for differently expressed miRNAs (Fig. 5). It has been previously reported that cyclic GMP (cGMP)-dependent protein kinase (PKG) is an important signaling for stress adaptation in

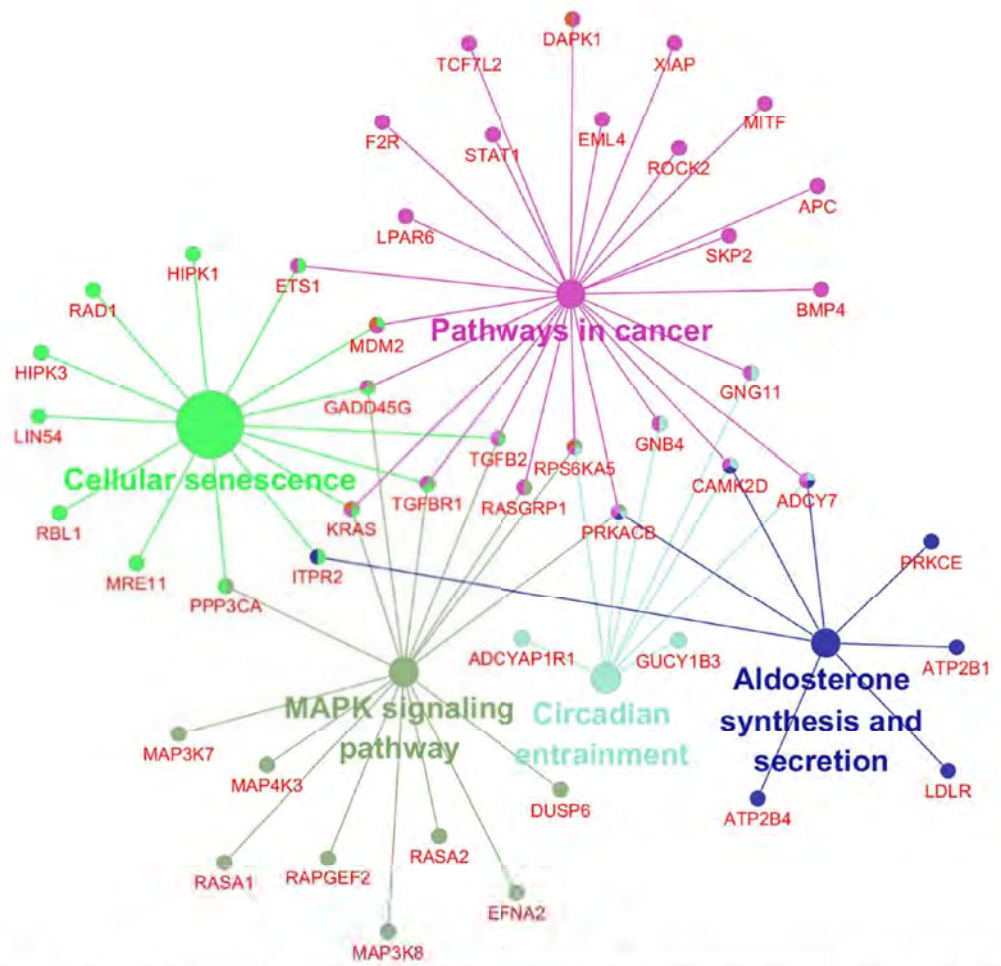


Fig. 4. KEGG pathway analyses of differentially expressed mRNAs. The network was created by cystoscope software with the ClueGO tool.

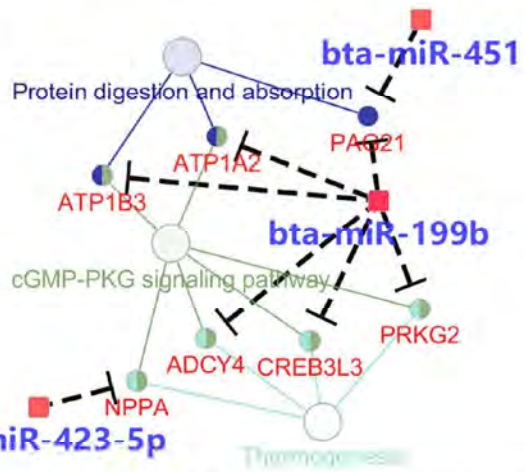


Fig. 5. KEGG pathway analyses of differentially expressed miRNAs. miRNA target genes were predicted by RNAhybrid, miRanda, and Targetscan. The target genes common to all three algorithms were selected for KEGG pathway analysis. The network was created by cystoscope software with the ClueGO tool.

the heart (Chai et al., 2011). It is also involved in the regulation of ROS generation (Ferreira et al., 2015). In the present study, the cGMP-PKG signaling pathway was enriched in cows under heat stress, suggesting that the cGMP-PKG signaling pathway may play an important role in heat-stress response.

Moreover, we found that bta-miR-423-5p could potentially target *NPPA*, a member of the cGMP-PKG signaling and thermogenesis pathways. A previous study reported that *NPPA* is an important factor in the regulation of heart development and stress response (Sergeeva et al., 2016). As a potential inhibitor of *NPPA*, the expression level of bta-miR-423-5p in the current study was significantly upregulated in cows under heat stress, indicating that bta-miR-423-5p might be a regulator of heat-stress response in cows. In addition, bta-miR-451 and bta-miR-199b were also found to potentially target the cGMP-PKG signaling, thermogenesis, and protein digestion and absorption pathways. Moreover, we performed a co-analyses of differently expressed genes and miRNAs and found that the change directions of 4 miRNAs' expression levels were opposite those of the mRNAs (Table 5). As an example, bta-miR-423-5p could potentially target *WIPF2*. miR-423-5p has also been shown to inhibit the proliferation and invasion of tumor (Wang et al., 2018; Tang et al., 2018). However, the target genes from the co-

Table 5
Co-analyses of differentially expressed mRNAs and miRNAs.

miRNAs	Expression level change direction (Summer -Winter)	Target mRNAs	Expression level change direction (Summer-Winter)
bta-miR-423-5p	Down	WIPF2	Up
bta-miR-296-3p	Down	DHX33	Up
bta-miR-199b	Up	ANGPTL6	Down
bta-miR-744	Down	USP42; CRAMP1; PDPR; NEDD9; CD1D	Up

analyses could not be enriched in the KEGG pathway. The functions of these miRNA and mRNA require further study.

5. Conclusions

In conclusion, the present study identified differently expressed mRNAs and miRNAs in blood from cows during the transition from heat-stress to thermoneutral conditions. The functional analyses showed that the MAPK signaling pathway, cellular senescence, circadian entrainment, aldosterone synthesis and secretion, and pathways in cancer were all enriched for differently expressed mRNAs and that the cGMP-PKG signaling pathway, thermogenesis, and protein digestion and absorption were enriched for miRNAs. Moreover, *GADD45G*, *TGFB2*, and *GNG11* may play roles under heat stress, and bta-miR-423-5p might be one of the regulators of heat-stress response in cows.

Funding

This research was funded by the National Natural Science Foundation of China (Grant Numbers 31872382 and 31501982) and by the Science and Technology Planning Project of Guangdong Province (Grant Numbers 2017B020201014 and 2017A020208050).

CRedit authorship contribution statement

Guangbin Liu: Conceptualization, Formal analysis, Writing - review & editing. **Yingxin Liao:** Formal analysis, Investigation, Writing - original draft. **Baoli Sun:** Investigation. **Yongqing Guo:** Investigation. **Ming Deng:** Resources. **Yaokun Li:** Project administration. **Dewu Liu:** Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank Professor Yves Boisclair (Department of Animal, Cornell University, Ithaca, USA) for editing the manuscript. We also thank WENS Company for their assistance in conducting our experiment (Guangdong, China).

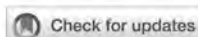
Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gene.2020.144550>.

References

- Agarwal, V., Bell, G.W., Nam, J.W., Bartel, D.P., 2015. Predicting effective microRNA target sites in mammalian mRNAs. *eLife* 4.
- Ain Baziz, H., Geraert, P.A., Padilha, J.C., Guillaumin, S., 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. *Poult. Sci.* 75 (4), 505–513.
- Armstrong, D.V., 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.* 77 (7), 2044–2050.
- Belhadj Slimen, I., Najjar, T., Ghram, A., Abdrrabba, M., 2016. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. *J. Anim. Physiol. Anim. Nutr.* 100 (3), 401–412.
- Bernabucci, U., Lacetera, N., Baumgard, L.H., Rhoads, R.P., Ronchi, B., Nardone, A., 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Anim. Int. J. Anim. Biosci.* 4 (7), 1167–1183.
- Bhanuprakash, V., Singh, U., Sengar, G., Sajjanar, B., Bhusan, B., Raja, T.V., Alex, R., Kumar, S., Singh, R., Ashish, K., et al., 2016. Differential effect of thermal stress on HSP70 expression, nitric oxide production and cell proliferation among native and crossbred dairy cattle. *J. Therm. Biol.* 59, 18–25.
- Bharati, J., Dangi, S.S., Chouhan, V.S., Mishra, S.R., Bharti, M.K., Verma, V., Shankar, O., Yadav, V.P., Das, K., Paul, A., et al., 2017. Expression dynamics of HSP70 during chronic heat stress in Tharparkar cattle. *Int. J. Biometeorol.* 61 (6), 1017–1027.
- Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W.H., Pages, F., Trajanoski, Z., Galon, J., 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics (Oxford, England)* 25 (8), 1091–1093.
- Chai, Y., Zhang, D.M., Lin, Y.F., 2011. Activation of cGMP-dependent protein kinase stimulates cardiac ATP-sensitive potassium channels via a ROS/calmodulin/CaMKII signaling cascade. *PLoS One* 6 (3), e18191.
- Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., et al., 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res.* 33 (20), e179.
- De, Zhou, Liu, D., Liao, Y., Liu, G., 2016. Study on adaptabilities of Hu sheep and Chuansong black goat in Southern China. *J. South China Agric. Univ.* 37 (5), 19–23.
- De Rensis, F., Garcia-Isperto, I., Lopez-Gatius, F., 2015. Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows. *Theriogenology* 84 (5), 659–666.
- Deb, R., Sajjanar, B., Singh, U., Kumar, S., Singh, R., Sengar, G., Sharma, A., 2014. Effect of heat stress on the expression profile of Hsp90 among Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* × *Bos taurus*) breed of cattle: a comparative study. *Gene* 536 (2), 435–440.
- Enright, A.J., John, B., Gaul, U., Tuschl, T., Sander, C., Marks, D.S., 2003. MicroRNA targets in *Drosophila*. *Genome Biol.* 5 (1), R1.
- Ferreira, R., Wong, R., Schlichter, L.C., 2015. KCa3.1/IK1 Channel Regulation by cGMP-Dependent Protein Kinase (PKG) via Reactive Oxygen Species and CaMKII in Microglia: An Immune Modulating Feedback System? *Front. Immunol.* 6, 153.
- Geraert, P.A., Padilha, J.C., Guillaumin, S., 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *Br. J. Nutr.* 75 (2), 195–204.
- Hagiya, K., Hayasaka, K., Yamazaki, T., Shirai, T., Osawa, T., Terawaki, Y., Nagamine, Y., Masuda, Y., Suzuki, M., 2017. Effects of heat stress on production, somatic cell score and conception rate in Holsteins. *Anim. Sci. J. = Nihon chikusan Gakkaiho* 88 (1), 3–10.
- Hao, Y., Feng, Y., Yang, P., Feng, J., Lin, H., Gu, X., 2014. Nutritional and physiological responses of finishing pigs exposed to a permanent heat exposure during three weeks. *Arch. Anim. Nutr.* 68 (4), 296–308.
- Hu, H., Zhang, Y., Zheng, N., Cheng, J., Wang, J., 2016. The effect of heat stress on gene expression and synthesis of heat-shock and milk proteins in bovine mammary epithelial cells. *Anim. Sci. J. = Nihon chikusan Gakkaiho* 87 (1), 84–91.
- Huang da, W., Sherman, B.T., Lempicki, R.A., 2009. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucl. Acids Res.* 37 (1), 1–13.
- Huang da, W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4 (1), 44–57.
- Kim, D., Langmead, B., Salzberg, S.L., 2015. HISAT: a fast spliced aligner with low memory requirements. *Nat. Meth.* 12 (4), 357–360.
- Kozomara, A., Griffiths-Jones, S., 2014. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucl. Acids Res.* 42 (Database issue), D68–73.
- Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Meth.* 9 (4), 357–359.
- Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinf.* 12, 323.
- Li, H., Liu, Y., Gu, Z., Li, L., Liu, Y., Wang, L., Su, L., 2018. p38 MAPK-MK2 pathway regulates the heat-stress-induced accumulation of reactive oxygen species that mediates apoptotic cell death in glial cells. *Oncol. Lett.* 15 (1), 775–782.
- Li, Q., Yang, C., Du, J., Zhang, B., He, Y., Hu, Q., Li, M., Zhang, Y., Wang, C., Zhong, J., 2018. Characterization of miRNA profiles in the mammary tissue of dairy cattle in response to heat stress. *BMC Genom.* 19 (1), 975.
- Li, R., Yu, C., Li, Y., Lam, T.W., Yiu, S.M., Kristiansen, K., Wang, J., 2009. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics (Oxford, England)* 25 (15), 1966–1967.
- Liu, Z.F., Zheng, D., Fan, G.C., Peng, T., Su, L., 2016. Heat stress prevents lipopolysaccharide-induced apoptosis in pulmonary microvascular endothelial cells by blocking calpain/p38 MAPK signalling. *Apoptosis* 21 (8), 896–904.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} Method. *Methods (San Diego, Calif)* 25 (4), 402–408.
- Lu, Q., Wen, J., Zhang, H., 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult. Sci.* 86 (6), 1059–1064.
- McCarthy, D.J., Chen, Y., Smyth, G.K., 2012. Differential expression analysis of multi-factor RNA-Seq experiments with respect to biological variation. *Nucl. Acids Res.* 40 (10), 4288–4297.
- Min, L., Cheng, J.B., Shi, B.L., Yang, H.J., Zheng, N., Wang, J.Q., 2015. Effects of heat stress on serum insulin, adipokines, AMP-activated protein kinase, and heat shock signal molecules in dairy cows. *J. Zhejiang Univ. Sci. B* 16 (6), 541–548.
- Niaudet, C., Bonnaud, S., Guillonnet, M., Gouard, S., Gaugler, M.H., Dutoit, S., Ripoche, N., Dubois, N., Trichet, V., Corre, I., et al., 2017. Plasma membrane reorganization links acid sphingomyelinase/ceramide to p38 MAPK pathways in endothelial cells apoptosis. *Cell. Signal.* 33, 10–21.
- O'Brien, M.D., Rhoads, R.P., Sanders, S.R., Duff, G.C., Baumgard, L.H., 2010. Metabolic adaptations to heat stress in growing cattle. *Domest. Anim. Endocrinol.* 38 (2), 86–94.
- Oceanic, N., Administration, A., 1976. *Livestock Hot Weather Stress*. Printing Office Washington, DC.
- Polsky, L., von Keyserlingk, M.A.G., 2017. Invited review: Effects of heat stress on dairy cattle welfare. *J. Dairy Sci.* 100 (11), 8645–8657.
- Rees, A., Fischer-Tenhagen, C., Heuwieser, W., 2016. Effect of heat stress on

- concentrations of faecal cortisol metabolites in dairy cows. *Reprod. Domestic Anim.* = *Zuchthygiene* 51 (3), 392–399.
- Rehmsmeier, M., Steffen, P., Hochsmann, M., Giegerich, R., 2004. Fast and effective prediction of microRNA/target duplexes. *RNA* (New York, NY) 10 (10), 1507–1517.
- Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* (Oxford, England) 26 (1), 139–140.
- Schüller, L., Burfeind, O., Heuwieser, W., 2014. Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices. *Theriogenology* 81 (8), 1050–1057.
- Sengar, G.S., Deb, R., Singh, U., Junghare, V., Hazra, S., Raja, T.V., Alex, R., Kumar, A., Alyethodi, R.R., Kant, R., et al., 2018. Identification of differentially expressed microRNAs in Sahiwal (*Bos indicus*) breed of cattle during thermal stress. *Cell Stress Chaperones* 23 (5), 1019–1032.
- Sergeeva, I.A., Hooijkaas, I.B., Ruljter, J.M., van der Made, L., de Groot, N.E., van de Werken, H.J., Creemers, E.E., Christoffels, V.M., 2016. Identification of a regulatory domain controlling the Nppa-Nppb gene cluster during heart development and stress. *Development* (Cambridge, England) 143 (12), 2135–2146.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13 (11), 2498–2504.
- Srikanth, K., Kwon, A., Lee, E., Chung, H., 2017. Characterization of genes and pathways that respond to heat stress in Holstein calves through transcriptome analysis. *Cell Stress Chaperones* 22 (1), 29–42.
- Sui, X., Kong, N., Ye, L., Han, W., Zhou, J., Zhang, Q., He, C., Pan, H., 2014. p38 and JNK MAPK pathways control the balance of apoptosis and autophagy in response to chemotherapeutic agents. *Cancer Lett.* 344 (2), 174–179.
- Tabiri, H.Y., Sato, K., Takahashi, K., Toyomizu, M., Akiba, Y., 2000. Effect of acute heat stress on plasma amino acids concentration of broiler chickens. *Jpn. Poultry Sci.* 37 (2), 86–94.
- Takauji, Y., Kudo, I., En, A., Matsuo, R., Hossain, M.N., Nakabayashi, K., Miki, K., Fujii, M., 2017. Ayusawa D: GNG11 (G-protein subunit gamma 11) suppresses cell growth with induction of reactive oxygen species and abnormal nuclear morphology in human SUSM-1 cells. *Biochem. Cell Biol.* = *Biochimie et biologie cellulaire* 95 (4), 517–523.
- Tang, X., Zeng, X., Huang, Y., Chen, S., Lin, F., Yang, G., Yang, N., 2018. miR-423-5p serves as a diagnostic indicator and inhibits the proliferation and invasion of ovarian cancer. *Exp. Ther. Med.* 15 (6), 4723–4730.
- Tao, S., Orellana, R.M., Weng, X., Marins, T.N., Dahl, G.E., Bernard, J.K., 2018. Symposium review: The influences of heat stress on bovine mammary gland function. *J. Dairy Sci.* 101 (6), 5642–5654.
- Temim, S., Chagneau, A.M., Peresson, R., Tesseraud, S., 2000. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens fed 20 or 25% protein diets. *J. Nutr.* 130 (4), 813–819.
- Wang, X., Peng, L., Gong, X., Zhang, X., Sun, R., Du, J., 2018. miR-423-5p inhibits osteosarcoma proliferation and invasion through directly targeting STMN1. *Cellular Physiol. Biochem.: Int. J. Exp. Cellular Physiol. Biochem. Pharmacol.* 50 (6), 2249–2259.
- Weitzel, J.M., Viergutz, T., Albrecht, D., Bruckmaier, R., Schmicke, M., Tuchscherer, A., Koch, F., Kuhla, B., 2017. Hepatic thyroid signaling of heat-stressed late pregnant and early lactating cows. *J. Endocrinol.* 234 (2), 129–141.
- Ying, J., Srivastava, G., Hsieh, W.S., Gao, Z., Murray, P., Liao, S.K., Ambinder, R., Tao, Q., 2005. The stress-responsive gene GADD45G is a functional tumor suppressor, with its response to environmental stresses frequently disrupted epigenetically in multiple tumors. *Clin. Cancer Res.: Off. J. Am. Assoc. Cancer Res.* 11 (18), 6442–6449.



OPEN ACCESS

EDITED BY

Shengguo Zhao,
Chinese Academy of Agricultural Sciences,
China

REVIEWED BY

Neeta Agarwal,
Indian Veterinary Research Institute (IVRI), India
Yu Pi,
Chinese Academy of Agricultural Sciences,
China

*CORRESPONDENCE

Guangbin Liu
✉ gblu@scau.edu.cn
Dewu Liu
✉ dwliu@scau.edu.cn

RECEIVED 08 July 2024

ACCEPTED 25 September 2024

PUBLISHED 06 November 2024

CITATION

Liu J, Chen J, Fang S, Sun B, Li Y, Guo Y,
Deng M, Zhou D, Liu D and Liu G (2024)
Effects of moringa polysaccharides on growth
performance, immune function, rumen
morphology, and microbial community
structure in early-weaned goat kids.
Front. Vet. Sci. 11:1461391.
doi: 10.3389/fvets.2024.1461391

COPYRIGHT

© 2024 Liu, Chen, Fang, Sun, Li, Guo, Deng,
Zhou, Liu and Liu. This is an open-access
article distributed under the terms of the
Creative Commons Attribution License
(CC BY). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication
in this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Effects of moringa polysaccharides on growth performance, immune function, rumen morphology, and microbial community structure in early-weaned goat kids

Jinyang Liu¹, Jinyu Chen¹, Sicheng Fang¹, Baoli Sun¹, Yaokun Li¹,
Yongqing Guo¹, Ming Deng¹, Duoan Zhou², Dewu Liu^{1*} and
Guangbin Liu^{1*}

¹College of Animal Science, South China Agricultural University, Guangzhou, China, ²Guangdong
Leader Intelligent Agriculture Co., LTD, Qingyuan, China

The aim of this research was to investigate the effects of adding moringa polysaccharides (MOP) on the growth performance, immune function, rumen tissue morphology, and rumen microbial community in early-weaned goat kids. Twenty-one 7-day-old Leizhou male goat kids weighing (3.05 ± 0.63) kg, were randomly divided into a control group (CON group), a low-dose group (LOW group), and a high-dose group (HIG group). MOP was added to the goat kids' milk replacer (MR) at 0, 0.15, and 0.3% (on dry matter basis), fed until 60 days of age, and four goat kids in each group with body weights close to the mean of each group were selected for slaughter. The results showed that, compared to the CON group, the MOP groups significantly improved final body weight, body measurements, daily weight gain, and feed intake of the early weaned goat kids; significantly reduced the content of propionic acid, butyric acid, valeric acid, and ammoniacal nitrogen; and in addition, the addition of MOP could significantly increase the height of rumen nipple, the content of immunoglobulin G (IgG) in the serum. The HIG group significantly increased rumen pH, rumen muscularis layer thickness, rumen wall thickness, and serum immunoglobulin A (IgA), and immunoglobulin M (IgM). In conclusion, the addition of MOP positively impacted the growth performance, serum immune function, and rumen tissue morphology in early-weaned goat kids.

KEYWORDS

moringa polysaccharide, goat kid, immune function, rumen morphology, rumen microbiota

1 Introduction

As a unique digestive organ of ruminants, the development of rumen plays an important role in the health and digestive function of young ruminants. Early weaning of lambs can shorten the reproductive cycle of ewes and increase lambing frequency (1), and at the same time promote the development of rumen structure and function (2, 3). However, mother lamb

separation and changes in feed type can easily cause stress in early weaned lambs (4), which not only reduces the immunity and growth rate of lambs, but also results in the underutilization of nutrients and blockage of the immune system during the weaning period, which can easily lead to diseases such as intestinal pathogenic bacterial infections, intestinal homeopathy imbalance and diarrhea, and even cause death (5–7). Antibiotics have been widely used to treat and prevent diarrhea in lambs (8), but the frequent use of antibiotics not only causes great damage to the balance of intestinal flora and immune system in lambs, but also the antibiotic residues in the intestinal tract and feces of the animals lead to the increased risk of bacterial microorganisms' resistance, and these antibiotic residues of fecal matter and animal products can cause great negative impacts on human beings and the ecological environment (9). Under the Chinese government's antibiotic ban, finding natural active substitutes for antibiotics to achieve healthy lamb farming is of great importance for the high-quality development of the goat industry.

Moringa is a perennial deciduous tree of the Moringaceae family, native to India, and nowadays it has been widely cultivated as a multipurpose plant in different countries and regions (10, 11). All parts of Moringa including leaves, rhizomes and seeds are rich in functional polysaccharides, which are not only of high nutritional value but also of medicinal value (10). The presence of ascorbic acid, carotenoids and flavonoids, phenols and other types of antioxidant compounds in the leaves of Moringa is a good source of natural antioxidants (12). Polysaccharides extracted from Moringa are highly bioactive and have anti-inflammatory, antioxidant, antimicrobial, immunomodulatory and gastrointestinal protective properties (13). Natural plant polysaccharides have strong immunomodulatory functions and are considered as ideal drugs to improve immunity and antitumor (14–16). The experimental results of Tian et al. (17) have showed that MOP enhanced immunity, improved intestinal flora and morphology, as well as modulated the metabolism in mice. Zhao et al. (18) demonstrated that MOP could improve calf diarrhea, improve immunity, improve intestinal flora as well as promote growth.

Therefore, we added different concentrations of MOP to explore its effects on growth performance, serum antioxidant capacity, immune capacity, gastrointestinal morphology and microbial composition of 7-day-old weaned goat kids. In order to evaluate the feeding value of MOP in early-weaned goat kids and determine the appropriate addition amount of MOP in the actual production of goat kids, it provides a theoretical basis for the more scientific and rational application of MOP in goat industry.

2 Materials and methods

2.1 Ethics statement

All experimental procedures in this study were approved by the Committee of Animal Experiments of South China Agricultural University (No. 2023g032).

2.2 Experimental design and treatments

The feeding experiment was conducted from November 2023 to January 2024 at a black goat farm in Qingyuan, Guangdong Province.

During the trial period, the average temperature was 16.4°C (ranging from 5 to 28.5°C) and the average humidity was 67.1% (ranging from 22 to 93%).

MOP was purchased from Xi'an Clover Biotech Co., Ltd. (Xi'an, China), brown powder with 70% purity. Milk replacer (MR) was purchased from Beijing Precision Animal Nutrition Research Center (Beijing, China). Nutritional levels (dry matter basis): crude protein 27%, crude fat 18%, crude ash 10%, calcium 1.5%, phosphorus 1.2%. The starter diet was a granular compound feed formulated with reference to the nutritional requirements of NRC (1994), and the composition and nutritional levels of the basal ration are shown in Table 1.

Twenty-one 7-day-old Leizhou goat male goat kids of close weight and good health were selected with an average initial weight of (3.05 ± 0.63) kg. The goat kids were randomly divided into three groups of seven replicates of one goat kid.

The amount of MOP added was referred to the experimental results of Guo et al. (5). The control group (CON group) was fed milk replacer. The low dose group (LOW group) was fed milk replacer supplemented with 0.15% of their daily dry matter intake (DMI) of MOP. The high dose group (HIG group) was fed milk replacer supplemented with 0.3% of their daily dry matter intake (DMI) of MOP. The goat kids were fed from 7 days of age to 60 days of age, with a total experimental period of 54 days.

2.3 Feeding management

The test goat kids were suckled with their mothers until 7 days of age, and were forced to wean at 7 days of age. The goat kids were fed in separate pens (3.5 m long, 2.5 m wide and 1.5 m high), with partitions between the pens to prevent the groups from coming into contact with each other. Each pen is equipped with a drinking trough and trough for goat kids to freely eat open food, hay and sufficient water. The troughs were cleaned and replaced every morning. Straw is used as bedding for goat kids to lie on and is replaced every 2 days. All goat kids were provided with free access to starter feed and received an equal amount of milk replacer powder (2% of body weight) daily at 08:00, 12:00, and 16:00. The milk replacer powder was reconstituted with warm water at a 1:7 (weight/volume) ratio, thoroughly mixed until fully dissolved, and then dried at room temperature to 40°C. The reconstituted milk replacer was administered via a 250 mL bottle.

TABLE 1 Composition and nutritional levels of the starter feed (air-dry basis).

Ingredients	Composition (%)
Corn	46.40
Soybean meal	15.60
DDGS	10.35
Dried whey	20.25
CaHPO ₄	1.00
Limestone	0.81
NaCl	4.09
Choline chloride	0.50
Premix	1.00
Total	100.00

The sanitation of the pens was carried out according to the management measures of the farm, and was cleaned and disinfected regularly. The goat pens were cleaned and disinfected regularly according to the farm management measures, and the goat were immunized according to the normal immunization procedures.

2.4 Experimental methods and parameter measurements

2.4.1 Feed intake measurement

Feed samples, including hay, milk replacer, and starter, were collected weekly during the trial and mixed at the end of the trial. The feed intake of each group of goat kids was recorded daily. The collected feed samples were oven dried at 65°C to constant weight, pulverized and then determined for dry matter, crude ash, crude protein, crude fat, acid detergent fiber, neutral detergent fiber, calcium and phosphorus with reference to the assay method of Horwitz et al. (19).

2.4.2 Growth performance measurement

Goat kid fasting weight and body measurements were determined at the beginning of the trial (i.e., when goat kids were 7 days old), and at the end of the trial (i.e., when goat kids were 60 days old) prior to morning feeding, and the average daily weight gain of the test goat kids was calculated.

2.4.3 Blood sample collection and analysis

Blood was collected from the jugular vein of the goat kids before morning feeding on the 60th day of the experiment using a procoagulant blood collection tube, centrifuging at 3,000g at 4°C for 15 min, and the serum was centrifuged and collected for the determination of biochemical, antioxidant, and immunological indices. Total protein (TP), albumin (ALB), globulin (GLB), urea nitrogen (BUN), glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, alanine aminotransferase (ALT), alanine oxalate aminotransferase (AST), and creatinine (CRE) were measured by using an automated biochemistry instrument (Zecheng CLS880); total antioxidant capacity (T-AOC) in serum was determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (Item No.: A015-2-1); catalase (CAT) activity was determined by ammonium molybdate method (Item No.: A007-1-1); and superoxide dismutase (SOD) activity was determined by Water-Soluble Tetrazolium Salt-1 (WST-1) method (Item No.: A001-3-2); Glutathione peroxidase (GSH-Px) activity (Cat. No.: A005-1-2); Malondialdehyde (MDA) content was determined by Thiobarbituric Acid (TBA) method (Cat. No.: A003-1-2); Serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) were determined by enzyme-linked immunosorbent assay (ELISA) kit; Serum The levels of pro-inflammatory factor interleukin-2 (IL-2), anti-inflammatory factor interleukin-6 (IL-6), tumor necrosis factor (TNF- α) and γ -interferon (IFN- γ) in serum were determined by using the kit of Nanjing Jianjian Institute of Biological Engineering, and the absorbance was measured by using an enzyme labeling instrument (RaytoRT-6100), and the specific procedures were carried out according to the instructions of the kit.

2.4.4 Collection and analysis of rumen tissue and rumen contents

Goat kids were euthanized and dissected for sampling within 15 min. Post-dissection rumen pH was determined using a pH meter (FE28, METTLER TOLEDO INSTRUMENTS CO., LTD., Shanghai, China). Goat kid rumen content samples were stored in liquid nitrogen for subsequent DNA extraction. Rumen fluid samples obtained by filtration using four layers of gauze were stored at -20°C for subsequent determination of fermentation parameters.

The rumen ventral sac tissue (1.5 × 1.5 cm²) of goat kids was collected after autopsy with reference to the method of Li et al. (20), and after rinsing with phosphate buffer solution (PBS), the rumen tissue was fixed for 24 h in 4% paraformaldehyde solution, dehydrated, transparent, and wax impregnated to obtain paraffin-embedded wax blocks of rumen tissue.

2.5 Morphology and measurement of rumen tissue

The embedded wax block was cut into 4 μ m thick slices with a paraffin slicer (RM2016), spread horizontally under 40°C warm water in a spreader, and the slide fished the tissue and baked the slices in a 60°C oven. After the water baking dry wax baked and removed, the slices were sequentially put into environmentally friendly dewaxing solution I 20 min—environmentally friendly dewaxing solution II 20 min—anhydrous ethanol I 5 min—anhydrous ethanol II 5 min—75% alcohol for 5 min, and washed with tap water. The slices were put into HD constant dye pretreatment solution for 1 min, hematoxylin dyeing solution for 3–5 min, washed with tap water, differentiation solution for differentiation, washed with tap water, return blue solution for return blue, rinsed with running water; dehydrated with 95% alcohol for 1 min, and stained into eosin dyeing solution for 15 s; anhydrous ethanol I 2 min—anhydrous ethanol II 2 min—anhydrous ethanol III 2 min—n-butanol I 2 min—n-butanol II 2 min—dimethyl 12 min—xylene I 2 min transparent, neutral gum sealing, microscopic observation of histological morphology and scanning and photographing using panoramic scanner scanning software. The rumen papillae, papillae width, muscular layer thickness, rumen wall thickness, cuticle thickness and calculated papillae density, rumen papillae specific surface area using (Image-ProPlus6.0, United States) analysis software, one field of view was selected for each section, and histomorphology was counted in each field of view.

2.6 Measurement of rumen fermentation parameters

The concentrations of acetic acid (AA), propionic acid (PA), butyric acid (BA), valeric acid (VA), isobutyric acid (IBA), and lactic acid (LA) were detected by gas chromatography (Agilent 7890B, NYSE: A, Palo Alto, CL, United States) with reference to the method of Wang et al. (21). A total of 0.2 mL of 25% metaphosphoric acid was added to 1 mL of rumen fluid sample and mixed properly. Subsequently, the mixed samples were stored at -20°C for more than 24 h. The samples were then centrifuged at 10,000 rpm for 10 min at 4°C, and 1.0 mL of the supernatant was filtered through a 0.45 μ m membrane. The filtrate was then injected into special gas phase vials where 0.4 μ L of the sample was automatically injected into the HP-INNOWax gas phase

capillary column. The injector and detector temperatures were set to 250°C and 280°C, respectively, and the split ratio was set to 40:1. The column was heated from 120°C to 250°C at 10°C/min. Ammonia-nitrogen concentration (NH₃-N in rumen fluid) was determined by a colorimetric method as described by Ma et al. (22).

2.7 Determination of rumen microbial flora in rumen contents

Total genomic DNA samples were extracted using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, United States), following the manufacturer's instructions, and stored at -20°C prior to further analysis. The quantity and quality of extracted DNAs were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the bacterial 16S rRNA genes V3-V4 region was performed using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 µL of buffer (5×), 0.25 µL of Fast pfu DNA Polymerase (5 U/µL), 2 µL (2.5 mM) of dNTPs, 1 µL (10 uM) of each Forward and Reverse primer, 1 µL of DNA Template, and 14.75 µL of ddH₂O. Thermal cycling consisted of initial denaturation at 98°C for 5 min, followed by 25 cycles consisting of denaturation at 98°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s, with a final extension of 5 min at 72°C. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, United States). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 × 250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6,000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The original 16S rRNA sequence data has been submitted to NCBI with the accession number PRJNA1159108.

2.8 Data processing and statistical analysis

Experimental data were organized and calculated using Excel 2019. Statistical analysis of the data was performed using SPSS 23.0 with one-way analysis of variance (ANOVA), and the results were expressed as means and standard error of the mean (SEM). Duncan's method of multiple comparisons was used for the test of variance, and $p < 0.05$ was used to indicate significant differences.

3 Results

3.1 Impact of moringa polysaccharides on the growth performance of goat kids

As shown in Table 2, initial body weight and milk replacer intake did not differ significantly among groups ($p > 0.05$); however, final body weight, average daily gain, openings, and green hay intake were significantly higher in the LOW and HIG groups compared to the CON group ($p < 0.05$).

TABLE 2 Effects of moringa oligosaccharides on the growth performance of early weaned goat kids.

Items	CON	LOW	HIG	SEM	p-Value
Initial BW (kg)	3.01	3.06	3.09	0.14	0.976
Final BW (kg)	5.40 ^b	6.95 ^a	7.36 ^a	0.30	0.009
ADG (g/d)	44.96 ^b	73.45 ^a	80.54 ^a	4.92	0.020
Milk replacer intake (g/d)	490.95	495.96	483.06	14.82	0.938
Starter intake (g/d)	284.11 ^b	519.24 ^a	582.39 ^a	49.11	0.032
Green hay intake (g/d)	90.79 ^b	165.29 ^a	166.32 ^a	10.68	0.003

CON, the milk replacer (n = 7); LOW, supplemented with 0.15% MOP in the milk replacer (n = 7); HIG, supplemented with 0.3% MOP in the milk replacer (n = 7); BW, body weight; ADG, average daily gain; \bar{x} represents mean; SEM standard error of the mean. Same lowercase letters within the same row indicate no significant difference ($p > 0.05$), while different lowercase letters indicate a significant difference ($p < 0.05$). The same applies to the following tables.

3.2 Effect of moringa polysaccharides on serum biochemical parameters of goat kids

As shown in Table 3, the addition of MOP had no significant effect on GLU, TP, ALB, BUN, TG, TC, HDL, LDL, ALT, AST and CRE contents of goat kids ($p > 0.05$), in which the TG content showed a decreasing trend with the increase of MOP additions ($p = 0.088$); GLB content of the HIG group was significantly lower than that of the CON group ($p < 0.05$), the difference in GLB content between the LOW group and the CON and HIG groups was not significant ($p > 0.05$); the A/C ratio of the CON group was significantly lower than that of the LOW and HIG groups ($p < 0.05$).

3.3 The effect of moringa polysaccharides on serum antioxidant indices in goat kids

As shown in Table 4, the addition of MOP had no significant effect on T-AOC and CAT contents of goat kids ($p > 0.05$); the GSH-Px content of the LOW group was significantly lower than that of the CON group ($p < 0.05$), and the difference was not significant compared with that of the HIG group ($p > 0.05$); and the contents of SOD and MDA in the LOW group and the HIG group were significantly lower than that of the CON group ($p < 0.05$).

3.4 Effect of moringa polysaccharides on the content of serum immunoglobulins and cytokines in goat kids

As shown in Table 5, the effects of adding MOP on IFN- γ , IL-2 and IL-6 contents of goat kids were not significant among the groups

TABLE 3 Effect of moringa polysaccharides on serum biochemical parameters of goat kids.

Items	CON	LOW	HIG	SEM	p-Value
GLU (mmol/L)	4.11	5.34	4.78	0.28	0.199
TP (g/L)	51.11	47.72	47.16	0.88	0.131
GLB (g/L)	32.81 ^a	29.87 ^{ab}	26.74 ^b	1.05	0.028
ALB (g/L)	19.06	20.26	20.42	0.49	0.522
A/G	0.52 ^b	0.71 ^a	0.76 ^a	0.04	0.021
BUN (mmol/L)	7.31	7.88	6.25	0.34	0.125
TG (mmol/L)	0.37	0.13	0.17	0.05	0.088
TC (g/L)	1.37	1.74	1.26	0.11	0.175
HDL (mmol/L)	0.97	0.97	1.01	0.04	0.930
LDL (mmol/L)	1.17	1.41	1.10	0.07	0.180
ALT (U/L)	27.83	28.19	31.41	0.78	0.105
AST (U/L)	106.54	90.40	92.87	3.61	0.137
CRE (μmol/l)	13.41	6.20	7.24	1.68	0.166

CON, the milk replacer ($n=3$); LOW, supplemented with 0.15% MOP in the milk replacer ($n=3$); HIG, supplemented with 0.3% MOP in the milk replacer ($n=3$).

TABLE 4 Effect of moringa polysaccharides on serum antioxidant indices in goat kids.

Items	CON	LOW	HIG	SEM	p-Value
T-AOC (U/L)	0.75	0.74	0.74	0.01	0.930
CAT (U/mL)	0.28	0.18	0.21	0.02	0.146
GSH-Px (mol/L)	105.67 ^a	79.40 ^b	97.91 ^{ab}	4.76	0.036
SOD (U/mL)	195.34 ^a	174.69 ^b	172.11 ^b	3.81	<0.001
MDA (nmol/mL)	2.55 ^a	1.98 ^b	2.02 ^b	0.12	0.049

CON, the milk replacer ($n=3$); LOW, supplemented with 0.15% MOP in the milk replacer ($n=3$); HIG, supplemented with 0.3% MOP in the milk replacer ($n=3$).

($p>0.05$); the IgA and IgM contents of the HIG group were significantly higher than those of the CON group ($p<0.05$), and the IgA and IgM contents of the LOW group were not significantly different from those of the CON and HIG groups ($p>0.05$); the IgG and TNF- α content was significantly higher than that of CON group ($p<0.05$), but the difference between the two groups was not significant ($p>0.05$).

3.5 The effect of moringa polysaccharides on rumen fermentation parameters in goat kids

Rumen fermentation parameters are summarized in Table 6. There were no significant differences among the Total VFA, AA, and IBA

groups ($p>0.05$). PA, BA, VA, and NH₃-N in the CON group were significantly higher than those in the LOW and HIG groups ($p<0.05$). The AA:PA ratio in the HIG group was significantly higher than in the LOW and CON groups ($p<0.05$). The pH value of the HIG group was significantly higher than that of the CON group ($p<0.05$), but there was no significant difference between the HIG and LOW groups ($p>0.05$).

3.6 Effect of moringa polysaccharides on the morphology of goat kid rumen tissue

As shown in Figure 1, the CON group displayed short, thick, irregular, and untidy rumen papillae. In contrast, the rumen papillae in the LOW and HIG groups were elongated, neat, and relatively regular. The effect of moringa polysaccharides on the morphology of lamb rumen tissue is shown in Table 7. The differences among groups in papilla width, cuticle thickness and specific surface area of rumen papillae were not significant ($p>0.05$); papilla height in the HIG and LOW groups was significantly higher than that in the CON group ($p<0.05$); the thickness of the muscularis propria and the thickness of the rumen wall in the HIG group was significantly higher than that in the LOW and CON groups ($p<0.05$); and the density of papillae in the CON group was significantly higher than that in the LOW and HIG groups ($p<0.05$).

3.7 Effect of moringa polysaccharides on the microbial flora of goat kids

In this experiment, 12 samples of rumen contents from goat kids were sequenced and analyzed using IlluminaNovaseqMiseq platform, and a total of 944,860 original sequences were obtained, and quality control was performed on the obtained sequences, and a total of 878,895 valid sequences were obtained after removing low-quality, short-length, and chimerism. The valid sequences were clustered into OTUs according to 100% similarity, and a total of 7,670 OTUs were obtained, of which 247 OTUs were common to the three treatments, and 2,457, 2,433 and 2,215 OTUs were specific to the CON, LOW and HIG groups, respectively (Figure 2A). The observed curves of the species of the 12 samples are shown below, and the Rank Abundance curves reflect the groups' Differences in species uniformity and abundance, as shown in Figure 2B, each sample was similar in uniformity and abundance, and the above results indicate that the sequencing depth of this study is sufficient and reasonable, and the results are stable and reliable, and can be used for subsequent analysis.

3.8 Effect of moringa polysaccharides on the alpha diversity of rumen microbiota in goat kids

Alpha diversity indices are commonly used to assess the diversity and richness of microbial species, and the value of Goods coverage reflects the coverage of OTUs in the samples, and the larger the value is, the more sufficient the sequencing data are, in this experiment, the value of Goods coverage of all the samples was not less than 0.996, which indicated that the depth of this sequencing could basically cover all the species. The results of the indexes in this experiment are shown

TABLE 5 Effect of moringa polysaccharides on the content of serum immunoglobulins and cytokines in goat kids.

Items	CON	LOW	HIG	SEM	p-Value
IgA (μg/mL)	34.38 ^b	39.87 ^{ab}	44.70 ^a	1.70	0.013
IgG (μg/mL)	1568.44 ^b	1932.73 ^a	1987.27 ^a	76.07	0.016
IgM (μg/mL)	17.61 ^b	19.96 ^{ab}	22.27 ^a	0.84	0.046
IFN-γ (pg/mL)	42.85	40.70	41.21	1.02	0.728
IL-2 (pg/mL)	110.59	113.28	120.67	6.61	0.852
IL-6 (pg/mL)	249.33	240.37	244.36	5.20	0.825
TNF-α (pg/mL)	549.02 ^b	972.87 ^a	865.95 ^a	67.58	0.001

CON, the milk replacer (n = 3); LOW, supplemented with 0.15% MOP in the milk replacer (n = 3); HIG, supplemented with 0.3% MOP in the milk replacer (n = 3).

in Figures 2D–J, and the alpha diversity index did not produce significant differences among treatment groups ($p > 0.05$).

3.9 Effect of moringa polysaccharides on the beta diversity of rumen microbiota in goat kids

PCoA is an unconstrained data dimensionality reduction method that reflects the similarity between samples based on their distribution distances on the graph. The closer the samples are on the graph, the more similar they are. NMDS analysis focuses more on the ordinal relationships between values and reflects the differences between samples based on the distances between points. In this test the CON group was not clearly distinguished from the other groups and the three groups overlapped each other (Figure 2C).

PERMANOVA analysis was further used in this trial to explore the beta diversity of rumen flora. The results are shown in Table 8, and the differences between the groups were not significant ($p > 0.05$).

3.10 Effect of moringa polysaccharides on the composition of rumen microbiota in goat kids

At the phylum level, Bacteroidetes and Firmicutes were the two main phyla, accounting for more than 67% of the rumen flora of goat kids (Table 9). The HIG group increased the relative abundance of Bacteroidetes by 5.4% compared to the CON group, but there was no significant difference at the phylum level of the phyla between the groups' differences ($p > 0.05$) (Figure 3A).

At the genus level, the top 15 abundant genera were *Prevotella*, *Olsenella*, *Ruminococcaceae_Ruminococcus*, *Sharpea*, *Treponema*, *Succinivibrio*, *Sphaerochaeta*, *Butyrivibrio*, *Fibrobacter*, *Megasphaera*, *Succinilasticum*, *Ruminobacter*, *YRC22*, *Anaerovibrio*, and *Schwartzia*.

TABLE 6 Effect of moringa polysaccharides on rumen fermentation parameters in goat kids.

Items	CON	LOW	HIG	SEM	p-Value
pH	5.54 ^b	5.83 ^{ab}	6.01 ^a	0.10	0.020
NH ₃ -N (G/L)	214.69 ^a	70.02 ^b	75.02 ^b	25.67	0.003
Total VFA (mmol/L)	327.13	148.74	204.09	41.32	0.206
Acetic acid (mmol/L)	96.12	61.64	139.92	27.94	0.564
Propionic acid (mmol/L)	169.87 ^a	65.01 ^b	33.55 ^b	20.00	0.001
Acetate: Propionic	0.53 ^b	1.36 ^b	4.89 ^a	0.68	0.004
Butyric acid (mmol/L)	38.13 ^a	12.96 ^b	19.94 ^b	4.68	0.059
Valeric acid (mmol/L)	15.22 ^a	5.50 ^b	3.77 ^b	1.98	0.019
Isobutyric acid (mmol/L)	7.79	3.63	6.90	0.85	0.103

CON, the milk replacer (n = 4); LOW, supplemented with 0.15% MOP in the milk replacer (n = 4); HIG, supplemented with 0.3% MOP in the milk replacer (n = 4).

The relative abundance results are shown in Table 10, the relative abundance of *Olsenella* genus. Increased in the high LOW group compared to the CON group, but there was no significant difference in the bacterial flora between the groups at the genus level ($p > 0.05$) (Figure 3B).

3.11 Stomach microbiota LEfSe analysis

LEfSe analysis is a statistical method commonly used to analyze differences between groups, which not only enables comparisons between different subgroups, but also finds biomarkers that are statistically different. In this study, LDA > 2 was used as the threshold value, and four differential bacteria were screened, which were three families and one genus. Among them, the differential bacteria with large LDA values mainly include the *Ruminococcaceae* family, *Bacillus* genus, *Staphylococcus* genus, and *Staphylococcaceae* family (Figure 4).

4 Discussion

The rumen of ruminant animals plays a crucial role in nutrient digestion and absorption, immune response, and host metabolism (23). Ruminal development in ruminants at a young age is crucial and may have long-term effects on their health and later growth and development. According to Wang et al. (24) the addition of fermented wheat bran polysaccharides (FWBPs) to milk replacer increased daily weight gain and decreased feed to weight ratio in early weaned lambs. Chen et al. (25) added Chinese medicinal polysaccharides (CMPs) to increase the average daily weight gain of lambs and improved the lamb



FIGURE 1
The histological sections of the rumen tissue in goat kids.

TABLE 7 Effect of moringa polysaccharides on the morphology of goat kid rumen tissue.

Items	CON	LOW	HIG	SEM	p-Value
Rumen papillae height (mm)	0.91 ^b	1.39 ^a	1.46 ^a	0.05	0.000
Rumen papillae width (mm)	0.49	0.48	0.48	0.01	0.819
Rumen muscular layer thickness (mm)	1.05 ^b	0.97 ^b	1.38 ^a	0.06	0.004
Rumen wall thickness (mm)	2.42 ^b	2.70 ^b	3.14 ^a	0.09	0.003
Stratum corneum thickness (mm)	0.0248	0.0237	0.0281	0.00	0.285
Rumen papillae density (items/mm ²)	2.12 ^a	1.53 ^b	1.48 ^b	0.09	0.004
Rumen papillae surface area ratio	2.14	2.02	2.04	0.08	0.825

CON, the milk replacer ($n=4$); LOW, supplemented with 0.15% MOP in the milk replacer ($n=4$); HIG, supplemented with 0.3% MOP in the milk replacer ($n=4$).

performance. Zhao et al. (18) added MOP to significantly increase the body weights of newborn calves. This is consistent with the significant increase in daily gain, body weight, and body size observed with MOP supplementation in this study. As the growth rate increases, nutritional demand also rises correspondingly. Additionally, in the MOP-added group, intake of green hay and other feeds increased significantly. This suggests that the improvement in growth performance is attributed to enhanced absorption and utilization of nutrients. Therefore, adding MOP can improve nutrient absorption and has a significant impact on promoting the growth and development of goat kids.

Changes in blood biochemical indicators can reflect alterations in the metabolic capacity of the organism. The serum A/G ratio and ALB content can reflect the protein synthesis of the organism, and the decrease of its ratio and content may be related to chronic inflammation caused by the accumulation of inflammatory cytokines (26, 27). The significant increase in the A/G ratio in this experiment indicated that the addition of MOP could reduce weaning stress in lambs and avoid stress-induced oxidative damage to proteins and lipids. Serum TG is a lipid metabolite (27). Addition of MOP in the present study reduced serum triglyceride levels in lambs. This is similar to the findings of Kwon et al. (28), which showed that plant polysaccharides such as MOP can affect lipid metabolism, leading to a decrease in serum TG content in goat kids. T-AOC, GSH-Px, SOD and other enzymes have important antioxidant functions, and T-AOC content can reflect the metabolic status of antioxidant free radicals, while MDA content can reflect the degree of oxidative stress, when the body undergoes oxidative stress, a large number of free radicals will be generated, and free radicals will react with lipids to produce MDA, which is the end product of lipid peroxidation, and the increase of MDA content represents the decrease of TG content. MDA is the end product of lipid peroxidation, and an increase in MDA content represents an increase in the level of oxidative stress in the body (29–31). In this study, there was no significant difference in the T-AOC

content in the serum of goat kids among the groups, whereas the addition of MOP decreased the GSH-Px, SOD and MDA content in the serum of goat kids, which may be attributed to the binding of polysaccharides to the surfaces of cell specific surface molecules and thus inhibiting the excess oxygen radicals (7) thus acting to alleviate the level of oxidative stress in the organism. Similarly, Su et al. (32) fed capsaicin also reduced MDA levels, which is consistent with the results of the present study, suggesting that the addition of capsaicin and MOP, a substance with a better biological function, can inhibit the serum levels of excess oxygen radicals in goat kids and protect the organism from oxidative damage.

Immune cells in animal bodies produce cytokines such as interleukins and anti-tumor factors, which have anti-disease functions. In this study, IL-2 and IL-6 had no significant effect among the groups, while TNF- α increased significantly with the increase of MOP addition. TNF- α is a protein produced by a variety of cells, and its main role is to regulate the immune response, promote inflammatory response, and regulate apoptosis (cell death) (33). When the organism is subjected to infection, tissue damage, or other pathological conditions, cells may release more TNF- α . Therefore, elevated levels of TNF- α in the blood or tissues are often considered as one of the indicators of inflammatory states. Elevated levels of TNF- α may indicate that the organism is experiencing an inflammatory response or immune system activation. However, high levels of TNF- α may also be associated with a number of other diseases and pathological conditions. Plant polysaccharides have been reported to improve phagocytosis by macrophages, leading to significantly higher levels of TNF- α (34, 35). It is possible that plant polysaccharides exert their antitumor effects by affecting tumor differentiation and apoptosis, altering intracellular signaling and immune regulation (36, 37). Immunoglobulins IgA, IgM, and IgG, as important immunoreactive molecules in animals, can specifically bind to the corresponding antigens and participate in the regulation

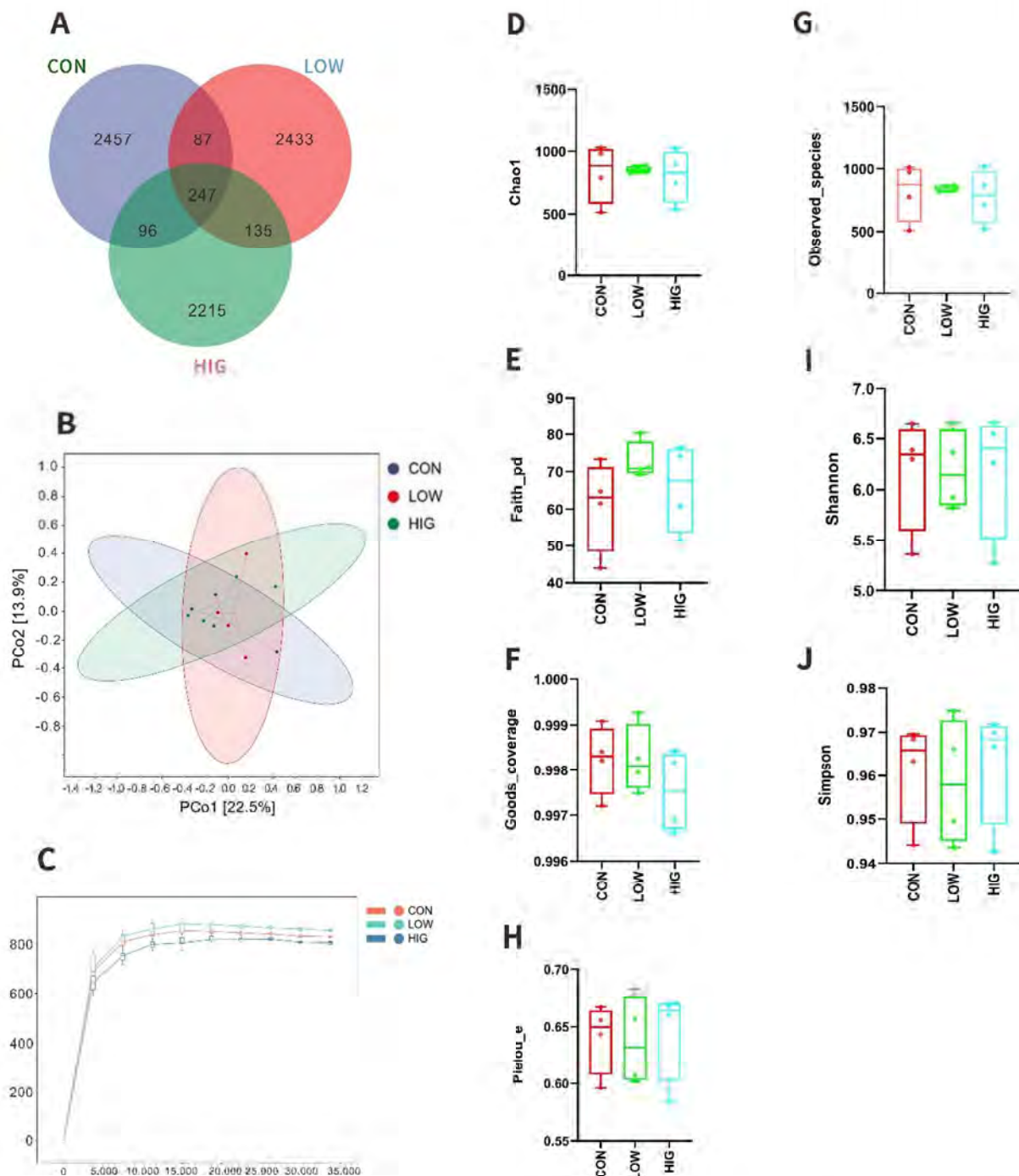


FIGURE 2

Effects of MOP on the alpha and beta diversity of the rumen microbiota in goat kids. Analysis of the alpha and beta diversity via (A) Venn figure of OTUs (B) Species observation curve in each group. (C) PCoA of weighted UniFrac distances, respectively (D) Chao1 index, (E) Faith_pd index, (F) Goods_coverage index, (G) Observed_species index, (H) Pielou_e index, and (I) Shannon index, (J) Simpson index; CON, the milk replacer ($n = 4$); LOW, supplemented with 0.15% MOP in the milk replacer ($n = 4$); HIG, supplemented with 0.3% MOP in the milk replacer ($n = 4$).

of body immunity, so that the elevation of the levels of IgA, IgM, and IgG indicates the improvement of immune function. The addition of MOP significantly increased the immunoglobulin level in serum in this study, which is consistent with the results of Chen et al. (25), indicating that plant polysaccharides such as MOP have

the effect of increasing immunoglobulin level to enhance the immunity of goat kids.

Chen et al. showed that pH in rumen fluid is closely related to VFA, and that an increase in rumen VFA concentration can lead to a decrease in rumen pH (25). This is similar to the results of the present

TABLE 8 PERMANOVA analysis results.

Items	PERMANOVA		
	F	P	q
CON vs LOW	1.165	0.190	0.467
CON vs HIG	1.128	0.311	0.467
LOW vs HIG	0.795	0.705	0.705

CON, the milk replacer ($n=4$); LOW, supplemented with 0.15% MOP in the milk replacer ($n=4$); HIG, supplemented with 0.3% MOP in the milk replacer ($n=4$).

TABLE 9 Effect of MOP on the relative abundance of rumen microbiota at the phylum level (%).

Items	CON	LOW	HIG	SEM	p-Value
Bacteroidetes	39.44	38.10	44.84	3.04	0.676
Firmicutes	34.28	29.45	32.69	1.93	0.627
Actinobacteria	7.04	13.24	9.56	1.74	0.375
Spirochaetes	7.24	9.04	8.36	0.80	0.695
Proteobacteria	8.17	6.11	1.75	1.78	0.356
Fibrobacteres	3.02	2.04	1.03	0.80	0.638
Tenericutes	0.16	0.40	0.34	0.11	0.697
Verrucomicrobia	0.06	0.14	0.28	0.09	0.645
Cyanobacteria	0.02	0.02	0.04	0.00	0.457
Synergistetes	0.02	0.03	0.02	0.00	0.344
Others	0.54	1.43	1.02	0.33	0.595

CON, the milk replacer ($n=4$); LOW, supplemented with 0.15% MOP in the milk replacer ($n=4$); HIG, supplemented with 0.3% MOP in the milk replacer ($n=4$).

study, where the VFA content was higher in the CON group than in the test group, while the pH was lower in the CON group than in the test group. In this study, according to the ratio of acetic acid to propionic acid, the majority of acetic acid fermentation could be judged. Meanwhile, the high level of green hay in the MOP group also proved that the cellulose in the rumen of goat kids was better decomposed to produce acetic acid. The energy produced by VFA in the rumen of lambs during its metabolism can directly stimulate the development of the rumen (38), with BA playing the most prominent role, followed by PA and AA. BA has been reported to have a promotional effect on the growth and development of rumen epithelial cells, and at the same time, it can reduce the apoptosis of rumen epithelial cells (39, 40). And the rumen epithelial tissue, as an important component of rumen function, has the function of transporting nutrients from the rumen to the bloodstream (40). IBA belongs to branched-chain fatty acids, which originate from the fermentation of protein feeds, and the fermentation process is accompanied by hazardous substances such as hydrogen sulfide and cresol, and a low amount of IBA can help to maintain the health of the rumen (24). VFA in the rumen is absorbed through the rumen epithelial cells, with an absorption rate depending on VFA concentration, rumen surface area and availability of transporter proteins (41). Another study showed that decreased serum GLU levels in young ruminants may be associated with incomplete development of the gastrointestinal tract (42). In this experiment, the serum GLU level, rumen papilla length density, muscularis propria thickness, and rumen wall thickness in the CON group were lower than those in the

test group with MOP addition. Additionally, the IBA content in the test group was lower than in the CON group, suggesting that the rumen of MOP-added goat kids was more developed, the rumen environment was healthier, and VFA absorption was more efficient. The relative abundance of *Succinivibrio* and *Megasphaera* was higher in the CON group than in the MOP-added group. Since *Succinivibrio* is involved in the synthesis of short-chain fatty acids (43), this may explain why the PA, BA, and VA contents were higher in the CON group compared to the test group. This was further supported by the lack of significant difference in total protein content in serum among the groups. $\text{NH}_3\text{-N}$ in the rumen is mainly produced by fermentation of ingested protein feeds and is also an important source of microbial protein synthesis. Lv et al. demonstrated that the addition of fermented wheat bran polysaccharides selectively increased rumen microbial populations, which led to a decrease in $\text{NH}_3\text{-N}$ content and its uptake by the rumen epithelial cells (44). The results of the present study showed that the addition of MOP reduced the $\text{NH}_3\text{-N}$ content in the rumen of goat kids, and the lower $\text{NH}_3\text{-N}$ content may be a result of the efficient transport of $\text{NH}_3\text{-N}$ into the bloodstream by the better developed rumen epithelial tissues to participate in microbial protein synthesis, thus promoting the growth and development of goat kids.

The degree of rumen development can be measured by the development status of rumen epithelial cells, such as rumen papilla length and width (44). The rumen epithelium consists of rumen, muscularis propria, and epithelial cells, which have the functions of nutrient absorption, transportation, and metabolism (24). The fibrous component in the early primary goat kid diet is considered a key factor influencing the rumen development of goat kids. The fiber-rich diet provides the necessary physical stimulation for the physiological development of the rumen of young ruminants (45–47). At the same time, a large number of studies have shown that only after the rumen nipple start to grow and the thickness of the rumen wall begins to increase. Bian et al. (48) show that feeding fiber-rich alfalfa hay promotes the rumen peristalsis, accelerates the fermentation of rumen microorganisms, promotes the development of the rumen muscle layer, and further improves the digestive capacity of goat kids. In this study, the intake of goat kid mouth and green hay in the MOP test group was significantly higher than that in the CON group, and the addition of high-dose MOP significantly increased the height, muscle layer thickness and rumen wall thickness of the goat kids, indicating that the improvement of hay and open feed promoted the development of rumen in lactating ruminants. The proliferation of ruminal epithelial cells can also promote the growth of ruminal papilla and width and improve the muscle layer thickness. Other studies showed that increasing the height and width of the rumen nipple increased the absorption area of the rumen epithelium, but decreased the rumen nipple density (49). This is consistent with the results that adding MOP significantly reduced the density of rumen nipple in this study, but the addition of MOP had no effect on the width, cuticle thickness and specific surface area, which is inconsistent with Wang (24) and other studies, which may have different effects of different types of polysaccharide on the development of goat kids, and its regulation mechanism needs further study.

The rumen, as a specialized digestive organ in ruminants, contains a large number of microbial communities, such as bacteria, fungi, and archaea, which can help the host digest cellulose and other carbohydrates (50, 51). Microbial community colonization is also relevant and important for rumen development in young animals (52).

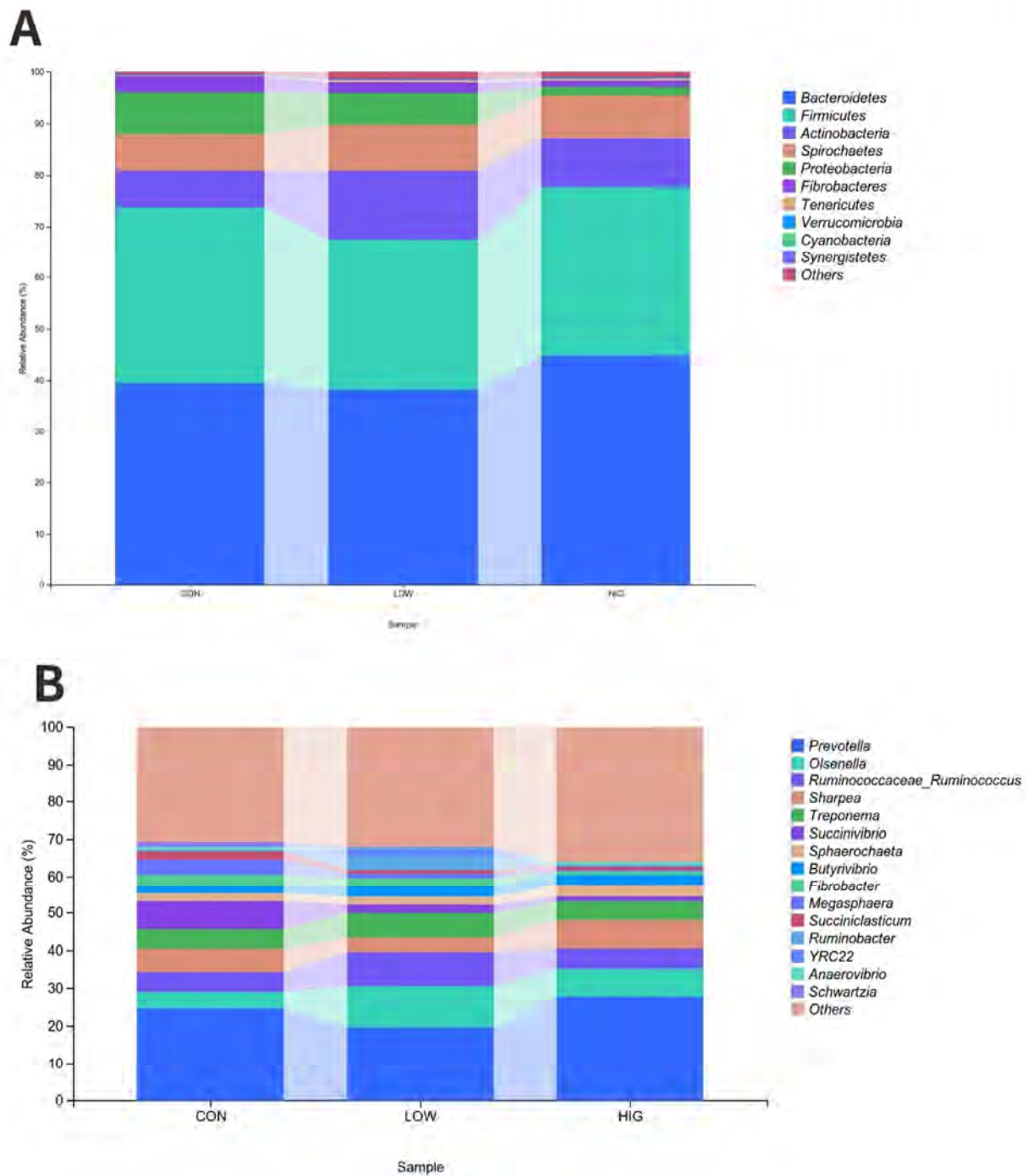


FIGURE 3
Effect of MOP on the composition of the rumen microbial community. (A) Phylum level (B) Genus level.

Therefore, the effect of MOP addition on the structure and composition of rumen microbiota in goat kids was analyzed by 16SrRNA sequencing. In the present study, there was no significant difference between the CON group and the MOP test group in terms of flora alpha and beta diversity. Wang et al. (24). found that the addition of fermented bran polysaccharides did not have a significant effect on the alpha and beta diversity of the goat kid flora; Chen et al.

(25) found that the addition of herbal polysaccharides did not have a significant effect on the alpha and beta diversity of the goat kid flora, which was in agreement with the present study. The current study showed that *Anaplasma* phylum, Thick-walled phylum and *Actinobacteria* phylum are the core phyla of the rumen with the highest relative abundance (1, 53). In this study, MOP had no effect on the structure and composition of the rumen microbiota. This

TABLE 10 Effect of MOP on the relative abundance of rumen microbiota at the genus level (%).

Items	CON	LOW	HIG	SEM	p-Value
<i>Prevotella</i>	24.88	19.71	27.82	1.91	0.228
<i>Olsenella</i>	4.09	11.05	7.58	1.44	0.140
<i>Ruminococcaceae_Ruminococcus</i>	5.39	9.06	5.24	1.20	0.372
<i>Sharpea</i>	6.39	3.73	7.71	1.52	0.600
<i>Treponema</i>	5.03	6.71	4.98	1.05	0.780
<i>Succinivibrio</i>	7.50	2.08	1.11	1.52	0.188
<i>Sphaerochaeta</i>	2.21	2.32	3.38	0.73	0.802
<i>Butyrivibrio</i>	1.93	2.70	2.60	0.67	0.900
<i>Fibrobacter</i>	3.02	2.04	1.03	0.80	0.638
<i>Megasphaera</i>	4.02	1.31	0.32	1.34	0.552
<i>Succiniclacticum</i>	2.18	1.13	0.99	0.28	0.159
<i>Ruminobacter</i>	0.05	3.61	0.00	1.20	0.408
<i>YRC22</i>	0.38	1.96	0.29	0.51	0.356
<i>Anaerovibrio</i>	0.91	0.32	0.74	0.15	0.303
<i>Schwartzia</i>	1.22	0.13	0.19	0.34	0.365
<i>Others</i>	30.81	32.14	36.01	2.41	0.701

CON, the milk replacer (n = 4); LOW, supplemented with 0.15% MOP in the milk replacer (n = 4); HIG, supplemented with 0.3% MOP in the milk replacer (n = 4).

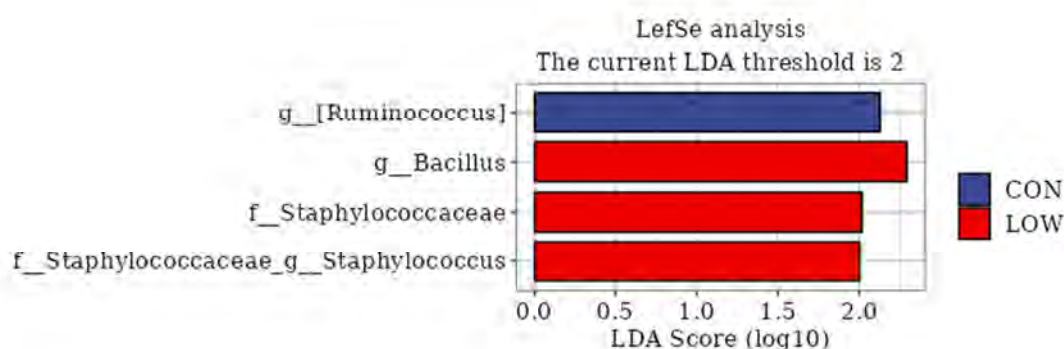


FIGURE 4

Bacterial taxa differences between control and LOW groups. Linear discriminant analysis (LDA) coupled with effect size (LEfSe) analysis was performed to identify the bacterial taxa differentially represented in control and LOW groups at different taxonomy levels. CON, the milk replacer (n = 4); LOW, supplemented with 0.15% MOP in the milk replacer (n = 4).

might be due to the fact that the amount of milk replacer administered or its passage through the esophageal groove into the abomasum and intestines resulted in minimal impact on rumen fermentation and microbiota composition in goat kids (1). In addition, LefSe analysis showed that goat kids in the LOW group were enriched with differential OTUs; most of these OTUs belonged to the genus *Bacillus*, and the *Bacillus* group includes many polysaccharide-degrading bacteria that contribute to the production of VFA in the gut. The cellulose secreted by *Bacillus* helps the animals to digest fiber, and produces various antimicrobial peptides that maintain the normal microbiota of the animals (54). Based on the present results and references, we hypothesize that MOP supplementation with MR stimulates the proliferation of fibrinolytic bacteria, such as *Bacillus*

spp., which are producers of VFAs. This, in turn, increases the production of VFAs and microbial proteins, accelerates rumen development, and consequently improves the growth performance of early-weaned goat kids.

5 Conclusion

The addition of MOP to milk replacer powder in early-weaned goat kids increased daily weight gain, feed intake, immunoglobulin G, tumor necrosis factor α levels, and rumen height, while decreasing the levels of propionic acid, butyric acid, valeric acid, ammonia nitrogen, and density. Specifically, high doses of MOP (0.3%) significantly

increased serum immunoglobulin A, immunoglobulin M, rumen muscle thickness, rumen wall thickness, and rumen contents pH, and also increased the relative abundance of Actinobacteria and Butyrivibrio species in the goat kid rumen. In summary, the addition of MOP to milk replacer powder for early-weaned goat kids can promote rumen growth and development, and improve immune function and growth performance.

Data availability statement

The data presented in the study are deposited in the Sequence Read Archive (SRA) repository, accession number PRJNA1159108.

Ethics statement

The animal study was approved by Committee of Animal Experiments of South China Agricultural University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JL: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Software, Validation, Visualization. JC: Writing – review & editing, Data curation, Formal analysis, Visualization. SF: Investigation, Writing – review & editing. BS: Formal analysis, Methodology, Writing – review & editing. YL: Formal analysis, Software, Writing – review & editing. YG: Methodology, Supervision, Writing – review & editing. MD: Formal analysis, Supervision, Writing – review & editing. DZ: Investigation, Writing – review & editing. DL: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. GL: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

References

- Zhang Q, Li C, Niu X, Zhang Z, Li F, Li F. The effects of milk replacer allowance and weaning age on the performance, nutrients digestibility, and ruminal microbiota communities of lambs. *Anim Feed Sci Technol*. (2019) 257:114263. doi: 10.1016/j.anifeedsci.2019.114263
- Carballo OC, Khan MA, Knol FW, Lewis SJ, Stevens DR, Laven RA, et al. Impact of weaning age on rumen development in artificially reared lambs1. *J Anim Sci*. (2019) 97:3498–510. doi: 10.1093/jas/skz148
- Wang S, Chai J, Zhao G, Zhang N, Cui K, Bi Y, et al. The temporal dynamics of rumen microbiota in early weaned lambs. *Microorganisms*. (2022) 10. doi: 10.3390/microorganisms10010144
- Li C, Wang W, Liu T, Zhang Q, Wang G, Li F, et al. Effect of early weaning on the intestinal microbiota and expression of genes related to barrier function in lambs. *Front Microbiol*. (2018) 9:1431. doi: 10.3389/fmicb.2018.01431
- Guo G, Yang W, Fan C, Lan R, Gao Z, Gan S, et al. The effects of fucoidan as a dairy substitute on diarrhea rate and intestinal barrier function of the large intestine in weaned lambs. *Front Vet Sci*. (2022) 9:1007346. doi: 10.3389/fvets.2022.1007346
- Zhang K, Xu Y, Yang Y, Guo M, Zhang T, Zong B, et al. Gut microbiota-derived metabolites contribute negatively to hindgut barrier function development at the early weaning goat model. *Anim Nutr*. (2022) 10:111–23. doi: 10.1016/j.aninu.2022.04.004
- Liu M, Liu W, Zhang W, Niu J, Yao J. Effect of marine red yeast *Rhodospiridium paludigenum* on diarrhea rate, serum antioxidant competence, intestinal immune capacity, and microflora structure in early-weaned lambs. *Biomed Res Int*. (2022) 2022:1–7. doi: 10.1155/2022/2228632
- Guo H, Cui J, Li Q, Liang X, Li J, Yang B, et al. A multi-omic assessment of the mechanisms of intestinal microbes used to treat diarrhea in early-weaned lambs. *mSystems*. (2024) 9:e00923–53. doi: 10.1128/mSystems.00953-23
- Bollen C, Louwagie E, Verstraeten N, Michiels J, Ruelens P. Environmental, mechanistic and evolutionary landscape of antibiotic persistence. *EMBO Rep*. (2023) 24:e57309. doi: 10.15252/embr.202357309
- Sharma K, Kumar M, Waghmare R, Suhag R, Gupta OP, Lorenzo JM, et al. Moringa (*Moringa oleifera* lam.) polysaccharides: extraction, characterization, bioactivities, and industrial application. *Int J Biol Macromol*. (2022) 209:763–78. doi: 10.1016/j.ijbiomac.2022.04.047
- Al Husnani LA, Alkahtani MDE. Impact of moringa aqueous extract on pathogenic bacteria and fungi in vitro. *Ann Agric Sci*. (2016) 61:247–50. doi: 10.1016/j.aos.2016.06.003
- Özcan MM. Moringa spp: composition and bioactive properties. *S Afr J Bot*. (2020) 129:25–31. doi: 10.1016/j.sajb.2018.11.017
- Li C, Dong Z, Zhang B, Huang Q, Liu G, Fu X. Structural characterization and immune enhancement activity of a novel polysaccharide from *Moringa oleifera* leaves. *Carbohydr Polym*. (2020) 234:115897. doi: 10.1016/j.carbpol.2020.115897
- Li C, You L, Fu X, Huang Q, Yu S, Liu RH. Structural characterization and immunomodulatory activity of a new heteropolysaccharide from *Prunella vulgaris*. *Food Funct*. (2015) 6:1557–67. doi: 10.1039/C4FO01039F
- Schepetkin IA, Quinn MT. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. *Int Immunopharmacol*. (2006) 6:317–33. doi: 10.1016/j.intimp.2005.10.005

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by Science and Technology Program from Forestry Administration of Guangdong Province (2023KJXC004); National Natural Science Foundation of China (32102557); Guangdong Provincial Department of Agriculture and Rural Affairs Project (2022-XPY-00-009).

Conflict of interest

DZ was employed by Guangdong Leader Intelligent Agriculture Co., LTD.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

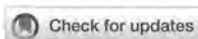
Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2024.1461391/full#supplementary-material>

16. Guo M, Li Z. Polysaccharides isolated from *Nostoc commune* Vaucher inhibit colitis-associated colon tumorigenesis in mice and modulate gut microbiota. *Food Funct.* (2019) 10:6873–81. doi: 10.1039/c9fo00296k
17. Tian H, Liang Y, Liu G, Li Y, Deng M, Liu D, et al. *Moringa oleifera* polysaccharides regulates caecal microbiota and small intestinal metabolic profile in C57BL/6 mice. *Int J Biol Macromol.* (2021) 182:595–611. doi: 10.1016/j.ijbiomac.2021.03.144
18. Zhao C, Li H, Gao C, Tian H, Guo Y, Liu G, et al. *Moringa oleifera* leaf polysaccharide regulates fecal microbiota and colonic transcriptome in calves. *Int J Biol Macromol.* (2023) 253:127108. doi: 10.1016/j.ijbiomac.2023.127108
19. Horwitz W, Latimer G. AOAC official methods of analysis of AOAC international. Gaithersburg, MD, USA: AOAC International (2005).
20. Li Y, Guo YL, Zhang CX, Cai XF, Liu P, Li CL. Effects of physical forms of starter feed on growth, nutrient digestibility, gastrointestinal enzyme activity, and morphology of pre- and post-weaning lambs. *Animal.* (2021) 15:100044. doi: 10.1016/j.animal.2020.100044
21. Wang Q, Wang Y, Wang X, Dai C, Tang W, Li J, et al. Effects of dietary energy levels on rumen fermentation, microbiota, and gastrointestinal morphology in growing ewes. *Food Sci Nutr.* (2020) 8:6621–32. doi: 10.1002/fsn3.15955
22. Ma T, Tu Y, Zhang NF, Deng KD, Diao QY. Effect of the ratio of non-fibrous carbohydrates to neutral detergent fiber and protein structure on intake, digestibility, rumen fermentation, and nitrogen metabolism in lambs. *Asian Australas J Anim Sci.* (2015) 28:1419–26. doi: 10.5713/ajas.15.0025
23. Lin L, Xie F, Sun D, Liu J, Zhu W, Mao S. Rumen microbiome-host crosstalk stimulates the development of the ruminal epithelium in a lamb model. *Microbiome.* (2019) 7:83. doi: 10.1186/s40168-019-0701-y
24. Wang W, Wang Y, Cui Z, Yang Y, An X, Qi J. Fermented wheat bran polysaccharides intervention alters rumen bacterial community and promotes rumen development and growth performance in lambs. *Front Vet Sci.* (2022) 9:841406. doi: 10.3389/fvets.2022.841406
25. Chen H, Guo B, Yang M, Luo J, Hu Y, Qu M, et al. Response of growth performance, blood biochemistry indices, and rumen bacterial diversity in lambs to diets containing supplemental probiotics and Chinese medicine polysaccharides. *Front Vet Sci.* (2021) 8:681389. doi: 10.3389/fvets.2021.681389
26. Abdelsattar M, Vargas-Bello-Pérez E, Zhang N. Age-related changes in blood biochemical composition of Hu sheep. *Ital J Anim Sci.* (2022) 21:1297–306. doi: 10.1080/1828051X.2022.2108730
27. Abdel-Salam AM, Zeitoun MM, Abdelsalam MM. Effect of synbiotic supplementation on growth performance, blood metabolites, insulin and testosterone and wool traits of growing lambs. *J Biol Sci.* (2014) 14:292–8. doi: 10.3923/jbs.2014.292.298
28. Kwon HJ, Kim Y, Choung SY. Effects of natural product extract on the fatty liver induced by alcohol diet in rats. *J Health Sci.* (2004) 50:466–73. doi: 10.1248/jhs.50.466
29. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem.* (1997) 43:1209–14. doi: 10.1093/clinchem/43.7.1209
30. Cheng Y, Chen Y, Li J, Qu H, Zhao Y, Wen C, et al. Dietary beta-sitosterol regulates serum lipid level and improves immune function, antioxidant status, and intestinal morphology in broilers. *Poult Sci.* (2020) 99:1400–8. doi: 10.1016/j.psj.2019.10.025
31. Yang W, Chen J, Guo G, Wang S, Peng S, Gao Z, et al. The effects of Fucoidan dietary supplementation on growth performance, serum antioxidant capacity, immune function indices and intestinal morphology in weaned kids. *Animals (Basel).* (2022) 12:574. doi: 10.3390/ani12050574
32. Su M, She Y, Deng M, Guo Y, Li Y, Liu G, et al. The effect of capsaicin on growth performance, antioxidant capacity, immunity and gut Micro-organisms of calves. *Animals (Basel).* (2023) 13. doi: 10.3390/ani13142309
33. Kushibiki S. Tumor necrosis factor- α -induced inflammatory responses in cattle. *Anim Sci J.* (2011) 82:504–11. doi: 10.1111/j.1740-0929.2011.00931.x
34. Yin M, Zhang Y, Li H. Advances in research on immunoregulation of macrophages by plant polysaccharides. *Front Immunol.* (2019) 10:10. doi: 10.3389/fimmu.2019.00145
35. Xie J, Wu D, Li W, Ning C, Tang Y, Zhao J, et al. Effects of polysaccharides in *Lycium Barbarum* berries from different regions of China on macrophages function and their correlation to the Glycosidic linkages. *J Food Sci.* (2017) 82:2411–20. doi: 10.1111/1750-3841.13813
36. Peng Y, Zhu X, Yang G, Zhang J, Wang R, Shen Y, et al. Ultrasonic extraction of *Moringa oleifera* seeds polysaccharides: optimization, purification, and anti-inflammatory activities. *Int J Biol Macromol.* (2024) 258:128833. doi: 10.1016/j.ijbiomac.2023.128833
37. Du X, Zhao Y, Ma Y, Xing H, Li X. Effect of Radix Hedysari polysaccharide on glioma by cell cycle arrest and TNF- α signaling pathway regulation. *Int J Polym Sci.* (2019) 2019:1–7. doi: 10.1155/2019/2725084
38. Sun D, Yin Y, Guo C, Liu L, Mao S, Zhu W, et al. Transcriptomic analysis reveals the molecular mechanisms of rumen wall morphological and functional development induced by different solid diet introduction in a lamb model. *J Anim Sci Biotechnol.* (2021) 12:33. doi: 10.1186/s40104-021-00556-4
39. Mentschel J, Leiser R, Mulling C, Pfarrer C, Claus R. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. *Arch Tierernähr.* (2001) 55:85–102. doi: 10.1080/17450390109386185
40. Malhi M, Gui H, Yao L, Aschenbach JR, Gabel G, Shen Z. Increased papillae growth and enhanced short-chain fatty acid absorption in the rumen of goats are associated with transient increases in cyclin D1 expression after ruminal butyrate infusion. *J Dairy Sci.* (2013) 96:7603–16. doi: 10.3168/jds.2013-6700
41. Petrič D, Mravčáková D, Kucková K, Kišidavová S, Cieslak A, Szumacher-Strabel M, et al. Impact of zinc and/or herbal mixture on ruminal fermentation, microbiota, and histopathology in lambs. *Front Vet Sci.* (2021) 8. doi: 10.3389/fvets.2021.630971
42. Belanche A, Palma-Hidalgo JM, Nejiam I, Jiménez E, Martín-García AI, Yáñez-Ruiz DR. Inoculation with rumen fluid in early life as a strategy to optimize the weaning process in intensive dairy goat systems. *J Dairy Sci.* (2020) 103:5047–60. doi: 10.3168/jds.2019-18002
43. Shao P, Sha Y, Liu X, He Y, Guo X, Hu J, et al. Astragalus additive in feed improved serum immune function, rumen fermentation and the microbiota structure of early-weaned lambs. *J Appl Microbiol.* (2023) 134:1–14. doi: 10.1093/jambio/lxad278
44. Lv F, Wang X, Pang X, Liu G. Effects of supplementary feeding on the rumen morphology and bacterial diversity in lambs. *PeerJ.* (2020) 8:e9353. doi: 10.7717/peerj.9353
45. Norouzzian MA, Valizadeh R, Vahmani P. Rumen development and growth of Balouchi lambs offered alfalfa hay pre- and post-weaning. *Trop Anim Health Prod.* (2011) 43:1169–74. doi: 10.1007/s11250-011-9819-z
46. Xie B, Huang W, Zhang C, Diao Q, Cui K, Chai J, et al. Influences of starter NDF level on growth performance and rumen development in lambs fed isocaloric and isonitrogenous diets. *J Anim Sci.* (2020) 98:1–8. doi: 10.1093/jas/skaa093
47. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2
48. Bian G, Yu S, Cheng C, Huang H, Liu J. Rumen microbiota-host crosstalks promote ruminal epithelial development in neonatal lambs with alfalfa hay introduction. *mSystems.* (2024) 9:e0103423. doi: 10.1128/mSystems.01034-23
49. Itnan R, Voigt J, Schönhusen U, Wegner J, Kokardova M, Hagemester H, et al. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. *Arch Anim Nutr.* (1998) 51:279–91. doi: 10.1080/17450399809381926
50. Edwards JE, McEwan NR, Travis AJ, Wallace RJ. 16S rDNA library-based analysis of ruminal bacterial diversity. *Antonie Van Leeuwenhoek.* (2004) 86:263–81. doi: 10.1023/B:ANTO.0000047942.69033.24
51. Mizrahi I, Wallace RJ, Morais S. The rumen microbiome: balancing food security and environmental impacts. *Nat Rev Microbiol.* (2021) 19:553–66. doi: 10.1038/s41579-021-00543-6
52. Arshad MA, Hassan FU, Rehman MS, Huws SA, Cheng Y, Din AU. Gut microbiome colonization and development in neonatal ruminants: strategies, prospects, and opportunities. *Anim Nutr.* (2021) 7:883–95. doi: 10.1016/j.aninu.2021.03.004
53. Yin X, Ji S, Duan C, Tian P, Ju S, Yan H, et al. Age-related changes in the ruminal microbiota and their relationship with rumen fermentation in lambs. *Front Microbiol.* (2021) 12:679135. doi: 10.3389/fmicb.2021.679135
54. Zhang K, Li B, Guo M, Liu G, Yang Y, Wang X, et al. Maturation of the goat rumen microbiota involves three stages of microbial colonization. *Animals (Basel).* (2019) 9:1028. doi: 10.3390/ani9121028



OPEN ACCESS

EDITED BY

Ran Di,
Institute of Animal Sciences (CAAS), China

REVIEWED BY

Jianning He,
Qingdao Agricultural University, China
Wei Luo,
Guangdong Academy of Agricultural
Sciences, China

*CORRESPONDENCE

Dewu Liu
✉ dwliu@scau.edu.cn
Guangbin Liu
✉ gbliu@scau.edu.cn

SPECIALTY SECTION

This article was submitted to
Livestock Genomics,
a section of the journal
Frontiers in Veterinary Science

RECEIVED 16 January 2023

ACCEPTED 14 March 2023

PUBLISHED 28 March 2023

CITATION

Hou B, Mao M, Dong S, Deng M, Sun B, Guo Y,
Li Y, Liu D and Liu G (2023) Transcriptome
analysis reveals mRNAs and long non-coding
RNAs associated with fecundity in the
hypothalamus of high-and low-fecundity goat.
Front. Vet. Sci. 10:1145594.
doi: 10.3389/fvets.2023.1145594

COPYRIGHT

© 2023 Hou, Mao, Dong, Deng, Sun, Guo, Li,
Liu and Liu. This is an open-access article
distributed under the terms of the [Creative
Commons Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Transcriptome analysis reveals mRNAs and long non-coding RNAs associated with fecundity in the hypothalamus of high-and low-fecundity goat

Biwei Hou, Min Mao, Shucan Dong, Ming Deng, Baoli Sun,
Yongqing Guo, Yaokun Li, Dewu Liu* and Guangbin Liu*

College of Animal Science, South China Agricultural University, Guangzhou, China

As an important organ that coordinates the neuroendocrine system, the hypothalamus synthesizes and secretes reproductive hormones that act on the goat organism, thereby precisely regulating follicular development and reproductive processes in goats. However, it is still elusive to explore the mechanism of hypothalamic effects on goat fertility alone. Therefore, RNA-seq was used to analyze the gene expression in hypothalamic tissues of goats in high fertility group (HFG: litter size per litter ≥ 2) and low fertility group (LFG: litter size per litter = 1), and identified the differential lncRNAs and mRNAs and their associated pathways related to their fertility. The results showed that a total of 23 lncRNAs and 57 mRNAs were differentially expressed in the hypothalamic tissue of high and low fertility goats. GO terms and KEGG functional annotation suggest that DE lncRNAs and DE mRNAs were significantly enriched in hormone-related pathways regulating ovarian development, hormone synthesis and secretion, regulation of reproductive processes, Estrogen signaling pathway, Oxytocin signaling pathway and GnRH signaling pathway. And we constructed a co-expression network of lncRNAs and target genes, and identified reproduction-related genes such as NMUR2, FEZF1, and WT1. The sequencing results of the hypothalamic transcriptome have broadened our understanding of lncRNA and mRNA in goat hypothalamic tissue and provided some new insights into the molecular mechanisms of follicle development and regulation of its fertility in goats.

KEYWORDS

Chuanzhong goat, hypothalamus, fecundity, mRNA, lncRNA

1. Introduction

The goat (*Capra hircus*) is one of the major livestock species, which is widely distributed in various regions due to its roughage resistance and strong adaptability. At the same time, it can provide people with daily necessities such as meat and milk, so it plays an important role in the animal husbandry (1). Fecundity is the ability of animals to produce live offspring, and high fecundity usually means higher economic efficiency in livestock species (2). Kidding trait is an important economic trait in goat reproduction, and kidding number is a key indicator of goat kidding trait, which is directly related to the economic benefits of goat farming (3). In addition, the number of lambs is not only affected by nutrient levels and environment, but also by a complex genetic component of quantitative traits controlled by

intrinsically micro-effective polygenes (4). Therefore, improving goat fecundity is the key way to change the status quo of the low level of goat breeding, and it is crucial to study its internal reproductive mechanism in depth.

Long non-coding RNAs (lncRNAs) are transcribed under the direction of RNA polymerase II/III and are longer than 200 nucleotides long-stranded RNAs, which are widely found in various eukaryotic organisms (5). Since lncRNAs do not encode any proteins, and most of the primary structures of lncRNAs are poorly conserved among different species, lncRNAs are generally considered by the scientific community as transcriptional “noise” (6). However, with the in-depth study of the genomic information of organisms, more and more scholars have paid attention to the special functions of lncRNAs in organisms. Studies have shown that lncRNAs play important regulatory roles in epigenetic control and regulation of transcription (7), translation (8), RNA metabolism, as well as in stem cell maintenance and differentiation (9), cell autophagy and apoptosis, and embryonic development (10). Recently, lncRNAs have been identified as an important RNA that regulates fertility-related RNAs in the sheep hypothalamus (11). Chen et al. (4) found that lncRNA LINC-676 and WNT3-AS acting in cis on DRD2 and WNT9B may induce gonadotropin-releasing hormone (GnRH) secretion at different stages by collecting hypothalamic tissues from wild-type and *FecB* genotyped sheep and performing whole transcriptome sequencing in ewes in follicular and luteal phases. However, there are few studies on mRNAs and long non-coding RNAs associated with fertility in the hypothalamus of high-and low-fecundity goats.

Early studies have shown that there is a high genetic correlation between ovulation rate and kidding rate (12). As the ovulation activity of animals is affected by the reproductive endocrine regulation (13), and the hypothalamus-pituitary-ovary (Hypothalamus-Pituitary-Ovary, HPO) axis plays a leading role in its reproductive regulation, the reproduction-related hormones released by various levels of the HPO axis participate in the whole regulation process as the key signal carrier (14). Where hypothalamic activation and gonadotropin-releasing hormone (GnRH) secretion are considered to be the decisive upstream factors in initiating the reproductive cascade to initiate pituitary and ovarian function. The hypothalamus regulates a large number of reproductive activities by integrating endocrine signals from the whole body and neural signals from the upstream brain, and its neuronal population is the central regulator of energy homeostasis and reproductive function (15). The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which reaches the pituitary gland *via* the pituitary portal system and binds to the corresponding receptors in the adenohypophyseal cells, stimulating them to secrete follicle stimulating hormone (FSH) or luteinizing hormone (LH), etc., to regulate reproductive activity downstream of the body (16). It has been shown that differential expression of hypothalamic genes can have an effect on reproductive traits in animals. Studies indicated that differential expression of the DRD1 gene in the hypothalamus may affect reproductive hormone secretion and ovulation numbers in ewes throughout the breeding season (17). In addition, DRD1 gene knockout in mice also revealed that DRD1 gene influences reproductive activity by regulating the secretion of GnRH, FSH, LH and PRL, suggesting that the differential expression of

DRD1 gene in hypothalamus is closely related to the fertility of animals (18).

So far, the most intensive research on lncRNAs has focused on the level of ovarian tissue in the HPO axis, but little is known about its role in the hypothalamus and its mechanism of regulating reproductive hormones in goats, and the synergistic action of hypothalamus, pituitary and ovary can promote the moderate development of sexual organs and the formation of gametes (19). Previous studies have confirmed that the expression levels of certain genes in the hypothalamus directly or indirectly significantly affect reproductive activities such as follicle formation, follicular development and ovulation, while the ovulation rate of goats directly affects the fecundity of goats (20). Therefore, in this study we performed differential expression analysis and gene function analysis using RNA-seq, and established an lncRNA-mRNA interaction network, in order that the correlation between the level of fertility and the expression of certain genes in the hypothalamus of goats could be explored in depth, thus laying the foundation for future studies on the molecular mechanisms regulating fertility in goats.

2. Materials and methods

2.1. Animals and sample collection

We selected 11 healthy Chuanzhong (CZ) female goats (3.5–4.5 years old) from a commercial farm with more than 3 litters were divided into high fecundity group and low fecundity group. The litter size of female goats in high fecundity group ($n = 6$) were more than 2 per litter, the litter size of female goats in low fecundity group ($n = 5$) were only 1 per litter. These ewes eat and drink freely under natural light conditions. After estrus synchronization, the selected goats were slaughtered and dissected, and the hypothalamus tissues were collected immediately and frozen in liquid nitrogen before being returned to the laboratory for RNA extraction.

2.2. RNA extraction and quality determination

Total RNA was extracted from the hypothalamus tissues of 11 CZ female goats using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA quality was estimated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and NanoDrop spectrophotometer (ND-2000, Thermo Fisher Scientific, Wilmington, DE, USA). The RNA integrity was evaluated by 1% agarose gel; purified RNA was stored at -80°C . High quality RNA (quantity $>6\ \mu\text{g}$, concentration $\geq 200\ \text{ng/mL}$, $1.8 < \text{OD}_{260/280} < 2.2$, and RNA integrity number > 8.5) was used for preparing the cDNA libraries.

2.3. RNA library construction and sequencing

The ribosomal RNAs (rRNAs) were removed from the total RNA using the Ribo-Zero rRNA Removal Kit (Illumina, Inc.), and

then the RNA was fragmented approximately 200–300 bp. The first-strand cDNA was synthesized using a random hexamer primer and reverse transcriptase. Using the first-strand cDNA as a template, the second-strand cDNA synthesis was subsequently performed. Then, the further hybridization was ligated with Sequencing Adaptor after adenylation of 3' ends of the DNA fragments. To select cDNA fragment preferentially 300–400 bp in length, the library fragments were purified with the AMPure XP system (Beckman Coulter, CA, USA). Subsequently, PCR amplification was performed to enrich cDNA libraries. The library quality was accessed on the Thermo Scientific StepOnePlus Real-Time System (Thermo Scientific, NY, USA). Finally, the cDNA libraries were sequenced on a HiSeq platform (Illumina HiSeq X-ten PE150) in Shanghai Personal Biotechnology Co. Ltd. (Shanghai, China), and 150 bp paired-end reads were generated.

2.4. Quality control, mapping, and transcriptome assembly

After converting the raw image data generated by the HiSeq platform (Illumina) into raw data in the FASTQ format, quality control was carried out. The clean data (clean reads) were obtained by removing the reads containing adapter or poly-N and other low-quality reads from the raw data using Cutadapt. Swine reference genome and gene model annotation files were downloaded from the genome website directly (asia.ensembl.org/index.html). Then, the clean reads were mapped to the goat reference genome using TopHat2 software. The transcripts obtained were assembled and the abundance estimation was performed.

2.5. Identification of lncRNAs

To reduce the false-positive rates, transcripts were assembled to obtain candidate lncRNAs by following steps: (1) Transcripts with a single exon and <200 bp in length were removed; (2) The transcripts with reads coverage <3 were removed; (3) Transcripts with protein coding potency were removed by three software: Coding Potential Calculator 0.9r2 (CPC), Coding Non-Coding Index v2 (CNCI) and Pfamscan (1.6). Subsequently, the number of candidate lncRNAs was obtained.

2.6. Differential expression analysis of lncRNAs and mRNAs

lncRNA and mRNA expression in each sample was evaluated based on the fragments per kilobase per million mapped reads (FPKM). Those lncRNAs and mRNAs with $|\log_2(\text{Fold change})| > 1$ and significant P -value < 0.05 were considered as DE between LF and HF groups. The R Pheatmap (1.0.8) software package was used for bidirectional cluster analysis of lncRNA and mRNA.

2.7. Target gene prediction and functional enrichment analysis

Gene transcript within 100 kb upstream or downstream of the DE lncRNAs was selected as cis target genes. To understand the potential roles of DE lncRNAs and DE mRNAs between LF and HF groups, Gene ontology (GO) enrichment analysis and the Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis were performed to investigate the biological function of the DE lncRNAs and DE mRNAs using DAVID (<http://david.abcc.ncifcrf.gov/>). $p < 0.05$ indicated statistical significance.

2.8. lncRNA-mRNA co-expression network analysis

The Pearson correlation test was used to calculate the correlation coefficient between DE lncRNAs and DE mRNAs in the hypothalamus. The Pearson correlation coefficient >0.95 and P -value < 0.05 were considered statistically significant. And the data was analyzed by Cytoscape 3.9.1 to visualize lncRNA-mRNA co-expression networks.

2.9. qRT-PCR verification

Three DElncRNAs and three DEMRNAs were randomly selected from hypothalamic tissues, and the accuracy of RNA-Seq was verified by qRT-PCR. Total RNA was reverse transcribed to cDNA using the PrimeScript RT kit and gDNA Eraser (TaKaRa, Guangzhou, China), and design primers for lncRNA and mRNA by Primer Premier 5.0. Then the qPCR reaction was performed in triplicate using an SYBR Premix Ex Taq TM (TaKaRa, Guangzhou, China) on the Thermo Scientific StepOnePlus Real-Time System (Thermo Scientific, NY, USA) as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s, respectively. Using β -actin as endogenous control, the relative expression of DE lncRNAs and DE mRNAs were quantified using the $2^{-\Delta\Delta Ct}$ method.

3. Results

3.1. Quality control of RNA sequencing data and read mapping

The raw reads from the high and low fecundity groups were analyzed for quality control before further analyses. In this study, 11 cDNA libraries in the hypothalamus-related tissues were constructed to identify the lncRNAs and mRNAs expressed in the HF and LF groups. The number of raw reads generated on the Illumina HiSeq platform was in the range of 101,379,018 to 106,945,128 (Supplementary Table 1). After discarding the reads containing adapter or poly-N and other low-quality reads, more than 99.10% of raw reads were filtered as clean reads and were used for the transcriptome assembly. The number of clean reads mapped to the CZ goats genome range from 86,849,768 to 92,341,252

in all libraries, and more than 97.62% were uniquely mapped. Additionally, the proportion of nucleotides with Q30 of each sample was greater than 92%. The detailed information about the genome distribution of the CZ goats genome was present in [Supplementary Table 1](#).

3.2. Identification of DE mRNAs and DE lncRNA in the hypothalamus of the high and low fecundity groups

In order to further explore the important regulators of the hypothalamic-pituitary-ovarian axis associated with goat prolificacy, we screened DE lncRNAs and DE mRNAs between LF group and HF group according to $|\log_2 \text{Fold Change}| > 1$ and P value < 0.05 . Consequently, a total of 23 DE lncRNA of which 14 lncRNA were up-regulated and 9 lncRNA were down-regulated were identified from the hypothalamus of HF groups compared to the hypothalamus of LF groups. The 9 down-regulated lncRNAs mainly include MSTRG.12755.2, MSTRG.20658.2 and ENSCHIT00000001669, and the 14 up-regulated lncRNAs mainly include ENSCHIT00000001894, ENSCHIT00000000939, and ENSCHIT000000009853, etc. Meanwhile, the analysis identified 57 DE mRNAs of which 27 mRNAs were up-regulated and 30 mRNAs were down-regulated between comparison groups in the hypothalamus. The 27 up-regulated mRNAs mainly included NUPR1, WT1, TP53I3 and FBLN7, and the 30 down-regulated mRNAs mainly included GNRH1, FEZF1, NPTX2, and CYP4X1 ([Supplementary Table 1](#)).

In addition, the volcano plot displayed the basic distribution of DE lncRNA and DE mRNAs, respectively ([Figures 1A, B](#)); Hierarchical cluster analysis was also used to determine the expression patterns of DE lncRNA and DE mRNAs under LF and HF conditions, and the heat maps of DE lncRNA and DE mRNAs showed a clear separation between LF and HF groups in the hypothalamus tissues, respectively ([Figures 1C, D](#)). The 6 samples (H1-H6) of the hypothalamus high fecundity group were clustered in the same cluster, and the 5 samples (L1-L5) of the low fecundity group were clustered in the same cluster. The results showed that the gene expression levels and patterns of the 6 samples in the high fecundity group were similar, and the gene expression levels and patterns of the 5 samples in the low fecundity group were similar. There were significant differences between the two groups of data, which confirmed the high reliability of the hypothalamic sequencing data.

3.3. Functional enrichment analysis in the hypothalamus

3.3.1. Functional enrichment analysis of DE lncRNAs

GO and KEGG enrichment analysis of DE lncRNAs expression was performed and found that 23 potential target genes were identified in the hypothalamus. For these genes, a total of 238 GO terms were significantly ($P < 0.05$) enriched in the hypothalamus. Of which biological processes, cellular components and molecular

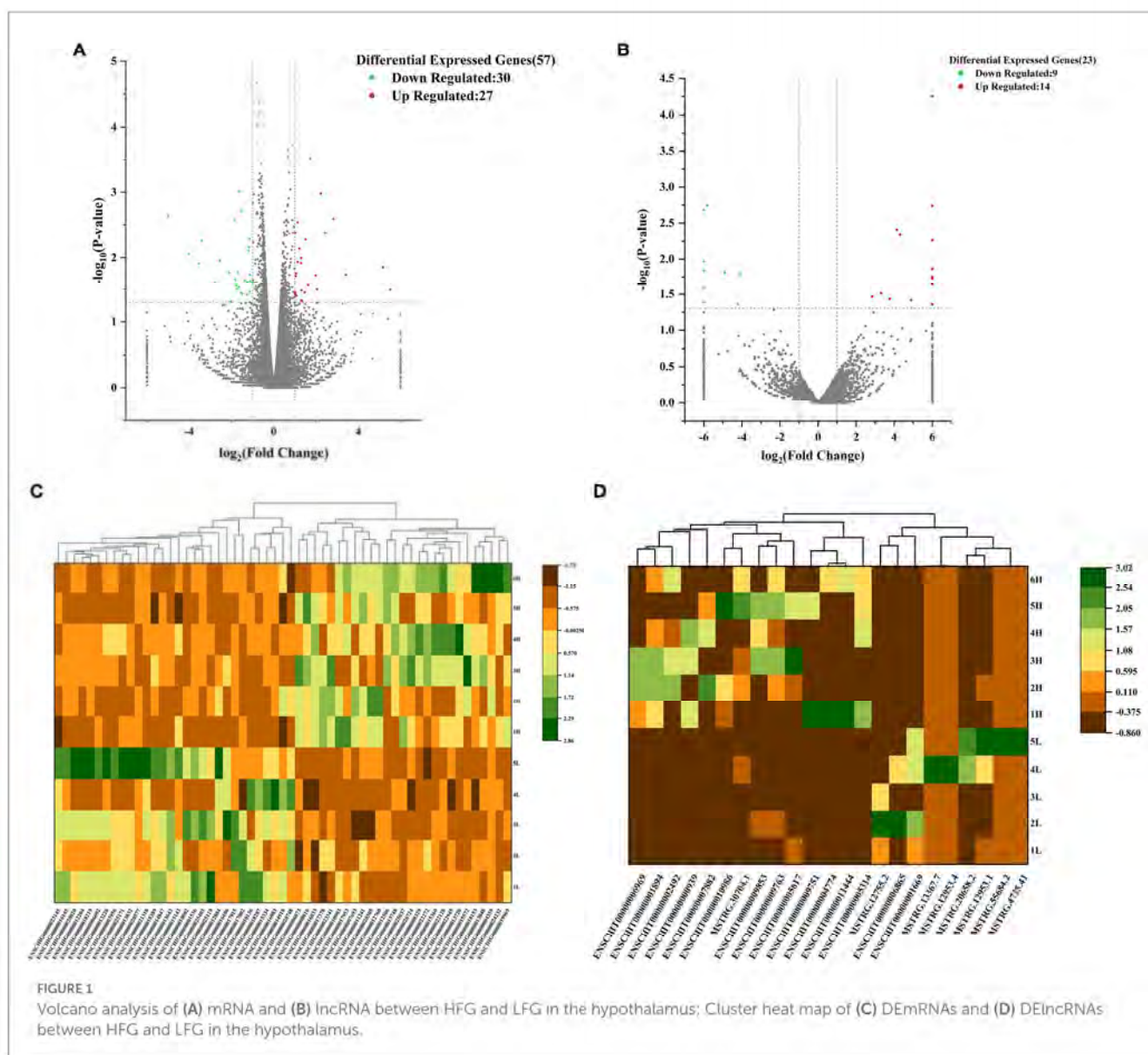
functions account for 188, 27, and 23 terms, respectively. And 5 KEGG pathways were enriched in the hypothalamus tissue ([Supplementary Table 1](#)).

We selected the GO terms with $p < 0.05$ as a significant condition in the database, and sorted the top 20 GO terms and drew them into a bubble chart. Among them, 10 GO terms belong to biological processes, and 5 terms each for cellular components and molecular functions. Therefore, In the hypothalamus, the GO enrichment analysis showed most target genes of DE lncRNAs were mainly significantly ($P < 0.05$) enriched in nucleus, chromosomal part, chromatin, chromosome, and regulation of sister chromatid cohesion. However, Starch and sucrose metabolism were the only pathway in KEGG pathway analysis of DE lncRNAs in the hypothalamus ($P < 0.05$) ([Figures 2A–D](#)).

3.3.2. Functional enrichment analysis of DE mRNAs

For DE mRNAs, a total of 57 hypothalamic DE mRNAs were significantly enriched in 301 GO terms ($P < 0.05$). Biological processes, cellular component, and molecular functions were occupied by 256, 1, and 44 terms, respectively. The top 20 GO terms were drawn into a bubble chart, and the results showed that there are 2 terms in molecular function, mainly hormone activity and receptor regulator activity. The remaining 18 terms belong to biological processes. Among them, 32 DEGs were specifically enriched in regulation of nervous system development, receptor regulator activity, neuropeptide signaling pathway and activation of phospholipase A2 activity by calcium-mediated signaling terms, such as the down-regulated genes GNRH1, NPTX1, NPTX2, VAX1, TNFRSF12A, and SST, suggesting that these genes may be involved in the regulation of nervous system and neurotransmitter receptor activity as well as the function of synaptic signaling. Secondly, 22 DEGs were mainly enriched in terms of regulation of gonad development, hormone activity, and regulation of reproductive process, including the upregulated gene NUPR1 and the downregulated genes GNRH1, NR5A1, SST, QRFP, and VIP, indicating that these genes are mainly associated with the fecundity of goats. Finally, there are 3 DEGs mainly enriched in terms of proline catabolic process and D-serine catabolic process, such as NMUR2 and DAO, which are mainly involved in cellular metabolic pathways ([Figures 3A–D](#)).

Besides, to further clarify the contribution of specific signaling pathways to goat fecundity, analysis of KEGG pathways in DE mRNAs from high- and low-fecundity Goats revealed 25 enriched pathways, which involved Neuroactive ligand-receptor interaction, Cell adhesion molecules (CAMs), Estrogen signaling pathway, Oxytocin signaling pathway and GnRH signaling pathway ($P < 0.05$). Among them, the Estrogen signaling pathway, the Oxytocin signaling pathway and the GnRH signaling pathway were the main KEGG pathways enriched in the hypothalamus. The genes regulating the Estrogen signaling pathway were mainly the down-regulated genes KCNJ5 and MMP9. The genes corresponding to the Oxytocin signaling pathway mainly included the up-regulated gene PRKAG3 and the down-regulated gene KCNJ5. The only gene that regulates the GnRH signaling pathway is GNRH1. The top 20 GO



terms and top 20 KEGG pathways for the DEMRNAs are shown in [Supplementary Table 1](#).

3.4. Construction of lncRNA-mRNA co-expression network

To further explore the interaction between DE lncRNAs and DE mRNAs in the hypothalamus of high and low fecundity goats, we constructed network of co-expression of DE lncRNAs and DE target mRNAs. A total of 14 DE lncRNAs and 8 DE mRNAs were involved in the network, which consists of 14 edges ([Figure 4](#), co-expression network diagram). Interestingly, 4 DE mRNAs were also found to interact with more than one DE lncRNA in the hypothalamus. The mRNAs with the largest number of interactions were NMUR2 and ENSCHIG00000010903. ENSCHIG00000010903 was negatively correlated with 3 different

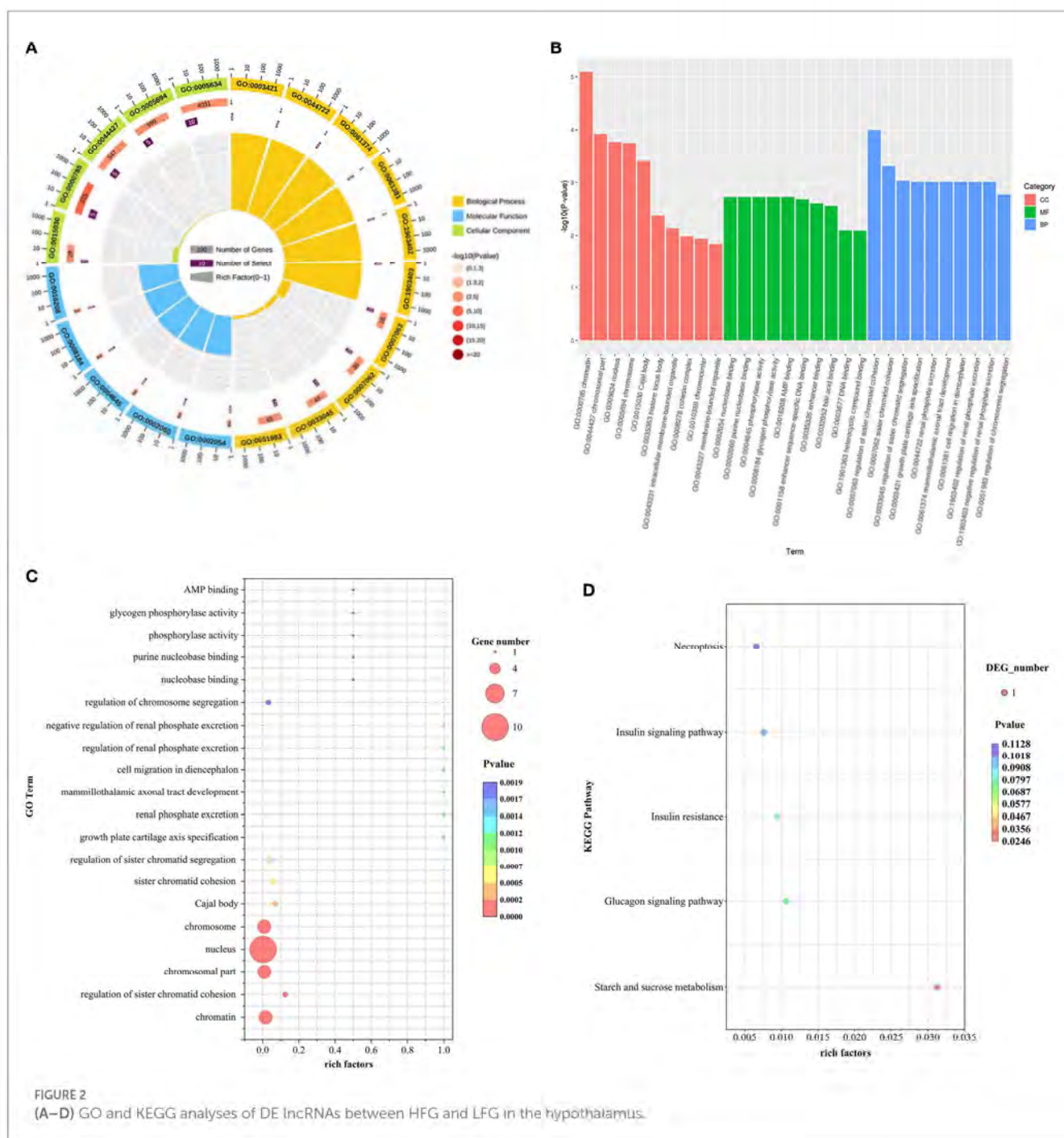
DE lncRNAs, while NMUR2 was positively correlated with 3 different DE lncRNAs.

3.5. The qPCR validation

Three DE lncRNAs and three DE mRNAs were randomly selected from the hypothalamus tissue for qPCR to verify their differential expression ([Figure 5](#)). The results were in concordance with the sequencing data.

4. Discussion

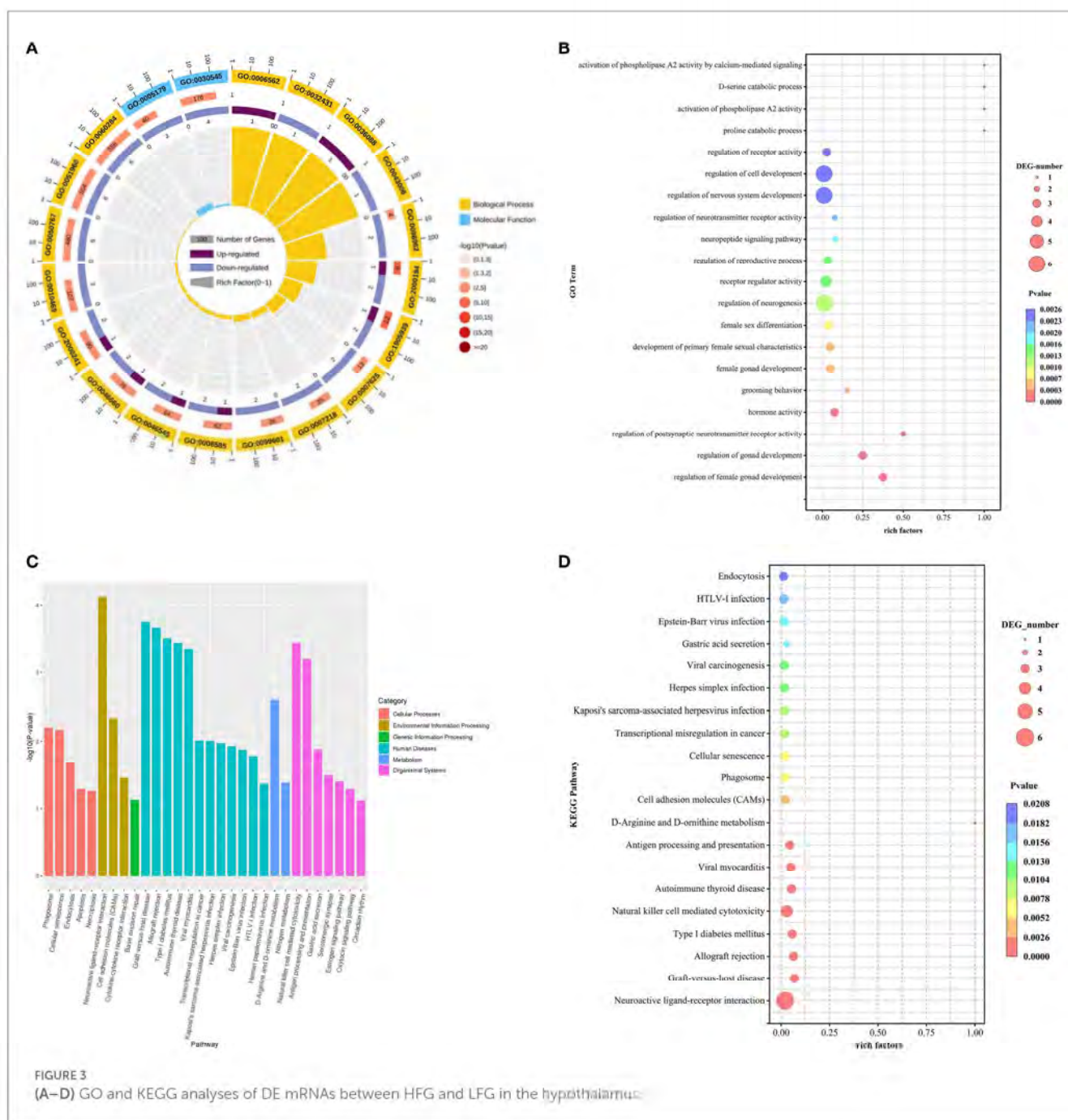
The reproductive performance of goats directly determines the productivity of animal husbandry (21). As the higher center of the neuroendocrine system, the hypothalamus releases hormones that play an indispensable role in the regulation of estrus and



follicular development in goats (22). For example, it can regulate the release of E2 and P4 from the ovary by releasing gonadotropin-releasing hormone (GnRH), while acting as a facilitator or inhibitor of FSH and LH secreted by the pituitary (23). It has been shown that lncRNAs have an increasingly prominent role in reproduction in goats and can be involved in a variety of reproductive activities, such as spermatogenesis (24), placental formation (25, 26), sex hormone response signaling pathways (27), and gonadogenesis (28). However, current studies on lncRNAs associated with fecundity in goats have mainly focused on ovarian tissues (11), ignoring the importance of hypothalamic tissues. Therefore, this study investigated the expression profiles of

reproduction-related lncRNAs and mRNAs in goat hypothalamic tissues by means of RNA-Seq technology, and explored the relationship between DE lncRNAs and mRNAs by generating co-expression networks, thus laying the foundation for a clear explanation of the expression and regulation of reproductive traits in goats.

In this study, we selected six high-breeding and five low-breeding healthy Chuanzhong female goats for identification and characterization of lncRNAs and mRNAs in their hypothalamic tissues to identify differential mRNAs, lncRNAs and pathways associated with reproductive performance. Finally, we screened 23 DE lncRNA transcripts and 57 DEMRNAs in the hypothalamus of



Chuanzhong Black Goats between high and low yielding goats. In total 57 of these DEGs were mainly enriched in the regulation of ovarian development, hormone synthesis and secretion, regulation of postsynaptic neurotransmitter receptor activity and regulation of reproductive processes. NUPR, GNRH1, NR5A1, SST, QRFP, and VIP DEGs were found to be involved in the release of hypothalamic hormones and thus could regulate reproductive performance in goats. An et al. (29) analyzed 641 goats of three breeds for *GNRH1* gene polymorphism and found that *GNRH1* was closely related to litter size in different breeds by association analysis and could be used as a genetic marker for litter size in goat breeding. Knight et al. (30) induced the release of LH and thus normalized estrus and

ovulation in goats with estrus difficulties by injecting the exogenous hormone GnRH. KEGG enrichment analysis showed that DEGs in the hypothalamus of high and low breeding goats were mainly involved in signaling pathways such as estrogen signaling pathway, oxytocin signaling pathway and GnRH signaling pathway. Among them, DEGs such as CALM3, GNRH1, ITPR2, ADCY4, PLD2, and MAP2K1 were significantly enriched in the GnRH signaling pathway, and these DEGs may affect the kidding power of goats. Early studies have shown that calcium plays an important role in the activation and regulation of signal transduction pathways in oogenesis and that the calcium signaling pathway is a key regulatory component of meiosis during oogenesis (31). Recent

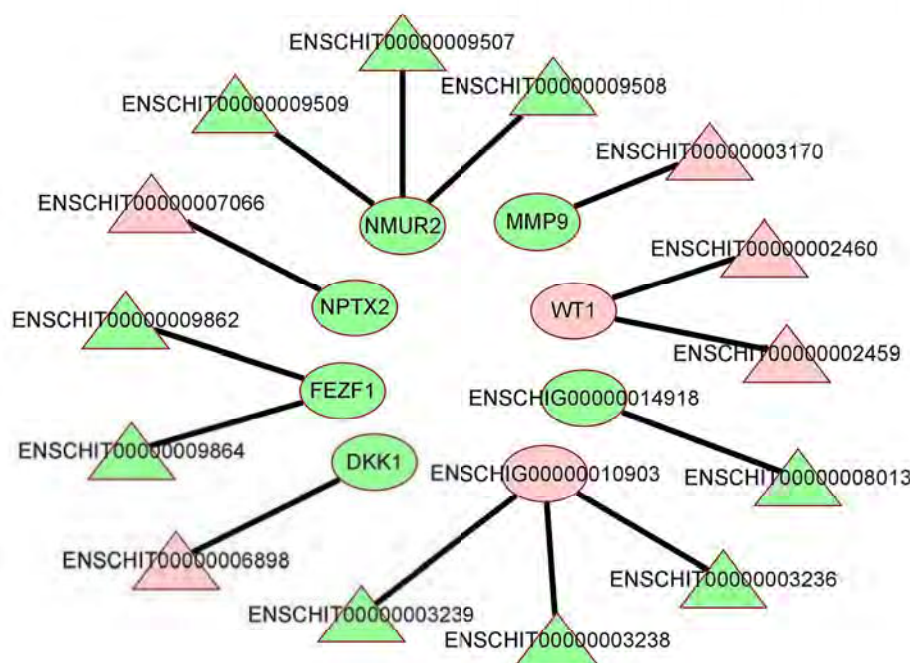


FIGURE 4

The co-expression network of lncRNA and mRNA between HFG and LFG in the hypothalamus. The red color represents upregulated expression, the green represents downregulated expression, the triangle represents lncRNA, and the ellipse represents mRNA.

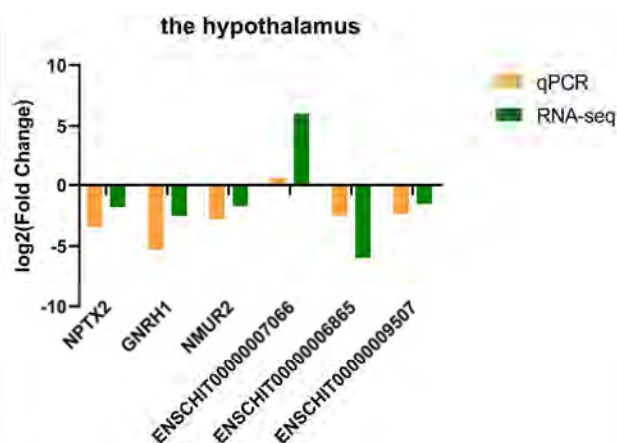


FIGURE 5

The qPCR validation results of DE lncRNAs and DE mRNAs for the hypothalamus.

studies suggest that CALM3 (calcium-modulated protein 3) may regulate oogenesis in tetraploid crucian carp through a calcium-associated pathway (32). ITPR2 is an important regulator of calcium channel activity, which is the basis of fertilization and embryonic development in animals (33, 34), and has been found to be involved in several reproduction-related pathways in sheep (35). In addition, DEGs such as NPR2, CALM3, CACNG2, KCNJ5, ITPR2, ADCY4, MAP2K1, CACNB1, and CAMK2B were mainly enriched in the oxytocin signaling pathway. C-type natriuretic

peptides (NPPC) in NPPC/NPR2 signaling and its high affinity receptor natriuretic peptide receptor 2 (NPR2) are considered to be related to female reproduction (36). They act as local factors by binding to NPR2, and then produce intracellular cGMP (37), cGMP through the guanylate cyclase catalytic domain of NPR2 to regulate oocyte meiosis arrest and ovarian follicular survival (38, 39). It can be seen that NPPC/NPR2 signal transduction is very important for oocyte meiosis arrest and cumulus formation, and cumulus can affect female fertility by producing developmental oocytes (36).

The co-expression analysis of lncRNA-mRNA showed that there was a certain interaction between lncRNA and mRNA. By regulating the expression of lncRNA, the mRNA related to the above signal pathways and biological processes could be regulated to affect the fecundity of goats. A total of 14 pairs of interaction between DElncRNAs and mRNAs were found in the hypothalamus of high and low breeding goats, some of which can directly or indirectly reflect the relationship between hypothalamus and goat fecundity. NMUR2, which is down-regulated in the hypothalamus, is the predictive target gene of DElncRNA ENSCHIT00000009507, ENSCHIT00000009508, and ENSCHIT00000009509. The translated protein can form a new autocrine system in membrane/interstitial cells after binding to its ligand, which plays a certain role in the ovary directly through cAMP signal transduction, in which the signal intensity is strictly controlled by gonadotropin (40). Romanov et al. (41) detected 62 neuronal subtypes in the hypothalamus by single cell RNA sequencing. The dopamine neuron gene of NMUR2 was placed in the periventricular nucleus with many synaptic afferents. It was found that these neuroendocrine dopamine cells may contribute

to the inhibition of dopaminergic secretion of prolactin during the day and night. Helfer et al. (42) injected NMUR2 into the hypothalamus by making the ligand NMU of NMU partially mimic the function of thyroid stimulating hormone (TSH), which can negatively regulate food intake and body weight of rats. At the same time, NMU increased the expression of Dio2 mRNA in the ependymal area of the hypothalamus. These results suggest that NMUR2 may affect the release of reproduction-related hormones in the neuroendocrine system and hypothalamus in different ways. In addition, DE lncRNA ENSCHIT00000009862 and ENSCHIT00000009864 in the hypothalamus are predicted to act on the target gene FEZF1, a family of Fez transcription factors that control neurogenesis and cellular fate in the developing mammalian nervous system (43). Zinc finger transcription factor FEZF1 is a highly conserved family of transcription factors that play a role in neurogenesis, developmental patterns, cell fate regulation and axonal guidance (44). And the function of the down-regulated target gene FEZF1 is consistent with the corresponding regulation of neurogenesis (GO:0050767) GO term in our sequencing results. In addition, we also found that the target gene WT1 corresponding to up-regulated DE lncRNA ENSCHIT00000002459 and ENSCHIT00000002460 was also closely related to ovarian follicular development in female animals. WT1 is a nuclear transcription factor, and Chen et al. (45) found that protein arginine methyltransferase 5 (PRMT5) deficient mouse follicular granulosa cells expressing steroid genes were reversed by WT1 overexpression, indicating that PRMT5 is necessary to prevent premature differentiation of granulosa cells by regulating WT1 expression. There is also a down-regulated DE lncRNA ENSCHIT00000003170 predicted target gene MMP9, as a matrix metalloproteinase regulated by LH or progesterone, which not only affects cell growth, differentiation, proliferation and apoptosis, but also plays an important role in ovulation and gonadal formation in female animals (46). Shah et al. (47) found that MMP9 is a key enzyme to maintain the normal physiology of the female reproductive system, and it can also participate in neuroendocrine regulation through gonadotropin-releasing hormone (GnRH). GnRH-mediated ERK1/2 activation in hypothalamic neurons and pituitary gonadotropins depends on MMP9. In addition, cell migration, division, differentiation, survival or death requires extracellular matrix (ECM) to provide the environment, and ECM homeostasis is strictly regulated by MMP9.

In conclusion, as the primary site in HPOA, the key function of the hypothalamus is to precisely regulate follicular development in goats by releasing hormones (48). The DE lncRNAs identified in this study cooperate with their target genes and DEGs to regulate hypothalamic function and reproductive processes through hormones and other regulators. However, we provided few indirect experimental results to infer the functional connectivity between lncRNA-mRNA network components, thus preventing a more complete and definitive proof of the obtained results, suggesting that there are still some limitations in our present study. In future studies, we will further demonstrate the linkage of our predicted lncRNAs and their potential target genes and elucidate how these differential lncRNAs and mRNAs play a role in goat fertility.

5. Conclusions

In this study, we revealed the regulation of differential lncRNAs and mRNAs in pathways associated with reproductive function in high- and low-fecundity goats through hypothalamic transcriptomics. Using the low-breeding group as a control, mRNA and lncRNA from the hypothalamus of goats in the high-breeding group were screened for differences and identified, and DE lncRNA and DEG were enriched and analyzed. And we also construct the interaction network between lncRNA and mRNA to provide some ideas for the follow-up study of the regulation mechanism of functional lncRNA in hypothalamus on goat follicular development.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Ethics Committees of the Laboratory Animal Center of South China Agricultural University (permit number: SYXK-2014-0136). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

Sample collection: SD and MM. Data curation and writing—original draft: BH. Methodology: BH and MM. Project administration: GL and DL. Software: GL and BS. Supervision: GL and YL. Validation: BH and SD. Writing—review and editing: GL. All authors have read and approved the manuscript.

Funding

This research was supported by the Modern Agricultural Industrial Technology System of Guangdong Province (2022KJ127) and Natural Science Foundation of Guangdong Province (2021A1515010636).

Acknowledgments

We thank Chuanzhong Sheep Farm for their care and management of goats. We also thank GL and DL for their help in collecting samples. Thanks to Novogene for collating the sequencing data and creating the diagrams.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2023.1145594/full#supplementary-material>

References

- Das A, Shaha M, Gupta MD, Dutta A, Miaz OF. Polymorphism of fecundity genes (BMP15 and GDF9) and their association with litter size in Bangladeshi prolific Black Bengal goat. *Trop Anim Health Prod.* (2021) 53:230. doi: 10.1007/s11250-021-02679-2
- Li Y, Xu X, Deng M, Zou X, Zhao Z, Huang S, et al. Identification and comparative analysis of long non-coding RNAs in high- and low-fecundity goat ovaries during estrus. *Front Genet.* (2021) 12:648158. doi: 10.3389/fgene.2021.648158
- Lian Z, Zou X, Han Y, Deng M, Sun B, Guo Y, et al. Role of mRNAs and long non-coding RNAs in regulating the litter size trait in Chuanshong black goats. *Reprod Domest Anim.* (2020) 55:486–95. doi: 10.1111/rda.13642
- Chen S, Guo X, He X, Di R, Zhang X, Zhang J, et al. Transcriptome analysis reveals differentially expressed genes and long non-coding RNAs associated with fecundity in sheep hypothalamus with different FecB genotypes. *Front Cell Dev Biol.* (2021) 9:633747. doi: 10.3389/fcell.2021.633747
- Golicz AA, Bhalla PL, Singh MB. lncRNAs in Plant and Animal Sexual Reproduction. *Trends Plant Sci.* (2018) 23:195–205. doi: 10.1016/j.tplants.2017.12.009
- Murillo-Maldonado JM, Riesgo-Escovar JR. The various and shared roles of lncRNAs during development. *Dev Dyn.* (2019) 248:1059–69. doi: 10.1002/dvdy.108
- Taylor DH, Chu ET, Spektor R, Soloway PD. Long non-coding RNA regulation of reproduction and development. *Mol Reprod Dev.* (2015) 82:932–56. doi: 10.1002/mrd.22581
- Yang CX, Wu ZW, Liu XM, Liang H, Gao ZR, Wang Y, et al. Single-cell RNA-seq reveals mRNAs and lncRNAs important for oocytes in vitro matured in pigs. *Reprod Domest Anim.* (2021) 56:642–57. doi: 10.1111/rda.13901
- Xiao H, Huang R, Chen L, Diao M, Li L. Integrating lncRNAs and mRNAs expression profiles in terminal hindgut of fetal rats with anorectal malformations. *Pediatr Surg Int.* (2018) 34:971–82. doi: 10.1007/s00383-018-4311-8
- Constanty F, Shkumatava A. lncRNAs in development and differentiation: from sequence motifs to functional characterization. *Development.* (2021) 148:dev182741. doi: 10.1242/dev.182741
- Zheng J, Wang Z, Yang H, Yao X, Yang P, Ren C, et al. Pituitary transcriptomic study reveals the differential regulation of lncRNAs and mRNAs related to prolificacy in different FecB genotyping sheep. *Genes.* (2019) 10:157. doi: 10.3390/genes10020157
- Smolucha G, Gurgul A, Jasiełczuk I, Kawecka A, Miksa-Cybulska A. A genome-wide association study for prolificacy in three Polish sheep breeds. *J Appl Genet.* (2021) 62:323–26. doi: 10.1007/s13353-021-00615-6
- Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci.* (2004) 82:461–477. doi: 10.1016/j.anireprosci.2004.05.013
- He J, Xu S, Ji Z, Sun Y, Cai B, Zhang S, et al. The role of miR-7 as a potential switch in the mouse hypothalamus-pituitary-ovary axis through regulation of gonadotropins. *Mol Cell Endocrinol.* (2020) 518:110969. doi: 10.1016/j.mce.2020.110969
- Hashem NM, Sallam SM. Reproductive performance of goats treated with free gonadorelin or nanoconjugated gonadorelin at estrus. *Domest Anim Endocrinol.* (2020) 71:106390. doi: 10.1016/j.domaniend.2019.106390
- Liang C, Han M, Zhou Z, Liu Y, He X, Jiang Y, et al. Hypothalamic transcriptome analysis reveals the crucial MicroRNAs and mRNAs affecting litter size in goats. *Front Vet Sci.* (2021) 8:747100. doi: 10.3389/fvets.2021.747100
- Tian D, Liu S, Tian F, Ding N, Li X, Zhao K. Comparative transcriptome of reproductive axis in Chinese indigenous sheep with different FecB genotypes and prolificacies. *Anim Reprod Sci.* (2020) 223:106624. doi: 10.1016/j.anireprosci.2020.106624
- Xu H, Shen X, Zhou M, Fang M, Zeng H, Nie Q, et al. The genetic effects of the dopamine D1 receptor gene on chicken egg production and broodiness traits. *BMC Genet.* (2010) 11:17. doi: 10.1186/1471-2156-11-17
- Refael T, Melamed P. Enhancing gonadotropin gene expression through regulatory lncRNAs. *Endocrinology.* (2021) 162:bqab116. doi: 10.1210/endo/bqab116
- Pramod RK, Sharma SK, Singh A, Pan S, Mitra A. Differential ovarian morphometry and follicular expression of BMP15, GDF9 and BMPRII influence the prolificacy in goat. *Reprod Domest Anim.* (2013) 48:803–9. doi: 10.1111/rda.12165
- Islam R, Liu X, Gebreselassie G, Abied A, Ma Q, Ma Y. Genome-wide association analysis reveals the genetic locus for high reproduction trait in Chinese Arbas Cashmere goat. *Genes Genomics.* (2020) 42:893–9. doi: 10.1007/s13258-020-00937-5
- Zi XD, Huang L, Wang Y, Lu JY. Comparative messenger RNA expression of FSHB, LHβ, FSHR, LHR, and ERβ in high and low prolific goat breeds. *Anim Biotechnol.* (2013) 24:307–11. doi: 10.1080/10495398.2013.790824
- Oyola MG, Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. *Stress.* (2017) 20:476–94. doi: 10.1080/10253890.2017.1369523
- Abdelmohsen K, Panda A, Kang MJ, Xu J, Selimyan R, Yoon JH, et al. Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. *Aging Cell.* (2013) 12:890–900. doi: 10.1111/ace.12115
- Gao WL, Liu M, Yang Y, Yang H, Liao Q, Bai Y, et al. The imprinted H19 gene regulates human placental trophoblast cell proliferation via encoding miR-675 that targets Nodal Modulator 1 (NOMO1). *RNA Biol.* (2012) 9:1002–10. doi: 10.4161/rna.20807
- Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, et al. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol.* (2012) 14:659–65. doi: 10.1038/ncb2521
- Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature.* (2013) 498:516–20. doi: 10.1038/nature12210
- Mulvey BB, Olcese U, Cabrera JR, Horabin JI. An interactive network of long non-coding RNAs facilitates the Drosophila sex determination decision. *Biochim Biophys Acta.* (2014) 1839:773–84. doi: 10.1016/j.bbarm.2014.06.007
- An XR, Hou JX, Zhao HB, Li G, Bai L, Peng JY, et al. Polymorphism identification in goat GNRH1 and GDF9 genes and their association analysis with litter size. *Anim Genet.* (2013) 44:234–8. doi: 10.1111/j.1365-2052.2012.02394.x
- Knight CH, Wilde CJ, McLeod BJ, Haresign W. Exogenous GnRH induces ovulation in seasonally anoestrous lactating goats (*Capra hircus*). *J Reprod Fertil.* (1988) 83:679–86. doi: 10.1530/jrf.0.0830679
- Lefèvre B, Nagyova E, Pesty A, Testart J. Acquisition of meiotic competence is related to the functionality of the phosphoinositide/calcium signaling pathway in the mouse oocyte. *Exp Cell Res.* (1997) 236:193–200. doi: 10.1006/excr.1997.3720
- Wang C, Qin H, Zhao C, Yang L, Yu T, Zhang Y, et al. Whole-genome re-sequencing and transcriptome reveal oogenesis-related genes in autotetraploid carassius auratus. *Mar Biotechnol (NY).* (2021) 23:233–41. doi: 10.1007/s10126-021-10018-7
- Miao YL, Williams CJ. Calcium signaling in mammalian egg activation and embryo development: the influence of subcellular localization. *Mol Reprod Dev.* (2012) 79:742–56. doi: 10.1002/mrd.22078
- Kuroda Y, Kaneko S, Yoshimura Y, Nozawa S, Mikoshiba K. Are there inositol 1,4,5-trisphosphate (IP3) receptors in human sperm? *Life Sci.* (1999) 65:135–43. doi: 10.1016/S0024-3205(99)00230-1

35. La Y, Tang J, Di R, Wang X, Liu Q, Zhang L, et al. Differential expression of circular RNAs in polytocous and monotocous uterus during the reproductive cycle of sheep. *Animals (Basel)*. (2019) 9:979. doi: 10.3390/ani9100797
36. Kiyosu C, Tsuji T, Yamada K, Kajita S, Kunieda T. NPPC/NPR2 signaling is essential for oocyte meiotic arrest and cumulus oophorus formation during follicular development in the mouse ovary. *Reproduction*. (2012) 144:187–93. doi: 10.1530/REP-12-0050
37. Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, et al. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology*. (1992) 130:229–39. doi: 10.1210/endo.130.1.1309330
38. McGee E, Spears N, Minami S, Hsu SY, Chun SY, Billig H, et al. Preantral ovarian follicles in serum-free culture: suppression of apoptosis after activation of the cyclic guanosine 3',5'-monophosphate pathway and stimulation of growth and differentiation by follicle-stimulating hormone. *Endocrinology*. (1997) 138:2417–24. doi: 10.1210/endo.138.6.5164
39. Törnell J, Billig H, Hillensjö T. Resumption of rat oocyte meiosis is paralleled by a decrease in guanosine 3',5'-cyclic monophosphate (cGMP) and is inhibited by microinjection of cGMP. *Acta Physiol Scand*. (1990) 139:511–7. doi: 10.1111/j.1748-1716.1990.tb08953.x
40. Lin TY, Wu FJ, Lee WY, Hsiao CL, Luo CW. Ovarian regulation of neuromedin U and its local actions in the ovary, mediated through neuromedin U receptor 2. *Am J Physiol Endocrinol Metab*. (2013) 304:E800–09. doi: 10.1152/ajpendo.00548.2012
41. Romanov RA, Zeisel A, Bakker J, Girach F, Helysaz A, Tomer R, et al. Molecular interrogation of hypothalamic organization reveals distinct dopamine neuronal subtypes. *Nat Neurosci*. (2017) 20:176–88. doi: 10.1038/nn.4462
42. Helfer G, Ross AW, Russell L, Thomson LM, Shearer KD, Goodman TH, et al. Photoperiod regulates vitamin A and Wnt/ β -catenin signaling in F344 rats. *Endocrinology*. (2012) 153:815–24. doi: 10.1210/en.2011-1792
43. Eckler MJ, Chen B. Fez family transcription factors: controlling neurogenesis and cell fate in the developing mammalian nervous system. *Bioessays*. (2014) 36:788–97. doi: 10.1002/bies.201400039
44. Shimizu T, Hibi M. Formation and patterning of the forebrain and olfactory system by zinc-finger genes *Fezf1* and *Fezf2*. *Dev Growth Differ*. (2009) 51:221–31. doi: 10.1111/j.1440-169X.2009.01088.x
45. Chen M, Dong F, Chen M, Shen Z, Wu H, Cen C, et al. PRMT5 regulates ovarian follicle development by facilitating Wt1 translation. *Elife*. (2021) 10:e68930. doi: 10.7554/eLife.68930
46. Zhu Y. Metalloproteases in gonad formation and ovulation. *Gen Comp Endocrinol*. (2021) 314:113924. doi: 10.1016/j.ygcen.2021.113924
47. Shah BH, Catt KJ. Matrix metalloproteinases in reproductive endocrinology. *Trends Endocrinol Metab*. (2004) 15:47–9. doi: 10.1016/j.tem.2004.01.004
48. Zhang Z, Tang J, Di R, Liu Q, Wang X, Gan S, et al. Comparative transcriptomics reveal key sheep (*Ovis aries*) Hypothalamus lncRNAs that affect reproduction. *Animals (Basel)*. (2019) 9:152. doi: 10.3390/ani9040152



Article

Integrating Analysis to Identify Differential circRNAs Involved in Goat Endometrial Receptivity

Wenjing Wang, Xupeng Zang, Yaokun Li, Dewu Liu, Linjun Hong and Guangbin Liu

Special Issue

Molecular Biology of RNA: Recent Progress

Edited by
Dr. Anne-Catherine Prats





Article

Integrating Analysis to Identify Differential circRNAs Involved in Goat Endometrial Receptivity

Wenjing Wang^{1,2,†}, Xupeng Zang^{1,2,†}, Yaokun Li^{1,2} , Dewu Liu^{1,2}, Linjun Hong^{1,2,*} and Guangbin Liu^{1,2,*}

¹ College of Animal Science, South China Agricultural University, Guangzhou 510642, China

² National Local Joint Engineering Research Center of Livestock and Poultry, South China Agricultural University, Guangzhou 510642, China

* Correspondence: linjun.hong@scau.edu.cn (L.H.); gblu@scau.edu.cn (G.L.); Tel.: +86-020-8528-1859 (L.H.)

† These authors have contributed equally to this work.

Abstract: Endometrial receptivity is one of the main factors underlying a successful pregnancy, with reports substantiating the fact that suboptimal endometrial receptivity accounts for two-thirds of early implantation event failures. The association between circRNAs and endometrial receptivity in the goat remains unclear. This study aims to identify potential circRNAs and regulatory mechanisms related to goat endometrial receptivity. Therefore, the endometrial samples on day 16 of pregnancy and day 16 of the estrous cycle were analyzed using high-throughput RNA-seq and bioinformatics. The results show that 4666 circRNAs were identified, including 7 downregulated and 11 upregulated differentially expressed circRNAs (DE-circRNAs). Back-splicing and RNase R resistance verified the identified circRNAs. We predicted the competing endogenous RNA (ceRNA) regulatory mechanism and potential target genes of DE-circRNAs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of these predicted target genes suggest that DE-circRNAs were significantly involved in establishing endometrial receptivity. Furthermore, Sanger sequencing, qPCR, correlation analysis and Fluorescence in Situ Hybridization (FISH) show that circ_MYRF derived from the host gene myelin regulatory factor (MYRF) might regulate the expression of interferon stimulating gene 15 (ISG15), thereby promoting the formation of endometrial receptivity. These novel findings may contribute to a better understanding of the molecular mechanisms regulating endometrial receptivity and promoting the maternal recognition of pregnancy (MRP).

Keywords: endometrium receptivity; goat; circRNAs; maternal recognition of pregnancy; ISG15



Citation: Wang, W.; Zang, X.; Li, Y.; Liu, D.; Hong, L.; Liu, G. Integrating Analysis to Identify Differential circRNAs Involved in Goat Endometrial Receptivity. *Int. J. Mol. Sci.* **2023**, *24*, 1531. <https://doi.org/10.3390/ijms24021531>

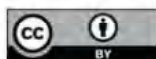
Academic Editor: Kanako Hayashi

Received: 31 October 2022

Revised: 27 December 2022

Accepted: 11 January 2023

Published: 12 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Successful embryo implantation is essential for normal pregnancy development in all mammals, while a receptive endometrium is a crucial prerequisite for embryo implantation [1]. Studies have shown that the acquisition of endometrial receptivity is a spatiotemporal process, and a large amount of crosstalk occurs between the endometrium and conceptus, which is also known as the “window of implantation” [2]. During this period, the proliferation of endometrial stromal cells and the differentiation of epithelial cells change the morphology and structure of the endometrium, resulting in the endometrium having a receptive capacity, thereby completing embryo implantation [3,4]. Previous studies have shown that endometrial receptivity is regulated by ovarian hormones, growth and transcription factors, lipid mediators and cytokines with paracrine signaling [5,6]. The dysfunctional receptive endometrium could cause infertility [7]. In ruminants, the establishment of endometrial receptivity accompanies the maternal recognition of pregnancy (MRP). MRP was successfully established due to the effect that embryonic-derived interferon τ (IFN τ) plays in corpus luteum roles by inhibiting the pulsatile release of prostaglandin F2 α (PGF2 α) in the goat endometrium [8,9]. During this period, and stimulated by these hormones and IFN τ , the endometrial epithelium undergoes dynamic changes to become

receptive, which is critical for anchoring the implanting embryo to the apical surface of the luminal epithelium [10]. Presently, ample evidence suggests that several other molecules regulate endometrial receptivity [11,12]. Remarkably, a dysfunctional receptive endometrium can cause infertility [7]. Current evidence suggests that 30% of implantation failures may be attributed to embryo quality, whereas the remaining 70% result from poor uterine receptivity [13,14]. Accordingly, it is necessary to thoroughly investigate the molecular mechanisms regulating endometrial receptivity.

As a large class of non-coding RNAs, circular RNAs (circRNAs) are produced by the back-splicing of precursor mRNAs and are characterized by the 3' and 5' ends covalently linked to form a covalently closed loop [15,16]. It is well-recognized that circRNAs, with a unique circular structure, are more stable and have longer half-lives than mRNAs [17]. Notably, previous studies found that most circRNAs composed of one or more exons are conserved among different species but exhibit temporal and spatial specificity in different tissues and developmental stages of the same species [18]. Although the functions of most circRNAs remain unclear, previous studies have shown that circRNAs have molecular functions of regulating gene expression. The competing endogenous RNA (ceRNA) hypothesis states that mRNAs, lncRNAs, circRNAs and transcribed pseudogenes can communicate with and regulate each other through miRNA response elements (MREs) [19]. Previous studies have shown that ciRS-7 is one of the highly expressed circRNAs in the brains of humans and mice, and acts as microRNA sponges to bind miR-7 in nerve tissue to hinder midbrain development [20,21]. Some circRNAs involved in endometrial receptivity were identified in goats [4]. For instance, circRNA-9119 can reportedly regulate the receptive endometrium development of dairy goats through a circRNA-9119-miR-26a-PTGS2 pathway [22]. In contrast, circRNA8073 is regarded as a miRNA sponge of miR-181a that can reduce its expression level, thereby indirectly increasing the abundance of neurotensin in the endometrium and promoting the establishment of endometrial receptivity [23]. The overall analysis on the regulation of endometrial receptivity by circRNAs in endometrium, nevertheless, is still lacking.

In this study, we performed RNA sequencing of the circRNAs present in goat endometrial samples on day 16 of pregnancy (P16) and nonpregnant goats on day 16 of the estrous cycle (C16). Subsequently, qRT-PCR combined with ceRNA interaction network construction were performed to identify potential circRNAs in the endometrium linked to endometrial receptivity. Our findings provide novel insights indicating that circMYRF is associated with the regulation of *ISG15* expression during the window of MRP in the doe, which may provide the foothold for improving the efficiency of RMP.

2. Results

2.1. Identification and Characterization of circRNAs in the Goat Endometrium

The Illumina paired-end RNA-seq approach was used to purify and sequence RNAs for identifying circRNAs and their corresponding changes in expression levels between the P16 and C16 goat endometrium. A total of over 400 million raw reads were obtained from the endometrium for these two stages, and the quality control results of the data are shown in Table S1. We obtained a total of 4666 circRNAs from these data, and the full-length distribution was mainly concentrated below 5000 nt (Figure 1A). The density of identified circRNAs among different chromosomes was not uniform (Figure 1B). After comparing with the database, we observed that the 95.74% and 95.49% of circRNAs from C16 and P16, respectively, were extensively transcribed from the exon region, and the remaining fraction were derived from the intron and intergenic region (Figure 1C). Following this, further analysis shows that most host genes could produce only one circRNA, although many genes still produced multiple circRNAs. In addition, more than 11% of host genes generated more than 3 circRNAs per gene, and even 7 host genes produced more than 12 circRNAs (Figure 1D).

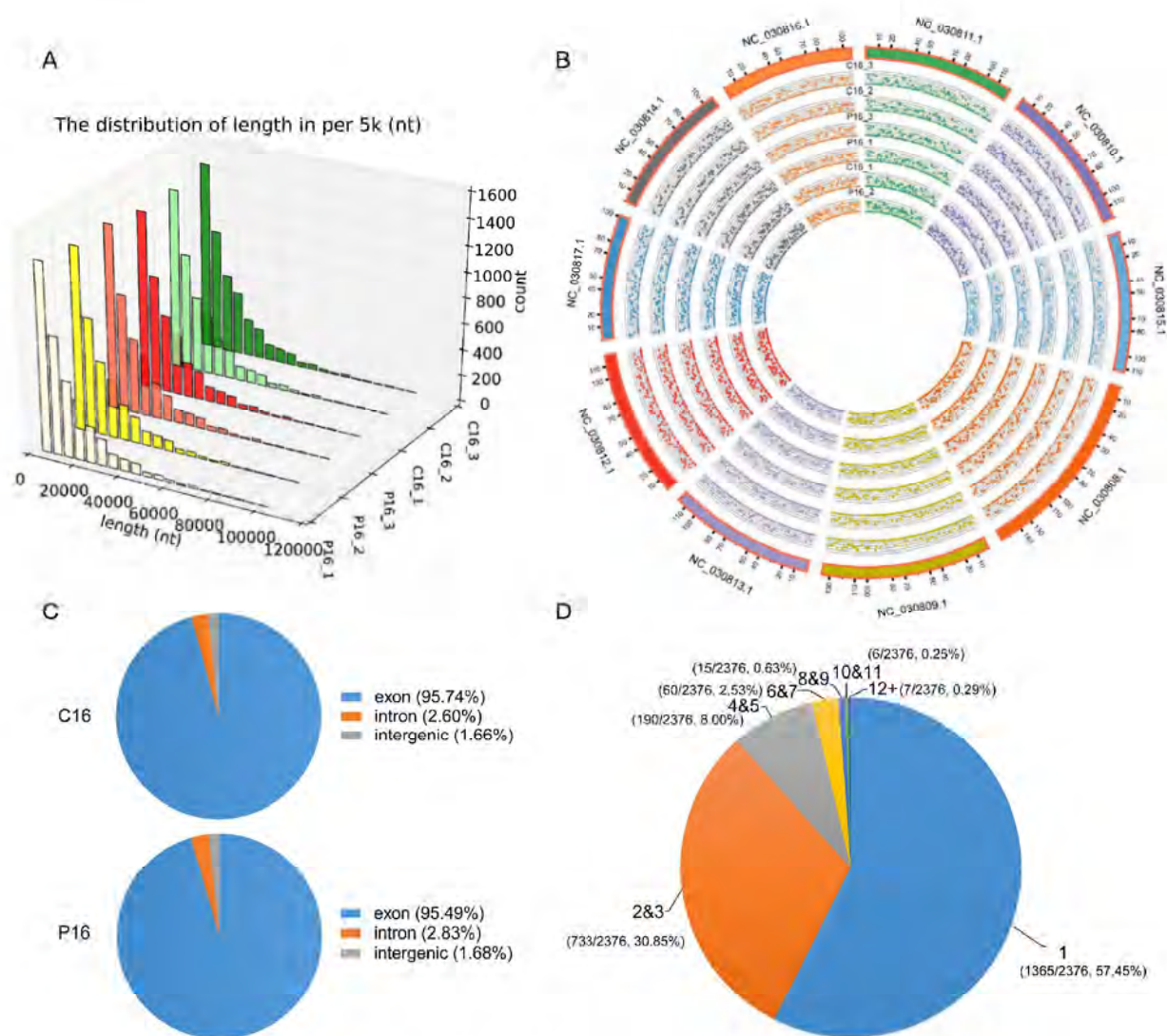


Figure 1. Identification of circRNAs. **(A)** The full-length distribution of circRNAs of all goat endometrium tissue samples. Each column represents 5000 nt. The x-axis length (nt) represents the length distribution of full-length circRNA; the y-axis represents different samples; the z-axis (count) represents the number of circRNAs. **(B)** The Circos plot shows the distribution of circRNAs on goat chromosomes. From the outside to the inside, the outside layer indicates the top 10 chromosome map of the goat genome, and the inside layers denote the distribution of circRNAs of each sample on these chromosomes. From outside to inside, the samples are C16_3, C16_2, P16_3, P16_1, C16_1 and P16_2, respectively. **(C)** The pie charts show the genic distribution of circRNAs in P16 and C16, respectively. **(D)** The amount of circRNAs produced by the host gene. Different colors represent different numbers of circRNAs produced by host genes. The values in parentheses represent the number and proportion of host genes that produce a corresponding number of circRNAs in total host genes.

As a result, among the 4666 circRNAs obtained in the two stages, 4500 circRNAs were co-expressed in these two stages, and 83 circRNAs were specifically expressed in each stage of P16 and C16 (Figure 2A,B). To study the molecular characteristics of circRNAs, we further analyzed the length of mature circRNAs after splicing, which primarily ranged from 200 to 500 bp (Figure 2C). Furthermore, it is widely acknowledged that RNA binding proteins (RBPs) play a major role in RNA metabolism, including regulating RNA splicing, maturation and function [24] and RBPs usually contain at least one RNA recognition motif (RRM) [25]. We hypothesized that RBPs in the flanking regions of the circRNA junction sites might potentially regulate circRNA biogenesis in different physiological processes;

therefore, we analyzed the potential RBPs of these identified circRNAs. Notably, we identified some RBPs, including EGR1, EGR3, ZNF684, INSM1, ZSCAN4, KLF9 and GLI2, whose binding motifs were enriched in the flanking regions of circRNA junction sites, implying that these RBPs may play functional roles in circRNA biogenesis (Figure 2D).

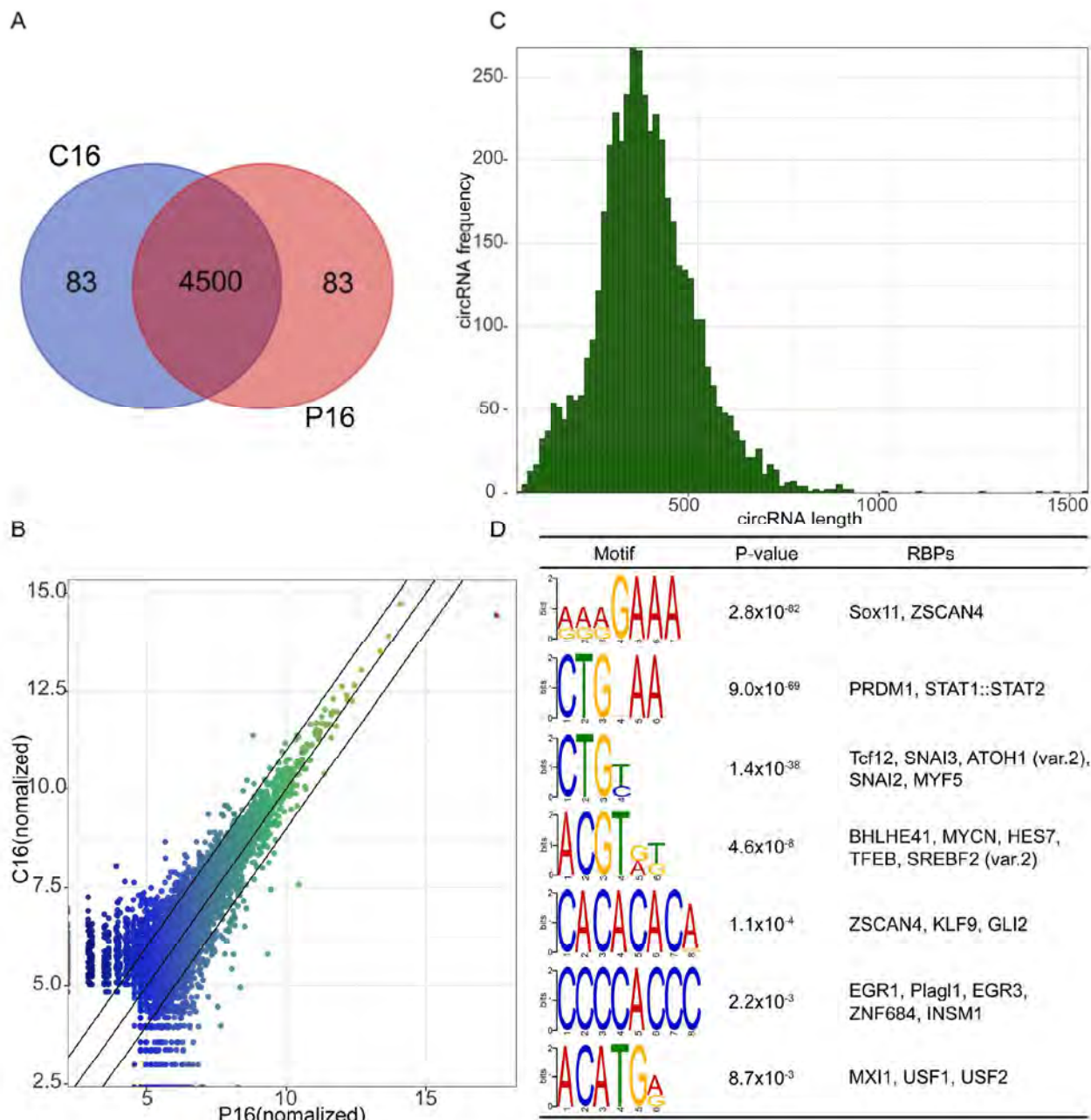


Figure 2. Characterization of circRNAs. (A) Venn diagram showing circRNAs co-expressed and specifically expressed in the goat endometrium of P16 and C16. (B) The overall analysis of the circRNA expression levels between the P16 and C16 endometrium. (C) The splice-length distribution of circRNAs. (D) The RRM of RBPs enriched in the flanking regions of the circRNA junction sites.

2.2. GO and KEGG Analysis of Host Genes of circRNAs

Previous studies have shown that circRNAs could exert biological functions by regulating the expression of their host genes. Therefore, we performed GO enrichment and KEGG pathway analyses on the host genes of circRNAs to explore their potential physiological functions. Five of the top 10 GO terms in biological processes were involved in the processes of cellular changes, including cellular processes (GO:0009987), regulation of cellular

processes (GO:0050794), positive regulation of cellular processes (GO:0048522), positive regulation of cell communication (GO:0010647) and cell adhesion (GO:0007155) (Figure 3A and Table S2). Furthermore, the KEGG pathway analysis yielded 296 enriched signaling pathways (Table S3). Among the top 20 signaling pathways, focal adhesion (chx04510), the MAPK signaling pathway (chx04010), the Ras signaling pathway (chx04014) and Adherens junction (chx04520) were associated with endometrium development (Figure 3B).

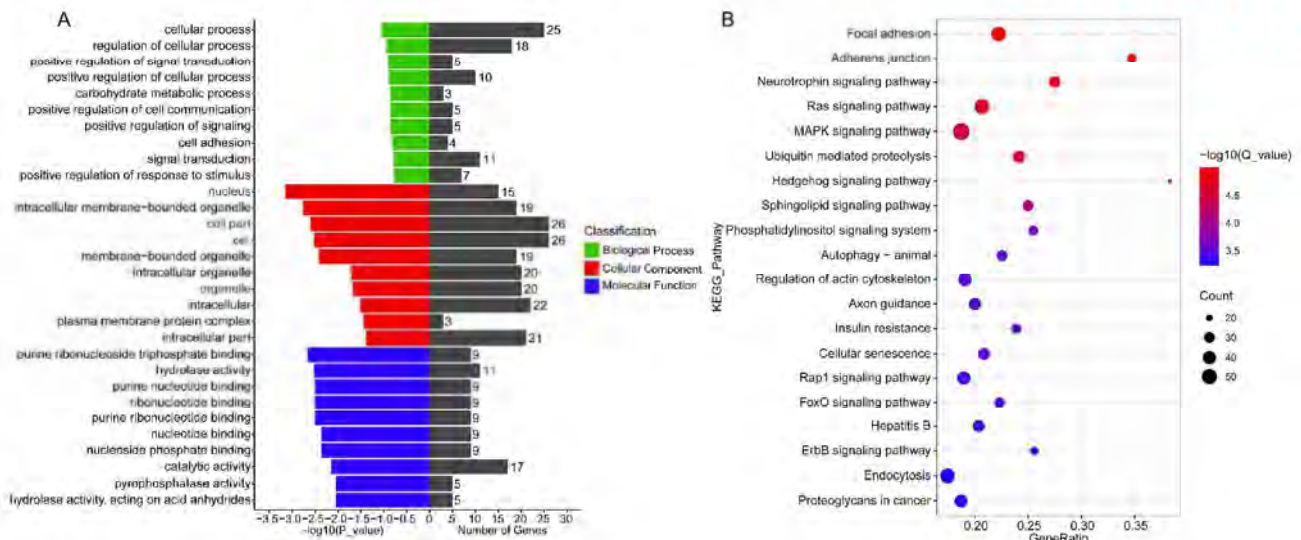


Figure 3. The biological function analysis of the host gene of circRNAs. (A) The GO enrichment analysis of host genes of circRNAs. The right x-axis indicates the number of gene in a category, and the left y-axis indicates the specific category of GO. Green: biological process, red: cellular component and blue: molecular function. (B) Scatter plot shows the KEGG pathway enrichment analysis of the host gene of circRNAs.

2.3. Identification of DE-circRNAs in the P16 and C16 Endometrium

To reveal DE-circRNAs in the goat endometrium during the two development stages, we focused on circRNAs. A total of 18 DE-circRNAs (11 upregulated and 7 downregulated) were obtained in the P16 and C16 endometrium using the criteria $FDR < 0.05$ and $|\log_2(\text{fold change})| > 1$ (Figure 4A and Table S4). Hierarchical clustering analysis shows a clear distinction of DE-circRNAs between the P16 and C16 endometrial samples (Figure 4B). Furthermore, 66.67% of DE-circRNAs were extensively spliced from exon regions, and the proportion of intergenic circRNAs in identified DE-circRNAs was higher than in the full list of circRNAs identified in goat endometrium (Figures 1C and 4C). Interestingly, the novel_circ_0003560 and novel_circ_0003562 in these DE-circRNAs were derived from one host gene, circ_LOC106502060 (Table S4).

2.4. Prediction and Construction of ceRNA Regulatory Network

Previous studies have confirmed that the circRNAs function as miRNA sponges competitively bind miRNAs, and indirectly regulate miRNA-mediated target gene expression [26,27]. To explore the functional mechanism of circRNAs involved in endometrial receptivity, we predicted the potential circRNAs-miRNAs interactions for DE-circRNAs. The result indicates that a total of 55 target miRNAs identified to the 15 DE-circRNAs while 1968 mRNAs were bound by the 55 miRNAs and suggests that a single circRNA regulates multiple miRNAs and mRNAs (Figure 5).

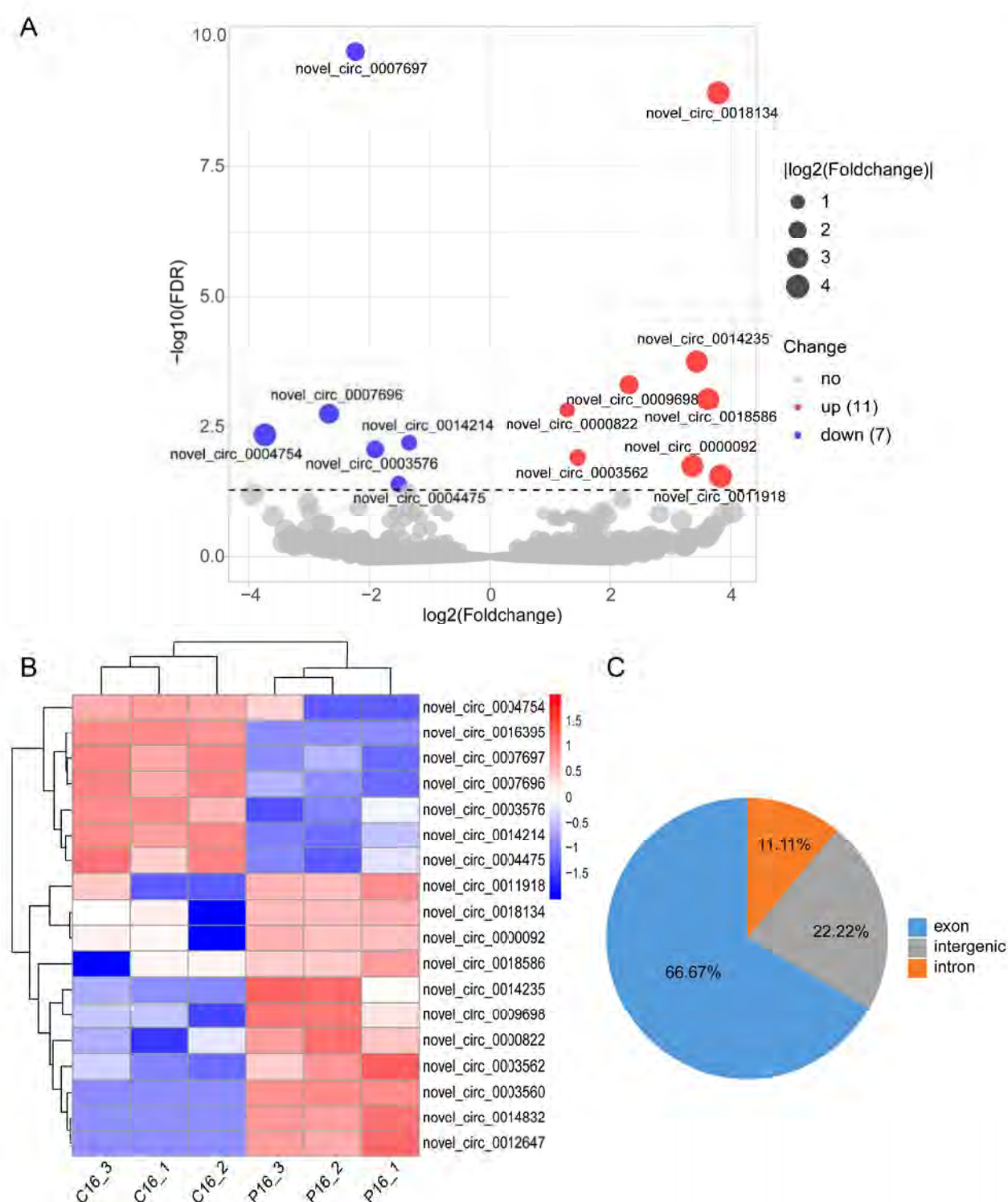


Figure 4. The detected DE-circRNAs in goat endometrium. **(A)** Volcano plot visualization of the statistical difference of the DE-circRNAs. The horizontal axis represents the fold-change of detected circRNAs, and the vertical axis represents the FDR. Red, up-regulated circRNAs; blue, down-regulated circRNAs; gray, not significantly changed circRNAs. **(B)** Hierarchical clustering shows the expression profiles of all DE-circRNAs. Each row represents one DE-circRNA, while columns represent different samples. The color scale is from -2.0 (blue, lower circRNA expression level) to 2.0 (red, higher circRNA expression level). **(C)** The pie chart shows the genic distribution of DE-circRNAs.

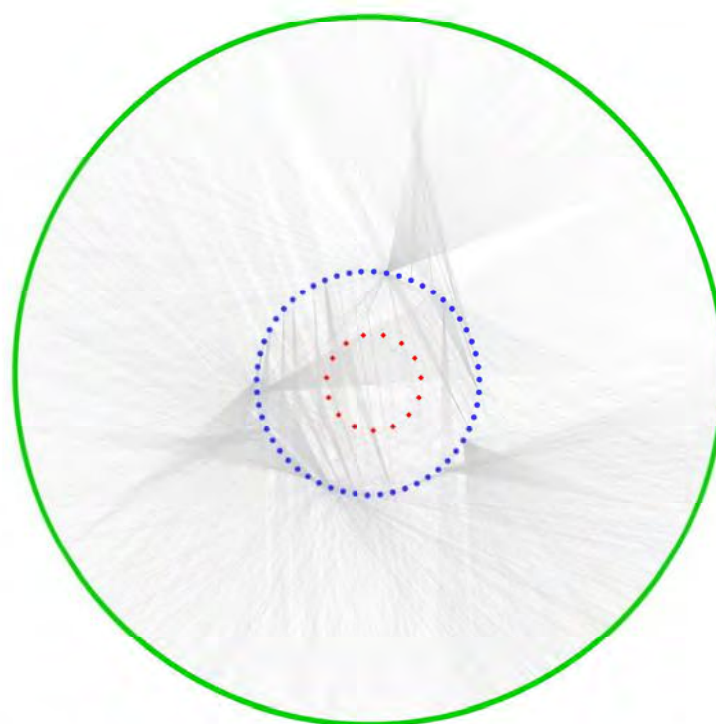


Figure 5. The ceRNA interaction network. The innermost red nodes represent DE-circRNAs. The blue nodes in the middle circle represent predicted miRNA targets. The green outer circle represents the predicted mRNAs.

2.5. Functional Annotation of DE-circRNAs

GO enrichment and KEGG pathway analyses were performed on the predicted target genes of DE-circRNAs to analyze the potential functions of DE-circRNAs in endometrial receptivity. During GO enrichment analysis, 612 GO terms were enriched, including a response to cytokine (GO:0034097), a cellular response to cytokine stimulus (GO:0071345), positive regulation of cell differentiation (GO:0045597), negative regulation of cell death (GO:0060548) and negative regulation of programmed cell death (GO:0043069) (Figure 6A and Table S5). Meanwhile, KEGG pathway analysis shows that DE-circRNAs were involved in regulating 316 signaling pathways, such as the MAPK signaling pathway (chx04010), TGF-beta signaling pathway (chx04350), Rap1 signaling pathway (chx04015), regulation of actin cytoskeleton (chx04810) and metabolic pathways (chx01100) (Figure 6B and Table S6), which significantly influenced the cellular processes involved in goat endometrium development.

2.6. Validation of DE-circRNAs in the Goat Endometrium

Six DE-circRNAs were randomly selected to design primers (Table S7) in their junction sites and validate the identified circRNAs from the RNA-seq data. The qPCR results revealed that the expression levels of novel_circ_0007697, circ_LOC106502447 and circ_ZNF568 were significantly lower, while circ_CRIM1, circ_MYRF and circ_LOC106502060 levels were significantly higher in P16 compared to C16, which is consistent with the RNA sequencing data (Figure 7A). In addition, the RNA samples of C16 and P16 were treated with exonuclease RNase R to verify the resistance. The results show that the expression levels of these circRNAs after RNase R treatment were not significantly different from controls, while the linear gene expressions were significantly decreased (Figure 7B). Meanwhile, Sanger sequencing confirmed the presence of head-to-tail splice junctions in the circRNAs (Figure 7C). These results further suggest that circRNAs have covalently closed circular structures and indicate that the RNA-seq data are reliable.

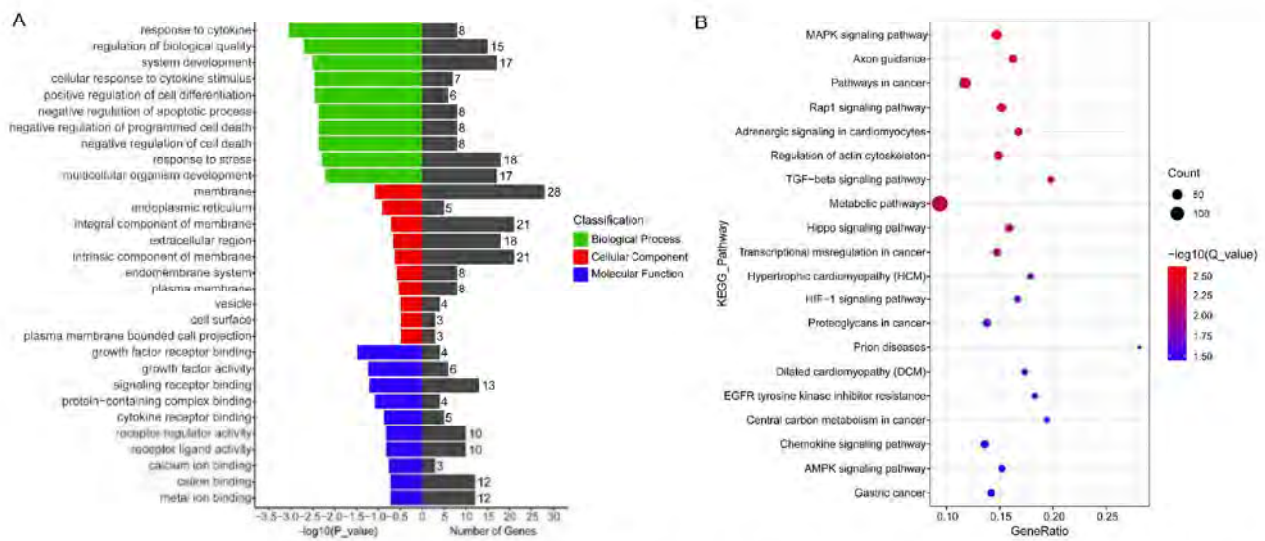


Figure 6. Functional analysis of the ceRNA network. (A) GO enrichment analysis of the ceRNA network. (B) KEGG pathways analysis of the ceRNA network.

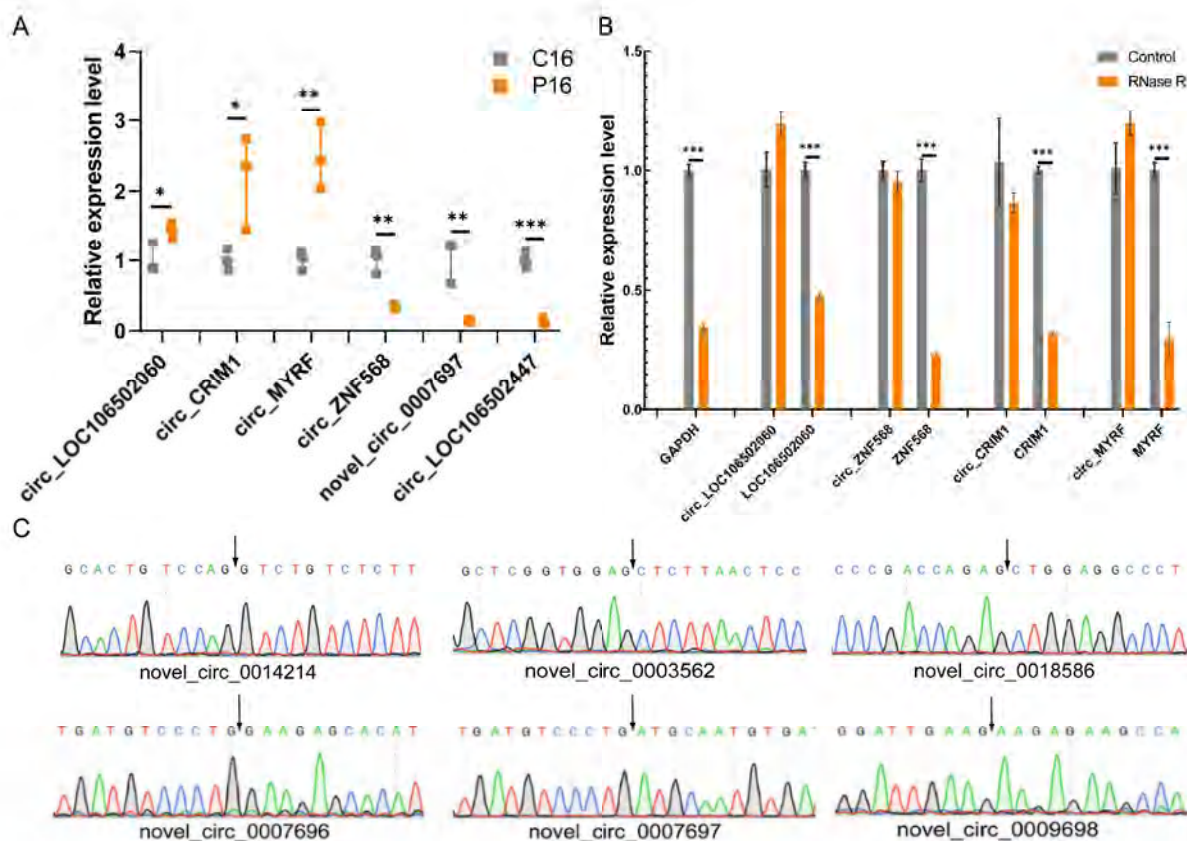


Figure 7. Validation of DE-circRNAs in the goat endometrium. (A) The relative expression level of DE-circRNAs were measured by qPCR in P16 and C16. (B) Validation of the resistance of DE-circRNAs and mRNAs to RNase R. There are three independent replicates per group, and the data are shown as the mean \pm standard error of the mean (SEM) values. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (C) The head-to-tail splice junctions for circRNAs were confirmed by Sanger sequencing. Black arrows represent the junction sites.

2.7. Functional Prediction of circ_MYRF in Goat Receptive Endometrium

During the screening of candidate circRNAs, circ_MYRF ($\log_2(\text{fold change}) = 3.63$; $\text{FDR} = 0.0009$, exonic circRNA) attracted our interest. We found that circ_MYRF was derived from 2 exons, including exons 9 (40573323–40573399) and 10 (40573581–40573691) of the host gene MYRF (NC_030836.1) (Figure 8A). We constructed one ceRNA network showing the relationship among circ_MYRF-miRNAs-mRNAs, including six putative miRNA sponges and 56 targeted genes (Figure 8B). Subsequently, we used qPCR to examine the expression levels of two randomly selected target genes, and the results were in line with the expression of circ_MYRF (Figure 8C). Interestingly, interferon stimulating gene 15 (ISG15) was one of the target genes of circ_MYRF in the network analysis. ISG15 is well-established as one of several proteins generated by conceptus-derived Type I and/or a Type II interferon and can reportedly regulate endometrial receptivity and conceptus development [28]. Correlation analysis confirmed that the expression of circ_MYRF was significantly associated with the ISG15 mRNA expression (Figure 8D). FISH analysis was performed to determine the circ_MYRF location in the endometrium tissues of P16 and C16 (Figure 9). It is abundantly expressed in the uterine glandular epithelium (GE) and stroma, which is consistent with ISG15 expression in the endometrial tissue, as reported in a previous study [29].

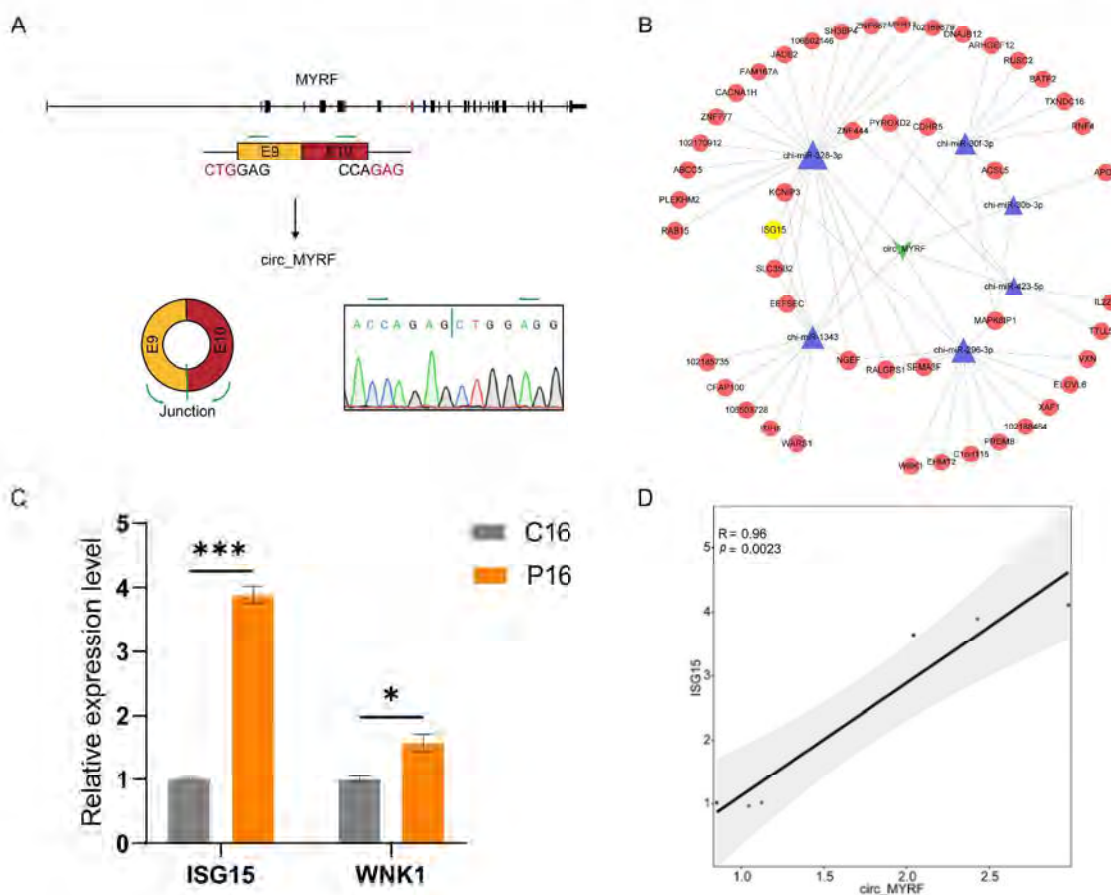


Figure 8. The characterization and putative function of circ_MYRF in the goat endometrium. (A) The genomic location of circ_MYRF in the host gene, MYRF. The expression of circ_MYRF was validated by qPCR and Sanger sequencing. Arrows represent divergent primer binding sites in the circular junction of circ_MYRF. (B) A putative ceRNA network of circ_MYRF. The green quadrilateral represents circ_MYRF, the blue triangles represent targeted miRNAs, and the red circles denote targeted mRNAs. The yellow circle represents the ISG15 mRNA. (C) Relative expression level of two randomly selected target genes in the ceRNA network determined by qPCR. Values represent mean \pm SEM. *, $p < 0.05$; ***, $p < 0.001$. (D) The correlation between circ_MYRF expression and ISG15 expression.

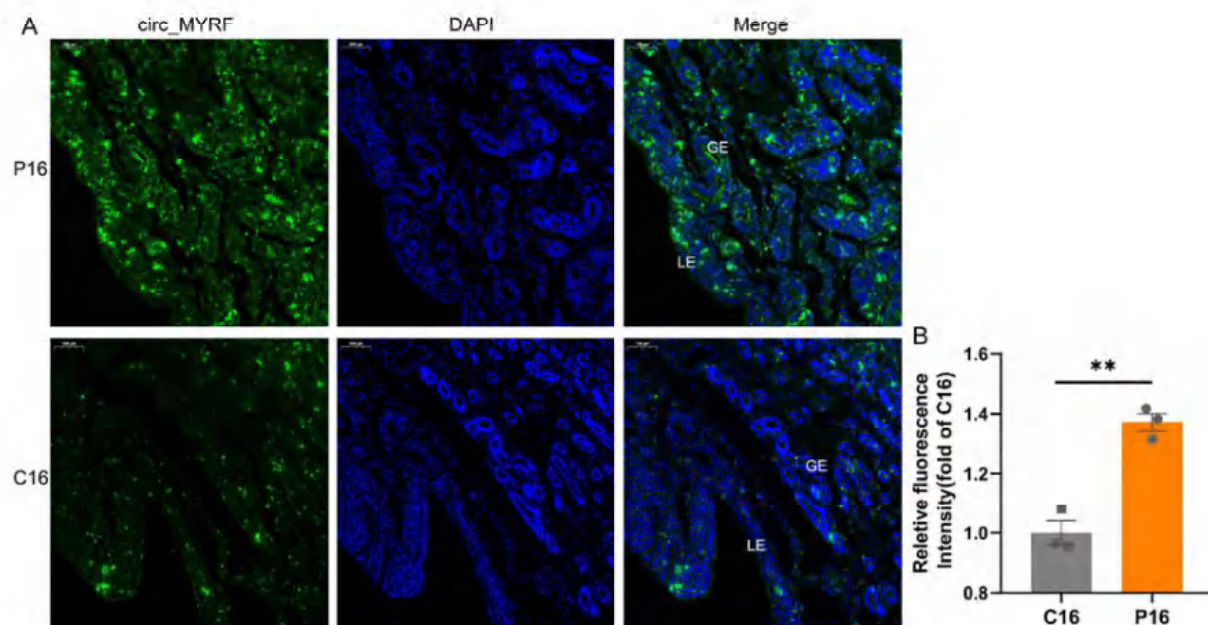


Figure 9. FISH analysis of circ_MYRF in the C16 and P16 uterus. (A) Representative images of circ_MYRF staining for two stages. The circ_MYRF was abundantly expressed in GE and stroma of P16, while it was slightly expressed in C16. LE, endometrial luminal epithelium; GE, glandular epithelium. Scale bar: 100 μ m. (B) Quantitative analysis was performed by measuring the fluorescence intensity of circ_MYRF in two stages. The data were shown as the mean \pm SEM. **, $p < 0.01$.

3. Discussion

Current evidence suggests that endometrium receptivity determines successful embryo implantation and embryonic mortality [30]. It is essential to conduct a comprehensive study of the molecular regulation underlying endometrial receptivity of specificity to pregnancy in the doe. The past decade witnessed the advent of high-throughput RNA sequencing, enabling us to better understand the molecular regulation processes of endometrial receptivity. In a previous study, it was revealed that hundreds of genes are involved in regulating endometrial receptivity by transcriptome studies [31]. In addition, it has reported that dozens of miRNAs found in both humans and mice were could potentially modulate endometrial receptivity [3]. In this study, we investigated the circRNA profiles of goat endometrium at C16 and P16 and identified circRNAs involved in regulating endometrial receptivity. Importantly, our research expanded the repertoire of goat endometrium-expressed circRNAs and provided information for future studies on endometrial development and embryo implantation. Notably, the RNAs used in this study were not treated with RNase R to remove all linear RNAs; however, they were predicted by the unique back-splicing structure of circRNAs, which may have some limitations in parsing all circRNA expression profiles.

A previous study has shown that circRNAs are formed by back-splicing the corresponding linear transcript [32]. In the present study, we found that novel_circ_0003560 and novel_circ_0003562 were derived from one host gene, indicating that diverse circRNAs could be expressed by a single gene locus, which is consistent with previous results [33]. Furthermore, growing evidence suggests that circRNAs could regulate the expression of their host gene to participate in the regulation of biological processes [32,34]. In this study, the host gene of the DE-circRNA, novel_circ_0009698 (circ_CRIM1), CRIM1, can be promoted by hormones and IFN τ in goat endometrium, and a deficiency of CRIM1 hindered cell proliferation, adhesion and prostaglandin secretion and thus disrupted normal endometrial receptivity [12,35,36], suggesting that it plays a vital role in the establishment of pregnancy. In contrast, circ_CRIM1 may contribute to cell proliferation, cell adhesion and the formation of normal endometrial receptivity by promoting CRIM1 expression. This

hypothesis, however, should warrant validation in further study. In the study by Song et al. [4], there were 334 DE-circRNAs identified in the endometrium from goats at gestational day 5 and goats at gestational day 15 using Illumina Solexa technology. Interestingly, both Song's study and ours found that the circRNA deriving from *CRIM1* gene was highly expressed in the receptive endometrium, which further suggests that circ_ *CRIM1* may be involved in regulating endometrial receptivity. In our study, most of the circRNAs were termed as exonic circRNAs. Intriguingly, we found that the proportion of intergenic circRNAs in identified DE-circRNAs was higher than in the full list of circRNAs identified in the goat endometrium. Substantial evidence suggests that exonic circRNAs can usually interact with host genes and regulate the roles of host genes in biological processes [37–39]. Since intergenic circRNAs do not have corresponding host genes, they might exert dominant functions by acting as miRNA regulators [40], suggesting that DE-circRNAs identified in this study may serve as miRNAs sponges to regulate the endometrial receptivity.

According to the ceRNA hypothesis, circRNAs are molecular sponges of miRNAs that ultimately regulate mRNA expression [19]. For instance, the ciR3175-miR182-TES pathway was identified in the endometrium of dairy goats; ciR3175 regulates the expression of TES by adsorbing miR182 and then decreases the expression of BCL-2/BAX through the MAPK pathway, thereby inhibiting EEC apoptosis [41]. This regulatory mechanism of circRNAs indicates that there are communication networks among RNAs. In our study, a circRNAs-miRNA-mRNA network analysis performed on DE-circRNAs shows that most DE-circRNAs predicted only one or two target sites for miRNAs, which is consistent with the literature [42]. This result suggests that circRNAs can act as a miRNA sponge and do not require many target sites.

It was reported that the receptive endometrium results from the normal development of the endometrium following successful pregnancy recognition. In addition to the dominant pregnancy recognition hormones, including PGF2 α and IFN τ , many growth factors, cytokines and inflammatory factors can coordinate the hormones mentioned above to co-regulate the process [43,44]. Notably, circ_0012647, which is specifically expressed in P16 endometrium, was predicted to upregulate OAS1 expression by sponging miR-671-5p. Previous studies confirmed that OAS1 expression in luteal cells could be increased under the function of IFN τ to maintain corresponding corpus luteum roles [45,46], suggesting that circRNAs may cooperate with IFN τ to act on the corpus luteum to establish a successful pregnancy.

Subsequently, a functional analysis of the putative target genes was performed. During GO annotation, most target genes were enriched in cell-related biological processes, including a response to cytokine (GO:0034097), a positive regulation of cell differentiation (GO:0045597) and a negative regulation of the apoptotic process (GO:0043066). Mounting evidence substantiates that the endometrial events are mediated by cell proliferation, differentiation and apoptosis [47,48]. In the present study, the KEGG pathway analysis of target genes shows that pathways such as the MAPK signaling pathway (chx04010), the Rap1 signaling pathway (chx04015) and regulation of actin cytoskeleton (chx04810) were enriched. Previous studies have confirmed that the MAPK signaling pathway could be involved in regulating EEC proliferation [49], the Rap1 signaling pathway was involved in regulating the function of endometrial stromal cells [50] and the regulation of actin cytoskeleton participated in regulating the remodeling of adherens junctions [51]. Overall, these results indicate that circRNAs may regulate goat endometrial receptivity through these pathways by ceRNA competition regulation mechanisms.

We further explored the detailed regulatory mechanism of circRNAs. ISG15 is a ubiquitin homolog whose expression is induced by the conceptus IFN in a temporal and cell-specific manner in the uterus, to degrade proteins detrimental to fetal/embryo survival [52]. Previous literature suggests that ISG15 plays a critical role in determining endometrial receptivity and regulating embryo development [28,53]. Chandrakar et al. consistently found an increase of ISG15 mRNA concentration in the goat endometrium during the early stages of pregnancy (16–24d) [54]. In this study, ceRNA network analysis and qPCR

analysis shows that, compared with C16, the mRNA expression level of ISG15 was higher in P16. Moreover, the FISH analysis results show that circ_MYRF was abundantly expressed in the GE and stroma of P16 and slightly expressed in C16. In contrast, the ISG15 mRNA was also mainly localized in GE and stromal cells and exhibited limited localization in the luminal epithelium (LE) [29]. Hence, we hypothesize that circ_MYRF may regulate goat endometrial receptivity by targeting ISG15 in GE and stroma. This also suggests that circ_MYRF may serve as a potential biomarker to identify non-receptive endometrium and a therapeutic target to improve human endometrial receptivity for the treatment of infertility. Future mechanistic studies should be warranted to confirm the precise roles of circ_MYRF and ISG15 in pregnancy establishment.

4. Materials and Methods

4.1. Animals and Sample Collection

This study complied with the Ethical Principles in Animal Research, and was performed in accordance with the ethical standards of the Animal Care and Use Committee of South China Agricultural University (permit number: SYXK-2022-0136). Six healthy and disease-free primiparous Chuanzhong black goats (*Capra hircus*) were provided by Guangdong Wen's Foodstuffs Group Co., Ltd. (Yunfu, China), and were randomly divided into a cyclic group ($n = 3$) and a pregnancy group ($n = 3$). Goats that belonged to the pregnant group were twice artificially inseminated using extended semen from one ram at the onset of estrus (day 0) and 12 h after. Subsequently, the goats were slaughtered at the local slaughterhouse on day 16 of the estrus cycle (C16) or pregnancy (P16). For each animal, the uterus was quickly removed and transported to the laboratory in an icebox, and pregnancy was confirmed by the presence of apparently normal filamentous conceptuses during uterine flushing [55]. The uteri were opened longitudinally along the antimesometrial side. Approximately 1 cm² of endometrial tissue samples were taken from the middle of each uterine horn at the antimesometrial side of the uterus, and endometrial samples were snap-frozen in liquid nitrogen and stored at -80°C for RNA extraction.

4.2. Library Preparation and RNA Sequencing

Total RNA was isolated from the endometrium using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's procedure. Total RNA quality and concentration was checked using the NanoDrop 2000 equipment (Thermo Scientific, Waltham, MA, USA), and integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). High throughput transcriptome sequencing was carried out by an Illumina HiSeq platform at Novogene (Beijing, China). Briefly, about 3 μg of total RNA per sample removed ribosomal RNAs using the Epicentre Ribo-zeroTM rRNA Removal Kit (Epicentre Madison, WI, USA), and rRNA free residue was cleaned up by ethanol precipitation. Then, the rRNA-depleted RNA was used to generate the sequencing library using the NEBNext[®] UltraTM Directional RNA Library Prep Kit (NEB, Ipswich, MA, USA), according to the recommendations of the manufacturer.

4.3. RNA-seq Data Analysis

During this step, clean reads were obtained by removing adapter sequences, reads with more than 10% poly-N and low-quality from the raw data. Simultaneously, Q20, Q30 and GC content of the clean reads were calculated, and all follow-up bioinformatics analyses were based on clean reads with high quality. Subsequently, the clean reads were mapped to the goat reference genome using Bowtie (v.0.12.9) [56]. Due to the high false-positive identification of circRNAs [57], we used two software, find_circ [21] and CIRI2 [58], to detect and identify circRNAs, and only circRNAs that were intersected between the two software were selected for further analyses.

4.4. Analysis of Differentially Expressed circRNAs (DE-circRNAs)

The expression level of circRNAs in each sample was counted and normalized with TPM [59]. The differential expression analysis between the P16 and C16 endometrium of goats was performed using the DESeq2 package [60]. The *p* values were adjusted using Benjamini-Hochberg's approach for controlling the false discovery rate [61]. Differentially expressed circRNAs were identified using $FDR < 0.05$ and $|\log_2(\text{foldchange})| > 1$ as screening criteria.

4.5. Motif Enrichment Analysis and ceRNA Network Construction

The 100 bp flanking region of the back-splicing site with circRNAs was retrieved from the goat genome, and then the short, ungapped motifs relatively enriched in these regions was compared with shuffled sequences and were detected using Dreme (v.5.1.1) [62]. The enriched motifs with $p < 0.05$ were selected for subsequent analysis. To associate the enriched motifs to potential RBPs, all selected motifs were compared against the JASPAR database [63] of known motifs using Tomtom (v.5.1.1) [64]. The top 5 target motifs with the most significant matches to the query motif were identified as potential RBPs, which might regulate the biogenesis of circRNAs.

The miRNA binding sites of the DE-circRNAs were predicted using the miRanda (v.3.3) software [65]. Then, miRNA-mRNA interactions were predicted using miRanda. Targetscan and RNAhybrid were used to determine the gene targets of each filtered miRNA. Using these data, the outline of the ceRNA regulatory network was generated using Cytoscape (v.3.7.2, <http://www.cytoscape.org/>, accessed on 30 October 2021) [66].

4.6. Functional Analysis of DE-circRNAs

To reveal the potential biological functions and principal pathways of DE-circRNAs of P16 and C16, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were used. The enriched GO terms of these target genes were identified using the clusterProfiler package in R, and the KEGG pathways were determined using KEGG Orthology Based Annotation System (KOBAS) [67].

4.7. Quantitative Real-Time PCR (qPCR)

Total RNAs were extracted from the same 6 samples of goat endometrium of P16 and C16 and used for RNA sequencing. Next, 2 µg of total RNA of each sample was incubated for 10 min at 37 °C with or without RNase R (3 U/µg RNA, GENESEED, Guangzhou, China), followed by inactivation for 10 min at 70 °C. All RNA samples were processed simultaneously to ensure equally effective RNase R treatment. The qPCR assay was conducted to quantify the amount of circRNA and mRNA. Initially, the first strand cDNA was synthesized using the Evo M-MLV RT Kit with gDNA Clean for qPCR II (Accurate Biology, Changsha, China). Then, qPCR reactions were conducted employing a SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Changsha, China). The PCR volume was 10 µL, consisting of 1 µL cDNA, 0.2 µM of each primer, 5 µL 2 × SYBR Green Pro Taq HS Premix, 0.4 µM of ROX Reference Dye and RNase free water to make up the total volume. The thermal cycling conditions were as follows: 95 °C for 30 s followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. Linear GAPDH was chosen as an internal reference to regulate the expression of circRNAs, and all reactions were performed in triplicate samples. To further identify the junction sequence of circRNAs, the RT-PCR products of divergent primers were analyzed by electrophoresis and Sanger sequenced at BGI Genomics Co., Ltd. (Shenzhen, China). The primer sequences used are listed in Table S7.

4.8. Fluorescence In Situ Hybridization (FISH) Analysis

The location of circ_MYRF in goat endometrium was determined by conducting a FISH analysis, as previously described [68]. In brief, micrometer sections (4 µm thick) were deparaffinized, digested with proteinase K and hybridized using FAM-labeled circ_MYRF

probes (green). Simultaneously, cell nuclei were stained with DAPI. Images were then photographed using a positive fluorescence microscope (Nikon, Tokyo, Japan).

4.9. Statistical Analysis

Data were statistically analyzed using SPSS (version 26.0, SPSS Inc., Chicago, IL, USA), GraphPad Prism (version 8.0, Graphpad Software, San Diego, CA, USA) and R programming language (version 3.6). All qPCR results of circRNAs and mRNAs in goat endometrium of P16 were normalized using the calibrator group, C16, and the circRNA and linear mRNA treated with RNase R were normalized using control values. The Kolmogorov-Smirnov test was used to assess the normality of the data. All experiments were performed in three independent replicates, and data were expressed as the mean \pm standard error of the mean (SEM). Differences between the two groups were analyzed using Student's *t*-test. A *p* value < 0.05 was statistically significant, and *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

5. Conclusions

In summary, this study identified 4666 novel circRNAs, including 18 significantly differentially expressed circRNAs (11 upregulated and 7 downregulated). The ceRNA network and functional analyses of circRNAs suggests the potential roles of circRNAs in endometrial receptivity. Our study provides the circRNA expression profiles during early pregnancy and data on the estrus periods to study the molecular regulation mechanism of mammalian early pregnancy and further promotes research in embryo implantation, cancer and gynecological diseases.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms24021531/s1>.

Author Contributions: Conceptualization, D.L., G.L. and L.H.; methodology, W.W., Y.L. and X.Z.; validation, W.W.; formal analysis, X.Z.; investigation, W.W., X.Z. and Y.L.; resources, D.L., G.L. and L.H.; writing—original draft preparation, W.W. and X.Z.; writing—review and editing, W.W., X.Z., G.L. and L.H.; visualization, W.W. and X.Z.; supervision, G.L. and L.H.; funding acquisition, D.L. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation Project of Guangdong Province, grant number 2021A1515010636, and Guangdong Provincial Promotion Project on Preservation and Utilization of Local Breed of Livestock and Poultry, grant number 2018143.

Institutional Review Board Statement: All animals were conducted under a protocol approved by the Ethics Committee of the Laboratory Animal Center of South China Agricultural University (permit number: SYXK-2022-0136).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, PRJNA578518.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Akbar, R.; Ullah, K.; Rahman, T.U.; Cheng, Y.; Pang, H.Y.; Jin, L.Y.; Wang, Q.J.; Huang, H.F.; Sheng, J.Z. miR-183-5p regulates uterine receptivity and enhances embryo implantation. *J. Mol. Endocrinol.* **2020**, *64*, 43–52. [CrossRef] [PubMed]
2. Karizbodagh, M.P.; Rashidi, B.; Sahebkar, A.; Masoudifar, A.; Mirzaei, H. Implantation Window and Angiogenesis. *J. Cell. Biochem.* **2017**, *118*, 4141–4151. [CrossRef] [PubMed]
3. Shekibi, M.; Heng, S.; Nie, G. MicroRNAs in the Regulation of Endometrial Receptivity for Embryo Implantation. *Int. J. Mol. Sci.* **2022**, *23*, 6210. [CrossRef]
4. Song, Y.; Zhang, L.; Liu, X.; Niu, M.; Cui, J.; Che, S.; Liu, Y.; An, X.; Cao, B. Analyses of circRNA profiling during the development from pre-receptive to receptive phases in the goat endometrium. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 34. [CrossRef]

5. Governini, L.; Luongo, F.P.; Haxhiu, A.; Piomboni, P.; Luddi, A. Main actors behind the endometrial receptivity and successful implantation. *Tissue Cell* **2021**, *73*, 101656. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Achache, H.; Revel, A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum. Reprod. Update* **2006**, *12*, 731–746. [\[CrossRef\]](#)
7. Chapron, C.; Marcellin, L.; Borghese, B.; Santulli, P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat. Rev. Endocrinol.* **2019**, *15*, 666–682. [\[CrossRef\]](#)
8. Roberts, R.M. Interferon-tau, a Type 1 interferon involved in maternal recognition of pregnancy. *Cytokine Growth Factor Rev.* **2007**, *18*, 403–408. [\[CrossRef\]](#)
9. Bazer, F.W.; Thatcher, W.W. Chronicling the discovery of interferon tau. *Reproduction* **2017**, *154*, F11–F20. [\[CrossRef\]](#)
10. Vasquez, Y.M.; Wang, X.Q.; Wetendorf, M.; Franco, H.L.; Mo, Q.X.; Wang, T.Y.; Lanz, R.B.; Young, S.L.; Lessey, B.A.; Spencer, T.E.; et al. FOXO1 regulates uterine epithelial integrity and progesterone receptor expression critical for embryo implantation. *PLoS Genet.* **2018**, *14*, 27. [\[CrossRef\]](#)
11. Liu, H.K.; Wang, C.X.; Li, Z.H.; Shang, C.M.; Zhang, X.Y.; Zhang, R.X.; Wang, A.H.; Jin, Y.P.; Lin, P.F. Transcriptomic Analysis of STAT1/3 in the Goat Endometrium During Embryo Implantation. *Front. Vet. Sci.* **2021**, *8*, 12. [\[CrossRef\]](#)
12. Yang, D.Q.; Liu, A.I.; Zhang, Y.Y.; Nan, S.; Yin, R.L.; Lei, Q.H.; Zhu, H.M.; Chen, J.G.; Han, L.; Ding, M.X.; et al. Essential Role of CRIM1 on Endometrial Receptivity in Goat. *Int. J. Mol. Sci.* **2021**, *22*, 5323. [\[CrossRef\]](#)
13. Zhang, H.; Qi, J.; Wang, Y.; Sun, J.; Li, Z.; Sui, L.; Fan, J.; Liu, C.; Shang, Y.; Kong, L.; et al. Progesterone Regulates Glucose Metabolism Through Glucose Transporter 1 to Promote Endometrial Receptivity. *Front. Physiol.* **2020**, *11*, 543148. [\[CrossRef\]](#)
14. Chen, Q.; Zhang, A.J.; Yu, F.; Gao, J.; Liu, Y.; Yu, C.L.; Zhou, H.; Xu, C. Label-Free Proteomics Uncovers Energy Metabolism and Focal Adhesion Regulations Responsive for Endometrium Receptivity. *J. Proteome Res.* **2015**, *14*, 1831–1842. [\[CrossRef\]](#)
15. Li, X.; Yang, L.; Chen, L.L. The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol. Cell* **2018**, *71*, 428–442. [\[CrossRef\]](#)
16. Kristensen, L.S.; Andersen, M.S.; Stagsted, L.V.W.; Ebbesen, K.K.; Hansen, T.B.; Kjems, J. The biogenesis, biology and characterization of circular RNAs. *Nat. Rev. Genet.* **2019**, *20*, 675–691. [\[CrossRef\]](#)
17. Jeck, W.R.; Sharpless, N.E. Detecting and characterizing circular RNAs. *Nat. Biotechnol.* **2014**, *32*, 453–461. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Jeck, W.R.; Sorrentino, J.A.; Wang, K.; Slevin, M.K.; Burd, C.E.; Liu, J.; Marzluff, W.F.; Sharpless, N.E. Circular RNAs are abundant, conserved, and associated with ALU repeats. *Rna* **2013**, *19*, 141–157. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Salmena, L.; Poliseno, L.; Tay, Y.; Kats, L.; Pandolfi, P.P. A ceRNA Hypothesis: The Rosetta Stone of a Hidden RNA Language? *Cell* **2011**, *146*, 353–358. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Hansen, T.B.; Jensen, T.I.; Clausen, B.H.; Bramsen, J.B.; Finsen, B.; Damgaard, C.K.; Kjems, J. Natural RNA circles function as efficient microRNA sponges. *Nature* **2013**, *495*, 384–388. [\[CrossRef\]](#)
21. Memczak, S.; Jens, M.; Elefsinioti, A.; Torti, F.; Krueger, J.; Rybak, A.; Maier, L.; Mackowiak, S.D.; Gregersen, L.H.; Munschauer, M.; et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* **2013**, *495*, 333–338. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Zhang, L.; Liu, X.; Che, S.; Cui, J.; Liu, Y.; An, X.; Cao, B.; Song, Y. CircRNA-9119 regulates the expression of prostaglandin-endoperoxide synthase 2 (PTGS2) by sponging miR-26a in the endometrial epithelial cells of dairy goat. *Reprod. Fertil. Dev.* **2018**, *30*, 1759–1769. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Zhang, L.; Liu, X.; Che, S.; Cui, J.; Ma, X.; An, X.; Cao, B.; Song, Y. Endometrial Epithelial Cell Apoptosis Is Inhibited by a ciR8073-miR181a-Neurotensin Pathway during Embryo Implantation. *Mol. Ther. Nucleic Acids* **2019**, *14*, 262–273. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Corley, M.; Burns, M.C.; Yeo, G.W. How RNA-Binding Proteins Interact with RNA: Molecules and Mechanisms. *Mol. Cell* **2020**, *78*, 9–29. [\[CrossRef\]](#)
25. Cassola, A.; Noé, G.; Frasch, A.C. RNA recognition motifs involved in nuclear import of RNA-binding proteins. *RNA Biol.* **2010**, *7*, 339–344. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Sand, M.; Bechara, F.G.; Gambichler, T.; Sand, D.; Bromba, M.; Hahn, S.A.; Stockfleth, E.; Hessam, S. Circular RNA expression in cutaneous squamous cell carcinoma. *J. Dermatol. Sci.* **2016**, *83*, 210–218. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Panda, A.C. Circular RNAs Act as miRNA Sponges. *Adv. Exp. Med. Biol.* **2018**, *1087*, 67–79.
28. Jain, A.; Baviskar, P.S.; Kandasamy, S.; Kumar, R.; Singh, R.; Kumar, S.; Agarwal, S.K.; Joshi, P.; Mitra, A. Interferon stimulated gene 15 (ISG15): Molecular characterization and expression profile in endometrium of buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.* **2012**, *133*, 159–168. [\[CrossRef\]](#)
29. Alak, I.; Hitit, M.; Kose, M.; Kaya, M.S.; Ucar, E.H.; Atli, Z.; Atli, M.O. Relative abundance and localization of interferon-stimulated gene 15 mRNA transcript in intra- and extra-uterine tissues during the early stages of pregnancy in sheep. *Anim. Reprod. Sci.* **2020**, *216*, 106347. [\[CrossRef\]](#)
30. Simmons, D.G.; Kennedy, T.G. Uterine sensitization-associated gene-1: A novel gene induced within the rat endometrium at the time of uterine receptivity/sensitization for the decidual cell reaction. *Biol. Reprod.* **2002**, *67*, 1638–1645. [\[CrossRef\]](#)
31. Altmäe, S.; Koel, M.; Vösa, U.; Adler, P.; Suhorutšenko, M.; Laisk-Podar, T.; Kukushkina, V.; Saare, M.; Velthut-Meikas, A.; Krjutškov, K.; et al. Meta-signature of human endometrial receptivity: A meta-analysis and validation study of transcriptomic biomarkers. *Sci. Rep.* **2017**, *7*, 10077. [\[CrossRef\]](#) [\[PubMed\]](#)
32. You, X.; Vlatkovic, I.; Babic, A.; Will, T.; Epstein, I.; Tushev, G.; Akbalik, G.; Wang, M.; Glock, C.; Quedenau, C.; et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat. Neurosci.* **2015**, *18*, 603–610. [\[CrossRef\]](#)

33. Holdt, L.M.; Kohlmaier, A.; Teupser, D. Molecular roles and function of circular RNAs in eukaryotic cells. *Cell. Mol. Life Sci.* **2018**, *75*, 1071–1098. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Venø, M.T.; Hansen, T.B.; Venø, S.T.; Clausen, B.H.; Grebing, M.; Finsen, B.; Holm, I.E.; Kjems, J. Spatio-temporal regulation of circular RNA expression during porcine embryonic brain development. *Genome Biol.* **2015**, *16*, 245. [\[CrossRef\]](#)
35. Zhang, Y.; Fan, J.; Ho, J.W.; Hu, T.; Kneeland, S.C.; Fan, X.; Xi, Q.; Sellarole, M.A.; de Vries, W.N.; Lu, W.; et al. Crim1 regulates integrin signaling in murine lens development. *Development* **2016**, *143*, 356–366.
36. Iyer, S.; Chhabra, Y.; Harvey, T.J.; Wang, R.; Chiu, H.S.; Smith, A.G.; Thomas, W.G.; Pennisi, D.J.; Piper, M. CRIM1 is necessary for coronary vascular endothelial cell development and homeostasis. *J. Mol. Histol.* **2017**, *48*, 53–61. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Xu, X.L.; Zhang, J.W.; Tian, Y.H.; Gao, Y.; Dong, X.; Chen, W.B.; Yuan, X.N.; Yin, W.N.; Xu, J.J.; Chen, K.; et al. CircRNA inhibits DNA damage repair by interacting with host gene. *Mol. Cancer* **2020**, *19*, 128. [\[CrossRef\]](#)
38. Zhou, J.Y.; Zhang, S.Z.; Chen, Z.M.; He, Z.F.; Xu, Y.; Li, Z.J. CircRNA-ENO1 promoted glycolysis and tumor progression in lung adenocarcinoma through upregulating its host gene ENO1. *Cell Death Dis.* **2019**, *10*, 885. [\[CrossRef\]](#)
39. Lv, T.; Miao, Y.F.; Xu, T.Q.; Sun, W.H.; Sang, Y.Z.; Jia, F.; Zhang, X.H. Circ-EPB41L5 regulates the host gene EPB41L5 via sponging miR-19a to repress glioblastoma tumorigenesis. *Aging* **2020**, *12*, 318–339. [\[CrossRef\]](#)
40. Zhao, X.L.; Duan, X.M.; Fu, J.P.; Shao, Y.N.; Zhang, W.W.; Guo, M.; Li, C.H. Genome-Wide Identification of Circular RNAs Revealed the Dominant Intergenic Region Circularization Model in *Apostichopus japonicus*. *Front. Genet.* **2019**, *10*, 603. [\[CrossRef\]](#)
41. Zhang, L.; Liu, X.; Ma, X.; Liu, Y.; Che, S.; Cui, J.; An, X.; Cao, B.; Song, Y. Testin was regulated by circRNA3175-miR182 and inhibited endometrial epithelial cell apoptosis in pre-receptive endometrium of dairy goats. *J. Cell. Physiol.* **2018**, *233*, 6965–6974. [\[CrossRef\]](#)
42. Zheng, Q.; Bao, C.; Guo, W.; Li, S.; Chen, J.; Chen, B.; Luo, Y.; Lyu, D.; Li, Y.; Shi, G.; et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat. Commun.* **2016**, *7*, 11215. [\[CrossRef\]](#)
43. Bauersachs, S.; Ulbrich, S.E.; Reichenbach, H.D.; Reichenbach, M.; Buttner, M.; Meyer, H.H.D.; Spencer, T.E.; Minten, M.; Sax, G.; Winter, G.; et al. Comparison of the Effects of Early Pregnancy with Human Interferon, Alpha 2 (IFNA2), on Gene Expression in Bovine Endometrium. *Biol. Reprod.* **2012**, *86*, 1–15. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Meidan, R.; Basavaraja, R. Interferon-Tau regulates a plethora of functions in the corpus luteum. *Domest. Anim. Endocrinol.* **2022**, *78*, 106671. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Basavaraja, R.; Przygodzka, E.; Pawlinski, B.; Gajewski, Z.; Kaczmarek, M.M.; Meidan, R. Interferon-tau promotes luteal endothelial cell survival and inhibits specific luteolytic genes in bovine corpus luteum. *Reproduction* **2017**, *154*, 559–568. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Basavaraja, R.; Madusanka, S.T.; Drum, J.N.; Shrestha, K.; Farberov, S.; Wiltbank, M.C.; Sartori, R.; Meidan, R. Interferon-Tau Exerts Direct Prosurvival and Antiapoptotic Actions in Luteinized Bovine Granulosa Cells. *Sci. Rep.* **2019**, *9*, 14682. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Hua, R.; Zhang, X.; Li, W.; Lian, W.; Liu, Q.; Gao, D.; Wang, Y.; Lei, M. Ssc-miR-21-5p regulates endometrial epithelial cell proliferation, apoptosis and migration via the PDCD4/AKT pathway. *J. Cell Sci.* **2020**, *133*, jcs248898. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Adiguzel, D.; Celik-Ozenci, C. FoxO1 is a cell-specific core transcription factor for endometrial remodeling and homeostasis during menstrual cycle and early pregnancy. *Hum. Reprod. Update* **2021**, *27*, 570–583.
49. Lim, W.; Bae, H.; Bazer, F.W.; Song, G. Fibroblast growth factor 2 induces proliferation and distribution of G(2) /M phase of bovine endometrial cells involving activation of PI3K/AKT and MAPK cell signaling and prevention of effects of ER stress. *J. Cell. Physiol.* **2018**, *233*, 3295–3305. [\[CrossRef\]](#)
50. Kusama, K.; Yoshie, M.; Tamura, K.; Nakayama, T.; Nishi, H.; Isaka, K.; Tachikawa, E. The role of exchange protein directly activated by cyclic AMP 2-mediated calreticulin expression in the decidualization of human endometrial stromal cells. *Endocrinology* **2014**, *155*, 240–248. [\[CrossRef\]](#)
51. Whitby, S.; Zhou, W.; Dimitriadis, E. Alterations in Epithelial Cell Polarity During Endometrial Receptivity: A Systematic Review. *Front. Endocrinol.* **2020**, *11*, 596324. [\[CrossRef\]](#)
52. Bazer, F.W.; Spencer, T.E.; Johnson, G.A. Interferons and uterine receptivity. *Semin. Reprod. Med.* **2009**, *27*, 90–102. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Zhao, S.; Wu, Y.; Gao, H.; Evans, A.; Zeng, S.M. Roles of interferon-stimulated gene 15 protein in bovine embryo development. *Reprod. Fertil. Dev.* **2017**, *29*, 1209–1216. [\[CrossRef\]](#)
54. Chandrakar, K.; Jain, A.; Khan, J.R.; Jain, T.; Singh, M.; Mishra, O.P. Molecular characterization and expression profile of interferon-stimulated gene 15 (ISG15) in the endometrium of goat (*Capra hircus*). *Theriogenology* **2020**, *142*, 348–354. [\[CrossRef\]](#)
55. Newton, G.R.; Lewis, S.K.; Avendano, J.; Williams, E.A.; Ribeiro, F.R.B.; Nuti, L.C.; Foxworth, W.B.; Ing, N.H. Fucosyltransferase gene expression in goat endometrium during the estrous cycle and early pregnancy. *Theriogenology* **2019**, *132*, 118–127. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **2009**, *10*, R25. [\[CrossRef\]](#)
57. Hansen, T.B.; Venø, M.T.; Damgaard, C.K.; Kjems, J. Comparison of circular RNA prediction tools. *Nucleic Acids Res.* **2016**, *44*, e58. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Gao, Y.; Zhang, J.; Zhao, F. Circular RNA identification based on multiple seed matching. *Brief. Bioinform.* **2018**, *19*, 803–810. [\[CrossRef\]](#)

59. Zhou, L.; Chen, J.; Li, Z.; Li, X.; Hu, X.; Huang, Y.; Zhao, X.; Liang, C.; Wang, Y.; Sun, L.; et al. Integrated profiling of microRNAs and mRNAs: microRNAs located on Xq27.3 associate with clear cell renal cell carcinoma. *PLoS ONE* **2010**, *5*, e15224. [[CrossRef](#)]
60. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [[CrossRef](#)]
61. Hochberg, Y.; Benjamini, Y. More powerful procedures for multiple significance testing. *Stat. Med.* **1990**, *9*, 811–818. [[CrossRef](#)] [[PubMed](#)]
62. Bailey, T.L. DREME: Motif discovery in transcription factor ChIP-seq data. *Bioinformatics* **2011**, *27*, 1653–1659. [[CrossRef](#)]
63. Fornes, O.; Castro-Mondragon, J.A.; Khan, A.; van der Lee, R.; Zhang, X.; Richmond, P.A.; Modi, B.P.; Correard, S.; Gheorghe, M.; Baranasic, D.; et al. JASPAR 2020: Update of the open-access database of transcription factor binding profiles. *Nucleic Acids Res.* **2020**, *48*, D87–D92. [[CrossRef](#)] [[PubMed](#)]
64. Gupta, S.; Stamatoyannopoulos, J.A.; Bailey, T.L.; Noble, W.S. Quantifying similarity between motifs. *Genome Biol.* **2007**, *8*, R24. [[CrossRef](#)] [[PubMed](#)]
65. Enright, A.J.; John, B.; Gaul, U.; Tuschl, T.; Sander, C.; Marks, D.S. MicroRNA targets in Drosophila. *Genome Biol.* **2003**, *5*, R1. [[CrossRef](#)]
66. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* **2003**, *13*, 2498–2504. [[CrossRef](#)]
67. Xie, C.; Mao, X.; Huang, J.; Ding, Y.; Wu, J.; Dong, S.; Kong, L.; Gao, G.; Li, C.Y.; Wei, L. KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res.* **2011**, *39*, W316–W322. [[CrossRef](#)]
68. Zang, X.; Zhou, C.; Wang, W.; Gan, J.; Li, Y.; Liu, D.; Liu, G.; Hong, L. Differential MicroRNA Expression Involved in Endometrial Receptivity of Goats. *Biomolecules* **2021**, *11*, 472. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

中国科技核心期刊

《中文核心期刊要目总览》核心期刊

中国科学引文数据库来源期刊

ISSN 0366-6964
CN 11-1985/S

畜牧兽医学报

ACTA VETERINARIA ET ZOOTECHNICA SINICA



中国畜牧兽医学会 主办

2024 | 05
Vol.55

畜牧兽医学报

XUMU SHOUYI XUEBAO

第 55 卷 第 5 期 2024 年 5 月

目次

综 述	
产单核细胞李氏杆菌噬菌体的应用研究进展	崇 倩,赵学慧,曹 青,薛惠文,苟惠天(1819)
动物遗传评估软件研究进展	张元旭,李 竟,王泽昭,陈 燕,徐凌洋,张路培,高 雪,高会江,李俊雅,朱 波,郭 鹏(1827)
多组学技术在畜禽重要经济性状研究中的应用	王亚鑫,王 璟,田学凯,杨公社,于太永(1842)
SNP 遗传力估计方法、影响因素及其在畜禽育种中的应用	段益欣,张林云,赵永聚(1854)
CD46 基因在家畜抗病育种中的应用研究进展	李剑南,袁利明,华进联(1866)
表观遗传学调控雌性动物初情期启动的研究进展	张 为,潘志豪,方富贵(1875)
鸡芦花羽性状遗传调控机制研究进展	牛佳佳,徐 丹,刘 洋,赵小玲(1883)
反刍动物瘤胃微生物 LuxS/AI-2 群体感应研究进展	龙唐晖,周江汇,詹彦波,张 健,赵向辉,李艳娇,欧阳克蕙,邱清华(1893)
肠道菌群介导次级胆汁酸及其受体调节肠黏膜免疫机制的研究进展	韩福珍,蔡李萌,李卓然,王雪莹,解伟纯,匡虹迪,李佳璇,崔 文,姜艳平,李一经,单智夫,唐丽杰(1904)
RNA 结合蛋白 ELAVL1 的功能及其调控病毒复制的研究进展	余祖华,高梦茹,齐志颖,张静雨,何 雷,陈 建,丁 轲(1914)
后生素调控动物肠道健康的作用机制及应用进展	张吉贤,范定坤,付域泽,焦 帅,马 涛,毕研亮,张乃锋(1926)
遗传育种	
大白猪生长、繁殖和体尺性状遗传参数估计	康佳威,黄宣凯,王志鹏,张爱珍,孟芳荣,盖 鹏,包军付,孙可心,宋少康,孙 攀,陈一川,张 蕾,高圣玥,常铭航(1936)
利用 GWAS 和 DNA 甲基化共定位鉴定猪肉品质性状的候选基因	崔晟頔,王 凯,赵真坚,陈 栋,申 琦,余 杨,王俊戈,陈子畅,禹世欣,陈佳苗,王翔枫,唐国庆(1945)
藏猪促炎因子(IL-1 β ,IL-6,TNF- α)多态性及其表达与免疫性状的关联分析	孙雯莉,王浩奇,泽里磋,高雨樊,张非凡,张 健,段梦琪,商 鹏,强巴央宗(1958)
不同出生季节对天津地区荷斯坦牛泌乳性能的影响	陈丽丽,赵 康,夏 敏,芦 娜,马 毅(1970)
基于转录组测序研究绿光影响鹅胚心脏早期发育的候选基因	陈 哲,曲小露,郭彬彬,孙雪峰,闫乐艳(1978)
保种场涪陵水牛及西南地区水牛品种间遗传结构与 ROH 分析	屠 芸,曾雅楠,张蒸豪,洪 瑞,王 震,吴 平,周泽洋,叶艺茹,杜亚楠,左福元,张龚炜(1989)
生物技术与繁殖	
细胞松弛素 B 改善冷冻引起的猪卵母细胞皮质颗粒迁移障碍	李婉君,徐皆欢,何孟纤,孔钰婷,张德福,戴建军(1999)
线粒体 tRNA-Lys(T7719G)基因变异影响绵羊颗粒细胞凋亡生理机制研究	吕世琪,周荣艳,田树军,陈晓勇(2011)
雄激素受体抑制剂恩杂鲁胺对山羊卵泡颗粒细胞增殖凋亡的影响	董书餐,毛帅翔,伍翠莹,李耀坤,孙宝丽,郭勇庆,邓 铭,刘德武,柳广斌(2022)
MNQ 的一种衍生物对 LPS 体外诱导的牛卵巢卵泡颗粒细胞炎性损伤的保护作用	杨小峰,秦小伟,吕丽华(2032)
营养与饲料	
饲料添加赖氨酸对肉牛粪便发酵参数和微生物菌群结构的影响	龙唐晖,詹彦波,廖观香,陈新锋,张 健,李艳娇,欧阳克蕙,邱清华(2042)

预防兽医

- 牛冠状病毒 S1 蛋白的真核表达及间接 ELISA 方法的建立与应用
..... 黄 金,李思远,毛 立,蔡旭航,谢玲玲,王 府,周 华,李基棕,李 彬(2050)
- 猪圆环病毒 3 型 Rep 蛋白的原核表达及酶活性分析
..... 韩 阳,关帅印,李 振,周赛赛,袁红根,宋云峰(2061)
- PCV4 Cap 抗体 ELISA 检测方法的建立及血清流行病学调查
..... 宋晓晴,邓瑞德,李 欣,李 姣,李润成,杜丽飞,董 伟,葛 猛(2072)
- 基于 Nanopore 测序技术的非洲猪瘟病毒全基因组测序方法建立
..... 周 扬,吴炜姿,曹伟胜,王福广,许秀琼,钟文霞,吴立炆,叶 健,卢受昇(2080)
- 不同猪源受体菌表达猪流行性腹泻病毒保护性抗原 S1 诱导免疫应答的比较研究
..... 马茹梦,赵玉梁,马明爽,国桂海,刘芯孜,李佳璇,崔 文,姜艳平,单智夫,
周 晗,王 丽,乔薪瑗,唐丽杰,王晓娜,李一经(2090)
- C8orf4 基因编码蛋白对猪流行性腹泻病毒体外复制的抑制效应
..... 徐 红,商红旗,张 雪,钱嘉莉,王传红,宋 旭,宝梅英,
刘诗雨,张格格,郭容利,赵永祥,范宝超,李 彬(2100)
- 三株鸡传染性支气管炎病毒优势流行毒株全基因组分析及致病性
..... 熊 挺,何献铭,赵希雅,庄婷婷,黄美珍,梁世金,余传照,梁雪静,陈瑞爱(2109)
- 共表达膜结合型与可溶性 H9N2 亚型禽流感病毒 HA 蛋白的重组基因Ⅷ型新城疫病毒的构建及免疫效果评价
..... 吕亚迪,杨 洁,谢文婷,徐 婷,陈瑞爱(2123)
- 牛种布鲁氏菌 A19ΔBtpA 缺失株生物学特性及其免疫原性研究
..... 徐朕宇,邓肖玉,王月丽,孙 灿,吴澳迪,曹 剑,易继海,王 勇,王 震,陈创夫(2135)
- 竞争 ELISA 和间接 ELISA 方法应用于牛布鲁氏菌病净化的研究
..... 赵灿奇,冯 宇,吕 浪,李彦军,魏玉磊,丁家波,陈 祥,蒋 卉(2146)
- 西藏牦牛源牛支原体 T10 株全基因组测序及其序列分析
..... 罗 婷,韩 著,徐业芬,蔡 林,索朗斯珠,徐晋花,牛家强(2154)
- 荚膜 B 型多杀性巴氏杆菌外膜囊泡生物学特性分析与免疫效果评价
..... 高 洁,李晓成,穆 杨,张 慧,魏 荣,李 劼(2168)

基础兽医

- CD44 通过影响猪流行性腹泻病毒复制调节钠氢交换体 3 活性
..... 王 静,张淑娟,胡 霞,刘向阳,张兴翠,宋振辉(2176)
- 伪狂犬病毒感染小鼠基质金属蛋白酶-9 介导紧密连接蛋白损伤血脑屏障的研究
..... 张 颖,宋春莲,张 莹,沈 鸿,舒相华,杨洪贵(2186)
- 猪肺炎支原体乳酸脱氢酶在诱导猪支气管上皮细胞凋亡中的作用
..... 王吉英,尹蕊如,谢 星,王海燕,刘胡栋,胡 辉,熊祺琰,冯志新,邵国青,于岩飞(2195)
- 花生茎源茉莉炭疽菌的分离鉴定及生物学特性研究
..... 郑 芮,刘紫石,张康友,颜 勇,魏 玲,泽仁翁姆,丁则德米,黄建钧,王 利,魏 勇(2206)
- 妊娠期奶牛黄体细胞的分离鉴定及培养特性
..... 费国庆,宁致远,赵泽芳,刘艳秋,刘腾飞,李 贤,丛日华,陈 鸿,陈树林(2214)
- SOCS2 对山羊鼻甲骨细胞增殖、周期及凋亡的影响
..... 李秋云,田芯源,廖文圣,张焕容,任玉鹏,杨发龙,朱江江,向 华(2226)

临床兽医

- 黄芪多糖、皂苷及益生菌复合物对感染大肠杆菌肉鸡肠道的保护作用
... 刘佳惠,吴开开,王 磊,张 康,韩松伟,陈富斌,徐国伟,郭志廷,古雪艳,张景艳,李建喜(2241)

研究简报

- 猫疱疹病毒 1 型的检测及一株分离毒株的致病性
... 邓楷男,张家祺,保志鹏,陈涛云,喻琦胜,丁 露,朱晨曦,王 怡,任玉鹏,贺 超,张 斌(2253)
- 山羊地方性鼻内肿瘤病毒 TaqMan 荧光定量 RT-PCR 检测方法的建立及应用
..... 李鹏飞,高桂琴,周广青,吴锦艳,颜新敏,曹小安,何继军,袁莉刚,尚佑军(2259)
- PEDV、PoRVA 和 PDCoV TaqMan 三重 RT-qPCR 检测方法的建立与初步应用
..... 胡泽奇,李润成,谭祖明,谢秀艳,王江平,秦乐娟,李 荣,葛 猛(2267)
- 七种蛋白酶抑制剂对多房棘球蚴 DNA 损伤诱导样 1 蛋白活性的影响
..... 张生英,刘仲葵,郭爱疆,王 帅(2273)



雄激素受体抑制剂恩杂鲁胺对山羊卵泡颗粒细胞增殖凋亡的影响

董书餐,毛帅翔,伍翠莹,李耀坤,孙宝丽,郭勇庆,邓 铭,刘德武,柳广斌*

(华南农业大学动物科学学院,广州 510642)

摘 要:旨在通过使用恩杂鲁胺探究雄激素受体(AR)对山羊卵泡颗粒细胞增殖凋亡的影响。本研究首先对山羊卵泡颗粒细胞进行体外分离培养,然后利用显微镜观察和 CCK8 探究颗粒细胞的恩杂鲁胺适合培养浓度;之后将试验分为 3 个组,即空白对照组(不做处理)、阴性对照组(DMSO 处理)和试验组(恩杂鲁胺处理),每个组均设置 3 个重复;然后利用 EDU 检测颗粒细胞的增殖情况,使用 Caspase3 活性与细胞凋亡检测试剂盒检测颗粒细胞的凋亡情况,并利用实时荧光定量 PCR(qRT-PCR)和蛋白免疫印迹(Western blot)检测颗粒细胞增殖凋亡相关基因和蛋白的表达情况。结果表明,AR 抑制剂恩杂鲁胺能够显著地抑制颗粒细胞的增殖和降低颗粒细胞中 *CCND1*、*PCNA* 和 *MKI67* 基因的 mRNA 水平,并且还能够显著抑制 *CCND1*、*PCNA* 和 β -catenin 蛋白的表达;此外,恩杂鲁胺还能够促进山羊卵泡颗粒细胞的凋亡,显著提高山羊卵泡颗粒细胞中 *BAX* 和 *BCL2* 的 mRNA 水平,但不影响 *BAX* 和 *BCL2* 的蛋白表达,另外恩杂鲁胺虽然没有影响 *CASP3* 基因的 mRNA 水平但能够提高 Caspase 3 的活性。这些结果表明,恩杂鲁胺抑制 AR 活性后能够影响颗粒细胞中 β -catenin、*PCNA*、*CCND1*、Caspase 3 等的表达,参与调控颗粒细胞的增殖凋亡。

关键词:山羊;颗粒细胞;AR;恩杂鲁胺;增殖凋亡

中图分类号: S827.3

文献标志码: A

文章编号: 0366-6964(2024)05-2022-10

The Effect of the Androgen Receptor Inhibitor Enzalutamide on Proliferation and Apoptosis of Goat Ovarian Granulosa Cells

DONG Shucan, MAO Shuaixiang, WU Cuiying, LI Yaokun, SUN Baoli, GUO Yongqing,
DENG Ming, LIU Dewu, LIU Guangbin*

(College of Animal Science, South China Agricultural University, Guangzhou 510642, China)

Abstract: The aim of this study was to investigate the effects of androgen receptor (AR) on goat granulosa cell proliferation and apoptosis using enzalutamide. Firstly, granulosa cells from goats were isolated and subjected to *in vitro* culture. The suitable concentration of enzalutamide for cell culture was determined by microscopic observation and CCK8 assay. The experiment was then divided into 3 groups: blank control group (no treatment), negative control group (treated with DMSO), and experimental group (treated with enzalutamide). Each group was set up with 3 replicates. Cell proliferation was detected using the EDU assay, while cell apoptosis was assessed using Caspase3 activity and apoptosis detection kits. The expression of genes and proteins related

收稿日期: 2023-09-18

基金项目: 广东省林业科技创新项目(2023KJ CX004); 广东省南方现代草牧业(羊)创新团队(2023KJ127); 广东省农业农村厅项目(2022-XPY-00-009); 韶关市仁化县牛羊产业园项目(GDSCYY2021-018)

作者简介: 董书餐(1999-), 男, 云南梁河人, 硕士生, 主要从事动物遗传育种与繁殖研究, E-mail: dsc15184989294@163.com

* 通信作者: 柳广斌, 主要从事动物遗传育种与繁殖研究, E-mail: gbliu@scau.edu.cn

to cell proliferation and apoptosis was evaluated using quantitative real-time PCR (qRT-PCR) and Western blot analysis. The results showed that the AR inhibitor, enzalutamide, significantly inhibited cell proliferation and reduced the mRNA levels of *CCND1*, *PCNA*, and *MKI67* in granulosa cells. It also suppressed the protein expression of *CCND1*, *PCNA*, and β -catenin. Additionally, enzalutamide promoted apoptosis in goat granulosa cells, as evidenced by increased mRNA levels of *BAX* and *BCL2*, without affecting their protein expression. Furthermore, enzalutamide, while not affecting the mRNA level of *CASP3*, enhanced Caspase3 activity. These findings suggest that enzalutamide, by inhibiting AR activity, can modulate the expression of β -catenin, *PCNA*, *CCND1*, Caspase 3, and other genes involved in the regulation of granulosa cell proliferation and apoptosis.

Key words: goat; granulosa cell; androgen receptor; enzalutamide; proliferation apoptosis

* **Corresponding author:** LIU Guangbin, E-mail: gblu@scau.edu.cn

繁殖性能是衡量家畜生产水平的重要经济指标,直接影响养殖户的经济效益,提高家畜的繁殖性能对于促进我国畜牧业的发展具有重要意义。卵泡发育是影响雌性哺乳动物繁殖性能的重要因素之一^[1]。在雌性哺乳动物刚出生时,卵巢拥有大量的卵泡储备,然而随着动物的生长发育,体内的卵泡逐渐凋亡闭锁,仅有少量的优势卵泡能够破裂排出可用于受精的卵母细胞,因此动物的卵泡发育并不是一个高效的过程^[2-4]。了解卵泡发育的机制对于提高动物的繁殖性能具有重要意义。卵泡主要由颗粒细胞、卵母细胞及膜细胞组成^[5]。颗粒细胞在卵泡的发育中发挥重要作用,其通过间隙连接与卵母细胞和膜细胞进行信号传递和物质交换,还可以通过旁分泌调节卵母细胞的生长^[6]。此外,颗粒细胞还能够表达促卵泡激素、黄体生成素/绒毛膜促性腺激素等多种激素的受体从而使初级卵泡对性腺轴激素具有应答功能^[7]。许多研究表明,颗粒细胞的凋亡是造成卵泡闭锁的重要因素之一^[8-9]。因此探究颗粒细胞的增殖凋亡机制对于全面了解卵泡发育具有重要意义。

雄激素受体(androgen receptor, AR)属于核受体超家族中的类固醇受体,能够与雄激素结合作为转录因子调控基因的表达^[10]。通过对不同发育阶段的卵泡进行测序,发现 AR 基因表达贯穿卵泡发育的全过程,并在卵泡发育不同阶段呈现出不同表达趋势,主要表现为在大卵泡中低表达,而在小卵泡中高表达,表明 AR 可能参与调控卵泡的发育^[11-12]。卵泡颗粒细胞 AR 特异性敲除小鼠表现出卵泡闭锁率高、繁殖力低等情况^[13]。此外,在猪卵泡颗粒细胞中 AR 能够与 miR-126 互作参与调控猪卵泡颗粒

细胞中促卵泡生成素受体的表达^[14]。这些结果表明 AR 在卵泡颗粒细胞中发挥着重要功能。恩杂鲁胺(enzalutamide)是一种 AR 拮抗剂,能够与雄激素竞争性结合 AR 使其失去转录活性,因此能够被用于 AR 功能的探索^[15]。

探究 AR 在卵泡颗粒细胞增殖凋亡中的作用对于全面了解卵泡发育的分子机制具有重要意义。本研究通过利用恩杂鲁胺探索 AR 在卵泡发育过程中对颗粒细胞增殖凋亡的影响,以期全面了解卵巢卵泡发育机制和卵泡相关疾病的治疗提供参考。

1 材料与方法

1.1 山羊卵泡颗粒细胞的分离与培养

山羊屠宰后找到卵巢组织并取下卵巢,然后分别在 75% 的酒精和含 2% 青霉素-链霉素双抗溶液的生理盐水中灭菌,之后放入 37 °C 含 2% 双抗溶液的生理盐水中保存运输。回到实验室后在已提前 30 min 紫外灭菌的生物安全柜中分离卵泡颗粒细胞。简而言之,首先,对运输回来的卵巢进行消毒,结束后将卵巢放入 37 °C 含 2% 双抗的 DMEM 培养基中,然后使用注射器刺破卵泡使卵泡中的颗粒细胞释放出来,之后收集 DMEM 培养液并过 70 μ m 的细胞筛,过筛结束后室温静置 15 min; 然后 1 000 $r \cdot \min^{-1}$ 条件下离心 10 min,离心结束后弃去上清,使用 3 mL 含 2% 双抗的 DMEM 培养基重悬细胞,再次离心并弃去上清;接着使用 10 mL 完全培养基(配方:90% DMEM/F12+10% FBS+1% 双抗+1% 100 \times ITS-A)重悬细胞,并将其接种于细胞培养皿中;最后将细胞放在 5% CO_2 ,饱和湿度和 37 °C 的细胞培养箱中静置培养,培养 24 h 后弃去未

贴壁的细胞并更换新的完全培养基,之后每 48 h 更换一次培养基直至细胞长到 80% 左右后进行细胞传代与冻存。

1.2 CCK8 细胞增殖检测

将生长状态良好的山羊卵泡颗粒细胞接种于 96 孔板中,其中 96 孔板外围一圈孔只加入完全培养基不加入细胞,防止中央液体的挥发影响试验结果,细胞铺板完成后置于细胞培养箱中静置培养 24 h 后进行试验。试验分为试验组与对照组,试验组分为 4 个不同浓度梯度处理的恩杂鲁胺培养液培养,恩杂鲁胺浓度分别为 20、40、60、80 $\mu\text{mol}\cdot\text{L}^{-1}$,对照组则采用恩杂鲁胺的溶剂 DMSO 处理,每组对照组的 DMSO 加入量与恩杂鲁胺溶液加入量相同,每个处理至少有 4 个生物学重复。然后在 24、48、72 h 时向每个细胞培养孔中分别加入 10 μL 的 CCK8 试剂,1 h 后用酶标仪检测培养液 450 nm 处的吸光度值,吸光度值越大代表细胞的增殖活力越强。

1.3 EDU 细胞增殖检测

按照 Cell-Light™ EDU Apollo In Vitor Kit 说明书进行 EDU 细胞增殖检测。简而言之,将配置好的 EDU 溶液加入细胞培养液中孵育 2 h,取出培养液,然后使用细胞固定液对细胞进行固定 30 min,通透液通透 10 min, PBS 洗涤。然后进行 Apollo 染色, PBS 洗涤之后,使用 Hoechst 染料进行细胞核 DNA 染色。倒置荧光显微镜下观察细胞染色情况。

表 1 实时荧光定量 PCR 基因引物序列

Table 1 Real-time fluorescence quantitative PCR gene primer sequences

基因 Gene	上游引物序列(5'→3') Forward primer sequence	下游引物序列(5'→3') Reverse primer sequence
BAX	CATGGGCTGGACATTGGAC	CACTTCAGCGACTCAGCCAA
BCL2	ATGACCGAGTACCTGAACCG	TCCACAAAGGCGTCGGAT
CASP3	TCTTCAGAGGGGACTGTTGC	GCCAGGAAAAGTAACCAGGTG
PCNA	GAACCTCACCAGCATGTCCA	TGCCAAGGTGTCCGCATTAT
CCND1	CACCTGTATGTTTCGTGGCCT	TGAACCTCACGTCTGTGGCA
MKI67	TGCGTGTGTTGGAAGGGGTAT	AGGTGACTTGCTTCCATCCTG
β -Actin	GATGATGATATTGCTGCGCTCG	GTCAGGATGCCTCTCTTGCT

1.7 实时荧光定量 PCR (qRT-PCR)

以 cDNA 为模板,按照诺维赞 ChamQ SYBR qPCR Master Mix (Low ROX Premixed)说明书进行实时荧光定量 PCR。反应体系如下,2×ChamQ SYBR qPCR Master Mix (Low ROX Premixed)

1.4 活细胞 Caspase3 活性与细胞凋亡检测

将细胞接种于 24 孔板中,使用恩杂鲁胺处理后,吸除细胞培养液,使用 PBS 洗涤细胞。然后加入 192 μL 的 Annexin V-mCherry Binding Buffer, 5 μL 的 Annexin V-mCherry, 2 μL 的 Hoechst 33342 和 1 μL 的 GreenNuc™ caspase3 Substrate, 轻轻混匀,室温避光孵育 30 min。倒置荧光显微镜下观察细胞荧光发光情况。

1.5 RNA 的提取与 cDNA 的合成

使用 Trizol 法提取细胞总 RNA。首先,使用 0.25% 的胰酶消化、离心收集细胞,弃去上清并加入 800 μL 的 Trizol,室温裂解 5 min,之后加入 160 μL 氯仿,离心收集上层水相,然后加入等体积的异丙醇,室温静置 10 min,离心弃去异丙醇,75% 乙醇洗涤沉淀,干燥,无菌无酶水溶解, -80 °C 保存。细胞总 RNA 按照艾科瑞生物公司的 EVO M-MLV 反转录试剂盒的说明书进行反转录,取 1 μg 总 RNA 使用 gDNA Clean Reaction Mix, EVO M-MLV RT Reaction Mix 进行反转录。反应条件: 37 °C, 15 min; 85 °C, 5 s。

1.6 引物的设计与合成

登录 NCBI 搜索山羊 PCNA、CCND1、MKI67、BAX、BCL2、CASP3 和 β -ACTIN 基因的 CDS 区序列,使用 NCBI 中的 pick primers 在线软件进行引物设计,设计时设置引物至少跨一个外显子,以防止基因组干扰。所有引物均由生工生物工程股份有限公司合成,合成引物及序列见表 1。

10 μL , 上、下游引物 (10 $\mu\text{mol}\cdot\text{L}^{-1}$) 各 0.4 μL , cDNA 1 μL , ddH₂O 8.2 μL 。反应程序为: 95 °C 30 s; 95 °C 10 s, 60 °C 30 s, 40 个循环; 95 °C 15 s, 60 °C 60 s, 95 °C 15 s。以 β -Actin 作为内参,结果使用 $2^{-\Delta\Delta\text{CT}}$ 法进行计算。

1.8 蛋白提取与 Western blot 检测

按照 RIPA 裂解缓冲液Ⅲ的说明提取细胞总蛋白,然后用改良型 BCA 蛋白检测试剂盒定量检测总蛋白浓度。根据测定的蛋白浓度使用 RIPA 裂解缓冲液Ⅲ调整每个样品的蛋白以使各样品浓度大概一致,然后向每个蛋白样品中加入 $4\times$ Protein SDS PAGE loading buffer,在 $100\text{ }^{\circ}\text{C}$ 下变性 10 min。变性蛋白质经 10% SDS-PAGE 凝胶电泳分离后转移至 $0.45\text{ }\mu\text{m}$ 聚偏二氟乙烯(PVDF)膜上。用 Quick block™ blocking buffer for western blot 封闭 10 min 后,使用 $1\times$ TBST 洗膜 5 次,每次 5 min。洗膜结束后,向 PVDF 膜中加入一抗, $4\text{ }^{\circ}\text{C}$ 冰箱中孵育过夜,洗膜,之后用相应的二抗在室温下孵育 1 h,洗膜,最后使用超敏 ECL 化学发光试剂盒在对应仪器中观察蛋白条带。

1.9 统计分析

所有数据均以“平均值 \pm 标准差(SD)”表示,

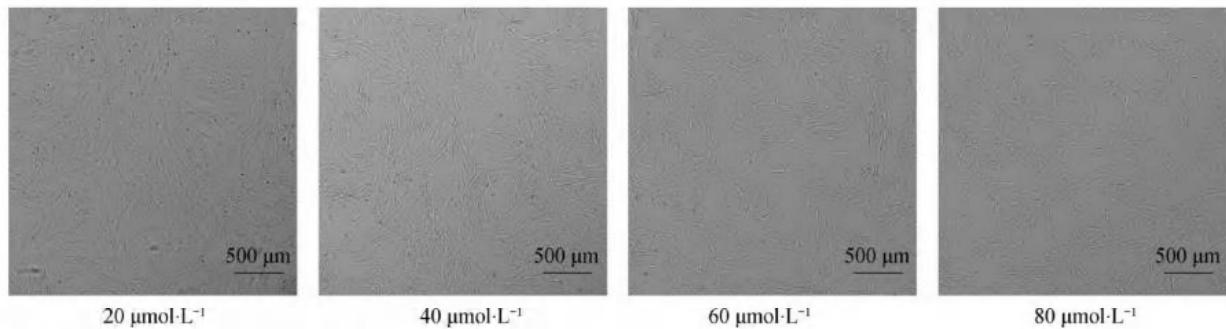


图1 48 h 时不同浓度梯度恩杂鲁胺培养条件下山羊卵泡颗粒细胞生长图(40 \times)

Fig. 1 Growth graph of goat ovarian granulosa cells under different concentration gradient enzalutamide culture conditions at 48 h (40 \times)

此外,为了探索不同时间点条件下恩杂鲁胺对山羊卵泡颗粒细胞增殖的影响,还使用 CCK8 对上述 4 个恩杂鲁胺培养浓度条件下的山羊卵泡颗粒细胞的增殖情况进行了检测。由图 2 可知,与对照组相比,不同浓度的恩杂鲁胺均能在不同程度上抑制山羊卵泡颗粒细胞的增殖。且随着浓度的提升,细胞的增殖活性逐渐降低,特别在 $80\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 浓度条件下,颗粒细胞的增殖活性最差,这可能是由于 DMSO 浓度过高影响了细胞的增殖。结合细胞生长状态观察及 CCK8 检测结果,后续试验选用 $40\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 的恩杂鲁胺细胞培养浓度对山羊卵泡颗粒细胞进行培养,并在 48 h 时收样。

2.2 EDU 检测结果分析

EDU 是一种胸腺嘧啶核苷类似物,能够在细胞

至少有 3 个独立重复。两组或多组之间的差异分析分别采用 t 检验和单因素方差分析,所有分析均在 GraphPad Prism9 中完成。“*”代表 $P<0.05$,“**”代表 $P<0.01$,“***”代表 $P<0.001$,“****”代表 $P<0.0001$ 。

2 结果

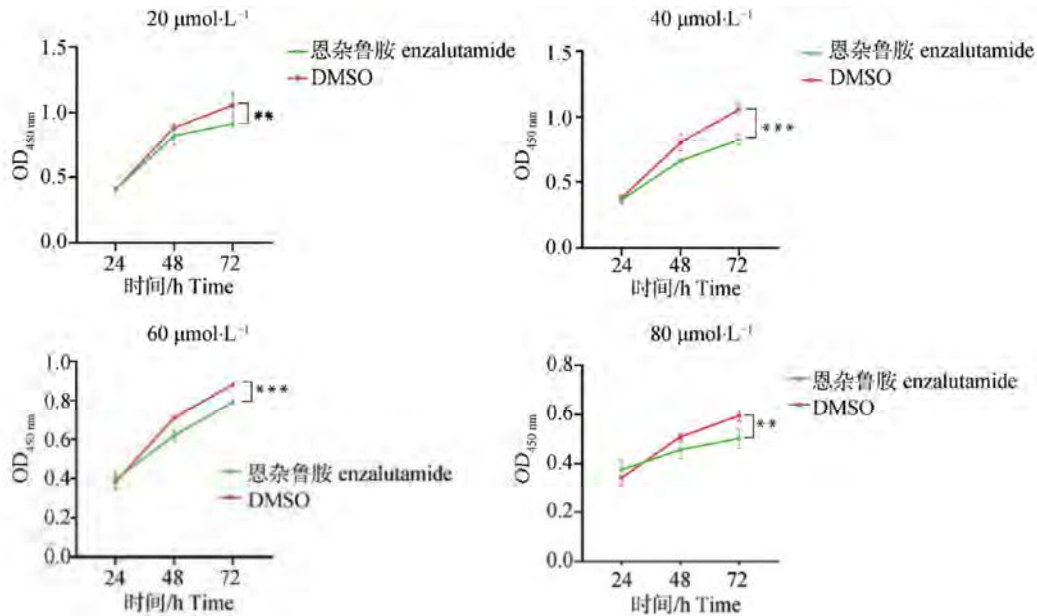
2.1 不同浓度 AR 抑制剂恩杂鲁胺对颗粒细胞增殖的影响

为了探索恩杂鲁胺最适细胞培养浓度,分别使用含 $20, 40, 60, 80\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 浓度的恩杂鲁胺对山羊卵泡颗粒细胞进行培养。药物处理 48 h 后,在显微镜下对山羊卵泡颗粒细胞的生长状况进行观察,由图 1 可知,随着恩杂鲁胺浓度的升高,山羊卵泡颗粒细胞的密度逐渐降低,说明在该试验中恩杂鲁胺对卵泡颗粒细胞的增殖具有一定的抑制作用。

增殖时替代胸腺嘧啶插入正在复制的 DNA 链中,通过使用 Apollo 荧光染料与结合到 DNA 双链上的 EDU 特异性反应,利用免疫荧光技术能够检测出细胞的增殖状况,其相较于 CCK8 技术,能够更直观的反映出细胞的增殖活力。由图 3 可知,在 48 h 时,与空白对照和 DMSO 对照组相比, $40\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞增殖活力被显著抑制($P<0.01$),表明 AR 活性能够影响山羊卵泡颗粒细胞的增殖能力。

2.3 山羊卵泡颗粒细胞增殖相关基因表达分析

细胞的增殖受到严格的基因表达调控,研究测定了 $40\text{ }\mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺培养液对卵泡颗粒细胞部分增殖相关基因表达的影响。如图 4A 所示,与空白对照和 DMSO 对照组相比,恩杂鲁胺处理显著



“**”和“***”代表 72 h 时, 恩杂鲁胺处理组与 DMSO 处理组 OD 值差异显著性, 其中“**”代表 $P < 0.01$, “***”代表 $P < 0.001$

“**” and “***” represent the significance of the difference in OD values between the enzalutamide-treated group and the DMSO-treated group at 72 h, where “**” represents $P < 0.01$ and “***” represents $P < 0.001$

图 2 不同浓度梯度恩杂鲁胺培养条件下 24、48 和 72 h 时山羊卵泡颗粒细胞 CCK8 增殖检测

Fig. 2 CCK8 proliferation assay of goat ovarian granulosa cells at 24, 48, and 72 h under different concentrations of enzalutamide culture conditions

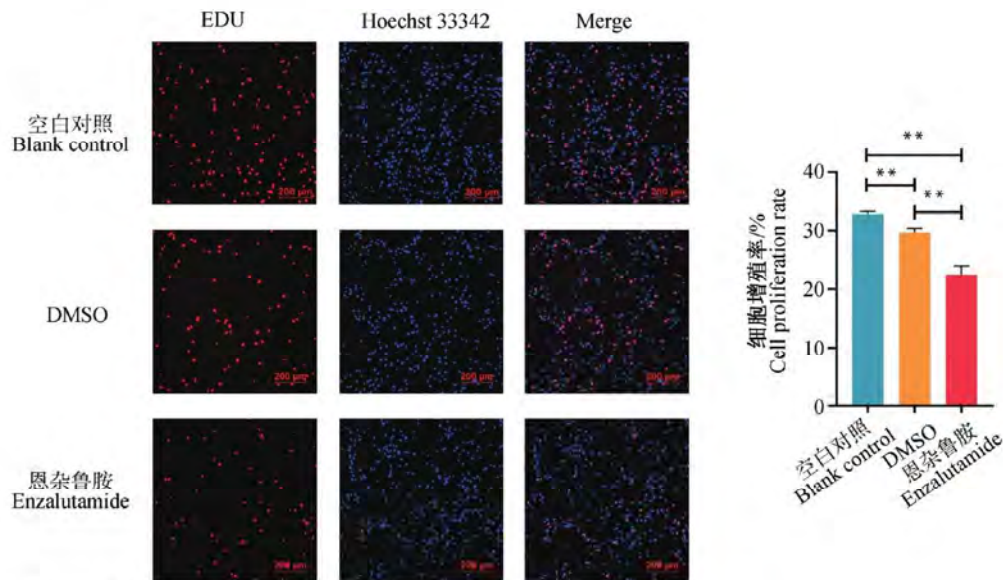


图 3 48 h 时 40 $\mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞 EDU 增殖检测

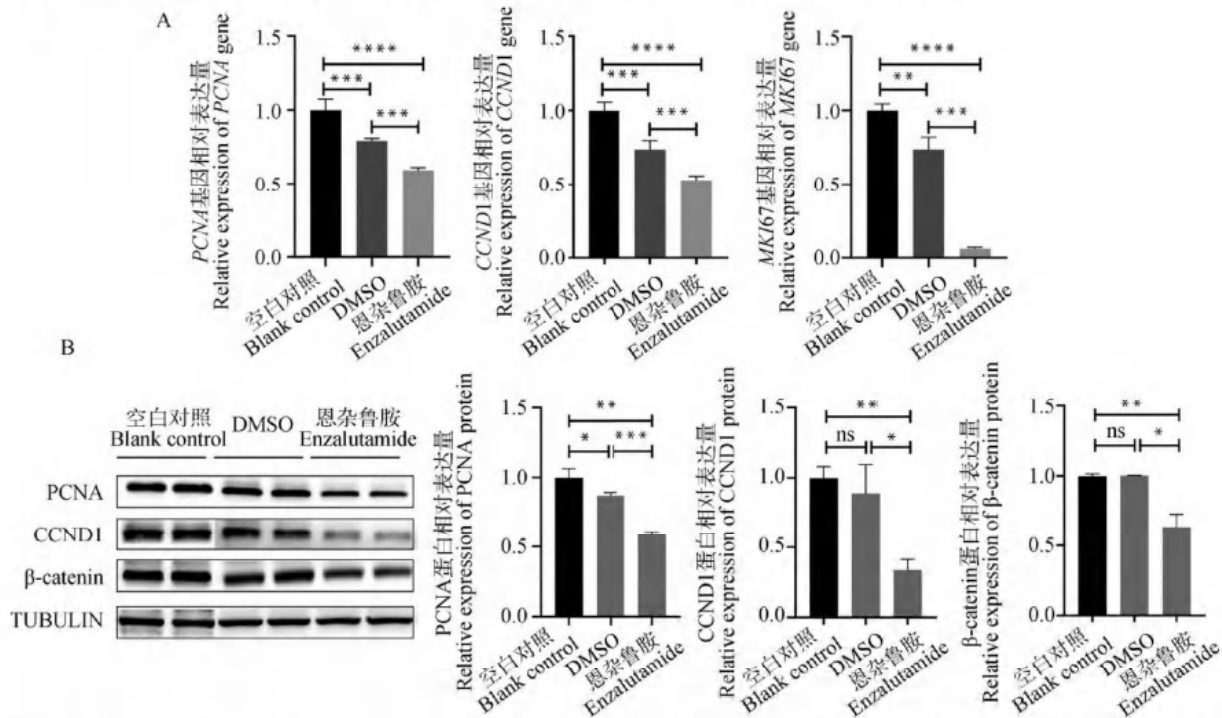
Fig. 3 Detection of EDU proliferation of goat ovarian granulosa cells treated with 40 $\mu\text{mol}\cdot\text{L}^{-1}$ enzalutamide at 48 h

降低了山羊卵泡颗粒细胞中 *MKI67* ($P < 0.01$)、*CCND1* ($P < 0.01$) 和 *PCNA* ($P < 0.01$) 基因 mRNA 水平。蛋白表达检测结果显示 (图 4B), 恩杂鲁胺能够显著抑制 *PCNA* ($P < 0.01$) 和 *CCND1* ($P < 0.05$) 蛋白的表达。WNT 信号通路在细胞的

增殖中发挥重要功能, β -catenin 作为 WNT 经典信号通路中的关键蛋白, 能够通过影响下游 TCF/LEF 家族基因的表达影响细胞的增殖^[16-18]。因此, 研究对 β -catenin 的蛋白水平变化进行了检测, 结果表明, 与空白对照和 DMSO 对照组相比, 40 $\mu\text{mol}\cdot\text{L}^{-1}$

恩杂鲁胺处理组的 β -catenin 蛋白表达量显著降低 ($P < 0.05$)。这些结果表明,AR 活性被抑制后,细

胞增殖相关基因的转录与翻译会被抑制,从而使颗粒细胞的增殖受到影响。



A. qRT-PCR 检测 $40 \mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞 *PCNA*、*CCND1* 和 *MKI67* 基因 mRNA 相对表达水平; B. Western blot 法检测 $40 \mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞 *PCNA*、*CCND1* 和 β -catenin 蛋白相对表达水平

A. qRT-PCR to detect the relative expression levels of *PCNA*, *CCND1* and *MKI67* genes in goat ovarian granulosa cells under $40 \mu\text{mol}\cdot\text{L}^{-1}$ enzalutamide treatment; B. Western blot to detect the relative expression levels of *PCNA*, *CCND1* and β -catenin proteins in goat ovarian granulosa cells under $40 \mu\text{mol}\cdot\text{L}^{-1}$ enzalutamide treatment

图 4 48 h 时 $40 \mu\text{mol}\cdot\text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞增殖相关基因表达检测

Fig. 4 Proliferation genes expression assay in goat ovarian granulosa cells under $40 \mu\text{mol}\cdot\text{L}^{-1}$ enzalutamide treatment condition at 48 h

2.4 恩杂鲁胺促进山羊卵泡颗粒细胞凋亡

了解卵泡颗粒细胞的凋亡调控对于理解卵泡发育具有重要意义^[39]。本研究使用了活细胞 Caspase3 活性与 Annexin V 细胞凋亡检测试剂盒来探究 AR 抑制剂对颗粒细胞凋亡的影响。由图 5 可知,与空白对照和 DMSO 处理组相比,恩杂鲁胺处理组的山羊卵泡颗粒细胞拥有较多的绿色荧光与红色荧光,Caspase3 活化比率显著上升 ($P < 0.05$),表明 AR 抑制剂恩杂鲁胺能够诱导山羊卵泡颗粒细胞发生凋亡。

2.5 山羊卵泡颗粒细胞凋亡相关基因表达分析

使用 qRT-PCR 探究恩杂鲁胺对山羊卵泡颗粒细胞凋亡标志基因 *BAX*、*BCL2* 和 *CASP3* 基因表达的影响,结果如图 6A 所示,与空白对照相比,恩杂鲁胺处能够显著提高 *BAX* ($P < 0.01$) 基因的 mRNA 水平,并降低 *BCL2* ($P < 0.01$) 基因的

mRNA 表达;而与 DMSO 对照相比,恩杂鲁胺能够同时提高 *BAX* ($P < 0.01$) 和 *BCL2* ($P < 0.01$) 基因的 mRNA 表达水平;此外,结果显示,*CASP3* 基因的 mRNA 水平在 3 组间无显著差异。研究还对 *BAX* 和 *BCL2* 蛋白表达水平进行了检测(图 6B),发现与空白对照组相比,DMSO 和恩杂鲁胺处理条件下 *BCL2* 蛋白表达均显著下降,但与 DMSO 处理组相比,恩杂鲁胺处理对 *BCL2* 蛋白表达无显著影响,表明,DMSO 的存在可能能够影响 *BCL2* 蛋白的表达。*BAX* 蛋白检测结果表明,*BAX* 蛋白的表达在 3 组间差异不显著。总的来说,这些结果表明, $40 \mu\text{mol}\cdot\text{L}^{-1}$ 的恩杂鲁胺培养液并不显著影响山羊卵泡颗粒细胞中 *BAX* 和 *BCL2* 蛋白表达。

3 讨论

卵泡的发育是一个十分复杂的过程,在这个过

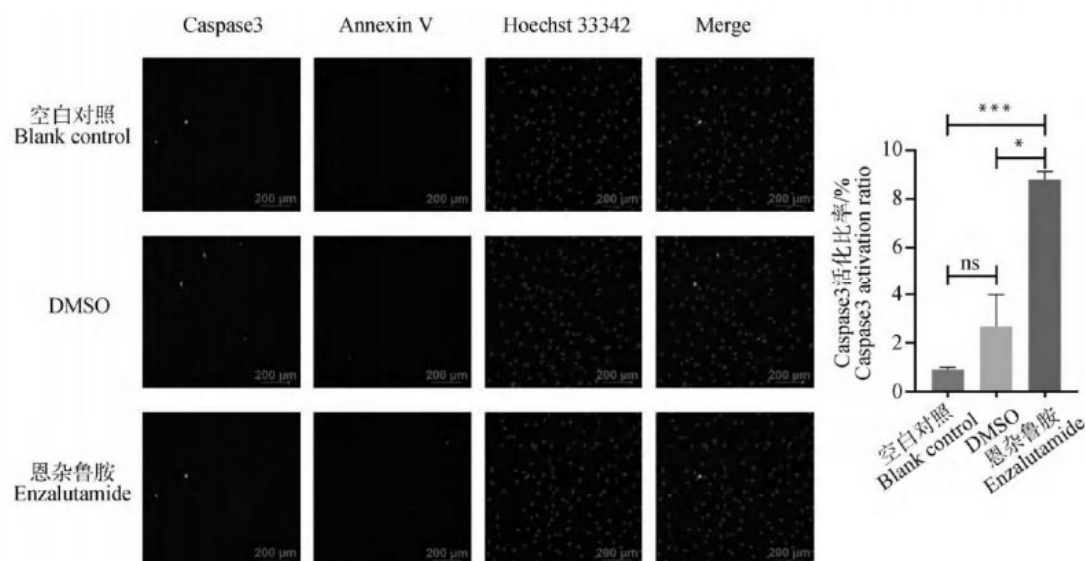
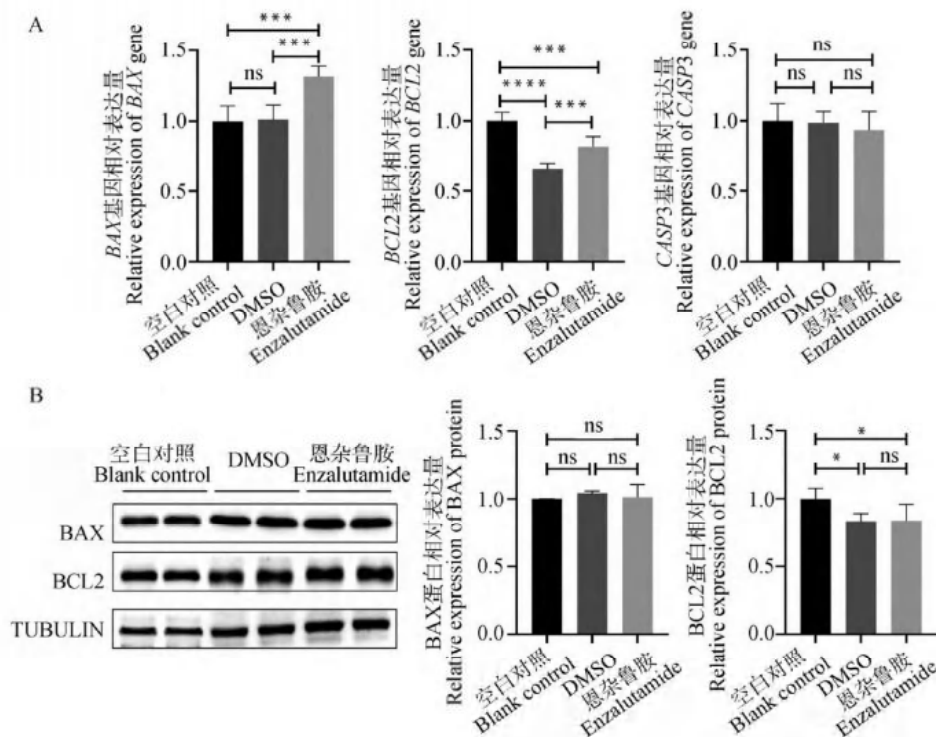


图 5 48 h 时 $40 \mu\text{mol} \cdot \text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞凋亡检测

Fig. 5 Detection of apoptosis in goat ovarian follicular granulosa cells under the treatment condition of $40 \mu\text{mol} \cdot \text{L}^{-1}$ enzalutamide for 48 h



A. qRT-PCR 检测 $40 \mu\text{mol} \cdot \text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞 BAX、BCL2 和 CASP3 基因 mRNA 相对表达量。

B. Western blot 法检测 $40 \mu\text{mol} \cdot \text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞 BAX 和 BCL2 蛋白相对表达量

A. qRT-PCR to detect the relative mRNA expression of BAX, BCL2 and CASP3 genes in goat ovarian granulosa cells under the condition of $40 \mu\text{mol} \cdot \text{L}^{-1}$ enzalutamide treatment; B. Western blot to detect the relative proteins expression of BAX and BCL2 in goat ovarian granulosa cells under the condition of $40 \mu\text{mol} \cdot \text{L}^{-1}$ enzalutamide treatment

图 6 48 h 时 $40 \mu\text{mol} \cdot \text{L}^{-1}$ 恩杂鲁胺处理条件下山羊卵泡颗粒细胞凋亡基因表达检测

Fig. 6 Detection of apoptotic genes expression in goat ovarian granulosa cells under $40 \mu\text{mol} \cdot \text{L}^{-1}$ enzalutamide treatment condition at 48 h

程中,颗粒细胞在不断的增殖分化与凋亡,从而响应卵泡的发育,颗粒细胞的增殖凋亡受到众多基因的调控^[20-22]。研究发现,AR 的表达贯穿卵泡发育的整个过程,但在小卵泡中表达量高,在大卵泡中表达量较低。此外,异常的 AR 活性可能导致卵泡发育障碍和多囊卵巢的形成^[23]。因此,这些研究表明,AR 可能参与卵泡发育的调控,且在其中发挥重要作用。

AR 在动物的生理活动中起着至关重要的调节作用。在雄性动物中,AR 能够调节精子的生成、睾丸功能的维持以及调控骨骼肌肉的发育等^[24-26];在雌性动物中,AR 参与调控卵泡发育和排卵过程,还在子宫内膜和子宫平滑肌中发挥作用,并参与调节月经周期、子宫收缩和妊娠等重要生理过程^[27-29]。AR 的功能发挥依赖于雄激素的存在,作为类固醇受体,其通过与雄激素结合后进入细胞核内并与受体形成复合物,这个复合物进一步调控基因的转录和翻译过程,从而影响细胞的功能和表型^[30-31]。恩杂鲁胺是一种 AR 抑制剂,其能够阻断雄激素与 AR 的结合,抑制 AR 的转录活性并促进 AR 的降解。在这项研究中,利用恩杂鲁胺抑制卵泡颗粒细胞中的 AR 的活性后,CCK8 和 EDU 结果显示卵泡颗粒细胞的增殖活性被抑制,表明 AR 能够参与调控卵泡颗粒细胞的增殖活性。MKI67 是一种标志细胞增殖的核蛋白,高 MKI67 表达通常表示细胞处于增殖状态,而低 MKI67 表达则可能表示细胞停止或减缓增殖^[32]。抑制 AR 活性后,细胞增殖相关基因 MKI67 的转录水平受到抑制,表明细胞的增殖状态受到抑制,这与 EDU 和 CCK8 的检测结果是相一致的。说明颗粒细胞中 AR 的活性能够调节细胞的增殖活性。

细胞增殖受到细胞周期的调控。研究表明,细胞周期进程由一个复杂的基因网络调控,其中 PCNA 在细胞周期中起到了重要的调控作用^[33]。PCNA 是 DNA 聚合酶 δ 和 ϵ 的辅助因子,在 S 期 DNA 复制过程中,PCNA 结合到 DNA 链上形成一个滑动夹持结构,促进 DNA 聚合酶的稳定结合并提高其活性^[34-35]。此外,PCNA 还能够与细胞周期蛋白依赖激酶、细胞周期抑制蛋白等蛋白相互作用调节细胞周期的进程^[36-37]。本研究发现抑制 AR 活性能够降低 PCNA 和 CCND1 基因的表达,说明 AR 能够调节 PCNA 和 CCND1 基因的表达,从而影响细胞周期的进程,进而参与调控颗粒细胞的增

殖。细胞的增殖还受到多条信号通路的调控,其中 WNT 信号通路在卵泡发育过程中扮演重要角色,抑制 WNT 信号通路会导致卵泡发育不良,早期闭锁卵泡增多,同时还会造成卵母细胞分化异常,影响卵泡质量^[38-40]。 β -catenin 是经典 WNT 信号通路的中心蛋白,当 WNT 信号被激活时, β -catenin 向细胞核转移,促进 CCND1、c-MYC 等基因的表达从而促进颗粒细胞的增殖,调控卵泡的生长发育^[41]。使用恩杂鲁胺抑制 AR 活性后,山羊卵泡颗粒细胞中的 β -catenin 蛋白表达水平降低,表明 AR 可能参与经典 WNT 信号通路的调控,但具体的调控机制还需要进一步探索。

卵泡发育的过程中常常伴随着颗粒细胞的凋亡,适度的颗粒细胞凋亡对于优势卵泡的选择、营养物质的供应以及生长因子的调节具有积极作用,但凋亡过多或凋亡缺失都可能会对卵泡发育产生不利影响^[42-43]。因此,维持适度的颗粒细胞凋亡水平是卵泡发育正常进行的关键之一。研究表明,AR 在细胞的生存和死亡中发挥重要调控作用。Huang 等^[44]发现沉默 AR 的表达能够促进肾缺血再灌注模型小鼠的肾脏 Caspase 3 的表达促进细胞凋亡;而 Lin 等^[45]研究表明 AR 能够通过 PIRH2-p53-p21 促进前列腺癌细胞的凋亡。因此,AR 对细胞凋亡的调节受到细胞环境和细胞外刺激影响。在我们的研究中,恩杂鲁胺抑制 AR 活性后,山羊卵泡颗粒细胞中 Caspase 3 蛋白活性增加,细胞膜上的磷脂酰丝氨酸外翻变多,细胞凋亡比率升高,表明恩杂鲁胺抑制 AR 活性后能够促进山羊卵泡颗粒细胞的凋亡。但 AR 对山羊卵泡颗粒细胞凋亡的调控似乎并不依赖于 BCL2 家族成员介导的线粒体凋亡途径,这是因为抑制 AR 活性后虽然上调了 BAX 和 BCL2 的 mRNA 水平,但并没有改变 BAX 和 BCL2 蛋白的表达。因此,AR 可能通过死亡受体凋亡途径或内质网主导的细胞凋亡途径调控山羊卵泡颗粒细胞凋亡。

4 结 论

抑制山羊卵泡颗粒细胞中 AR 的活性能够影响 β -catenin、PCNA、CCND1 和 Caspase 3 等基因的表达从而抑制山羊卵泡颗粒细胞的增殖和促进山羊卵泡颗粒细胞凋亡。

参考文献 (References):

- [1] ZHANG T J, BASANG W D, CHANG W H, et al.

- Dynamics of apoptosis-related gene expression during follicular development in yak[J]. *J Anim Physiol Anim Nutr (Berl)*, 2021, 105(6): 1002-1013.
- [2] KUMARIYA S, UBBA V, JHA R K, et al. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective[J]. *Autophagy*, 2021, 17(10): 2706-2733.
- [3] 刘杰, 许香萍, 邓铭, 等. miR-144-5p 靶向 WNT5a 对山羊卵巢颗粒细胞增殖、凋亡的影响[J]. *畜牧兽医学报*, 2023, 54(6): 2421-2435.
LIU J, XU X P, DENG M, et al. Effect of miR-144-5p targeting WNT5a on the proliferation and apoptosis of goat ovarian granulosa cells[J]. *Acta Veterinaria et Zootechnica Sinica*, 2023, 54(6): 2421-2435. (in Chinese)
- [4] TELFER E E, GROSOBOIS J, ODEY Y L, et al. Making a good egg; human oocyte health, aging, and *in vitro* development[J]. *Physiol Rev*, 2023, 103(4): 2623-2677.
- [5] LI H, HUANG X, CHANG X W, et al. S100-A9 protein in exosomes derived from follicular fluid promotes inflammation via activation of NF- κ B pathway in polycystic ovary syndrome[J]. *J Cell Mol Med*, 2020, 24(1): 114-125.
- [6] CLARKE H. Control of mammalian oocyte development by interactions with the maternal follicular environment [M]//KLOC M. Oocytes: Maternal Information and Functions. Cham; Springer, 2017: 17-41.
- [7] DUFFY D M, KO C, JO M, et al. Ovulation: parallels with inflammatory processes[J]. *Endocr Rev*, 2019, 40(2): 369-416.
- [8] DU X, LI Q Q, YANG L, et al. SMAD4 activates Wnt signaling pathway to inhibit granulosa cell apoptosis[J]. *Cell Death Dis*, 2020, 11(5): 373.
- [9] 李碧筠, 黄思艺, 王钰锟, 等. SMAD7 对山羊卵泡颗粒细胞增殖、凋亡的影响[J]. *畜牧兽医学报*, 2022, 53(8): 2548-2557.
LI B J, HUANG S Y, WANG Y K, et al. Effects of SMAD7 on proliferation and apoptosis of goat follicular granulosa cells [J]. *Acta Veterinaria et Zootechnica Sinica*, 2022, 53(8): 2548-2557. (in Chinese)
- [10] WASMUTH E V, BROECK A V, LACLAIR J R, et al. Allosteric interactions prime androgen receptor dimerization and activation[J]. *Mol Cell*, 2022, 82(11): 2021-2031. e5.
- [11] ASTAPOVA O, MINOR B M N, HAMMES S R. Physiological and pathological androgen actions in the ovary[J]. *Endocrinology*, 2019, 160(5): 1166-1174.
- [12] BURGER H G. Androgen production in women[J]. *Fertil Steril*, 2002, 77 Suppl 4: S3-S5.
- [13] PRIZANT H, GLEICHER N, SEN A. Androgen actions in the ovary; balance is key[J]. *J Endocrinol*, 2014, 222(3): R141-R151.
- [14] DU X, LI Q Q, PAN Z X, et al. Androgen receptor and miRNA-126* axis controls follicle-stimulating hormone receptor expression in porcine ovarian granulosa cells [J]. *Reproduction*, 2016, 152(2): 161-169.
- [15] STERNBERG C N. Enzalutamide, an oral androgen receptor inhibitor for treatment of castration-resistant prostate cancer [J]. *Future Oncol*, 2019, 15(13): 1437-1457.
- [16] KATOH M, KATOH M. WNT signaling and cancer stemness[J]. *Essays Biochem*, 2022, 66(4): 319-331.
- [17] COLOZZA G, KOO B K. Wnt/ β -catenin signaling: structure, assembly and endocytosis of the signalosome[J]. *Dev Growth Differ*, 2021, 63(3): 199-218.
- [18] MUKHERJEE S, LUEDEKE D M, MCCOY L, et al. SOX transcription factors direct TCF-independent WNT/ β -catenin responsive transcription to govern cell fate in human pluripotent stem cells[J]. *Cell Rep*, 2022, 40(8): 111247.
- [19] GAO Y, CHEN J, JI R, et al. USP25 regulates the proliferation and apoptosis of ovarian granulosa cells in polycystic ovary syndrome by modulating the PI3K/AKT pathway via deubiquitinating PTEN[J]. *Front Cell Dev Biol*, 2021, 9: 779718.
- [20] LI H Y, WANG X F, MU H B, et al. Mir-484 contributes to diminished ovarian reserve by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis[J]. *Int J Biol Sci*, 2022, 18(3): 1008-1021.
- [21] XING J S, QIAO G, LUO X, et al. Ferredoxin 1 regulates granulosa cell apoptosis and autophagy in polycystic ovary syndrome [J]. *Clin Sci (Lond)*, 2023, 137(6): 453-468.
- [22] WEI X L, ZHENG L P, TIAN Y P, et al. Tyrosine phosphatase SHP2 in ovarian granulosa cells balances follicular development by inhibiting PI3K/AKT signaling[J]. *J Mol Cell Biol*, 2022, 14(7): mjac048.
- [23] RODRIGUEZ PARIS V, BERTOLDO M J. The

- mechanism of androgen actions in PCOS etiology[J]. *Med Sci (Basel)*, 2019, 7(9):89.
- [24] CHRISTIN-MAITRE S, YOUNG J. Androgens and spermatogenesis[J]. *Ann Endocrinol (Paris)*, 2022, 83(3): 155-158.
- [25] WALKER W H. Androgen actions in the testis and the regulation of spermatogenesis[M]//CHENG C Y, SUN F. *Molecular Mechanisms in Spermatogenesis*. Cham:Springer, 2021:175-203.
- [26] MARCHIORETTI C, ZANETTI G, PIRAZZINI M, et al. Defective excitation-contraction coupling and mitochondrial respiration precede mitochondrial Ca^{2+} accumulation in spinobulbar muscular atrophy skeletal muscle[J]. *Nat Commun*, 2023, 14(1):602.
- [27] YU K, HUANG Z Y, XU X L, et al. Estrogen receptor function: impact on the human endometrium[J]. *Front Endocrinol (Lausanne)*, 2022, 13:827724.
- [28] WALTERS K A, SIMANAINEN U, HANDELSMAN D J. Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models[J]. *Hum Reprod Update*, 2010, 16(5):543-558.
- [29] ZHANG Y H, HU M, YANG F, et al. Increased uterine androgen receptor protein abundance results in implantation and mitochondrial defects in pregnant rats with hyperandrogenism and insulin resistance[J]. *J Mol Med (Berl)*, 2021, 99(10):1427-1446.
- [30] JAMROZE A, CHATTA G, TANG D G. Androgen receptor (AR) heterogeneity in prostate cancer and therapy resistance[J]. *Cancer Lett*, 2021, 518:1-9.
- [31] AHMAD I, NEWELL-FUGATE A E. Role of androgens and androgen receptor in control of mitochondrial function[J]. *Am J Physiol Cell Physiol*, 2022, 323(3):C835-C846.
- [32] ZHANG A L, WANG X J, FAN C F, et al. The role of Ki67 in evaluating neoadjuvant endocrine therapy of hormone receptor-positive breast cancer[J]. *Front Endocrinol (Lausanne)*, 2021, 12:687244.
- [33] ARBEL M, CHOUDHARY K, TIFILIN O, et al. PCNA loaders and unloaders-one ring that rules them all[J]. *Genes (Basel)*, 2021, 12(11):1812.
- [34] GONZÁLEZ-MAGAÑA A, BLANCO F J. Human PCNA structure, function and interactions[J]. *Biomolecules*, 2020, 10(4):570.
- [35] ACHARYA N, PATEL S K, SAHU S R, et al. 'PIPs' in DNA polymerase:PCNA interaction affairs[J]. *Biochem Soc Trans*, 2020, 48(6):2811-2822.
- [36] MANSILLA S F, DE LA VEGA M B, CALZETTA N L, et al. CDK-independent and PCNA-dependent functions of p21 in DNA replication[J]. *Genes (Basel)*, 2020, 11(6):593.
- [37] SHENG C B, MENDLER I H, RIEKE S, et al. PCNA-mediated degradation of p21 coordinates the DNA damage response and cell cycle regulation in individual cells[J]. *Cell Rep*, 2019, 27(1):48-58. e7.
- [38] LI L Y, SHI X J, SHI Y, et al. The signaling pathways involved in ovarian follicle development[J]. *Front Physiol*, 2021, 12:730196.
- [39] ABEDINI A, ZAMBERLAM G, LAPOINTE E, et al. WNT5a is required for normal ovarian follicle development and antagonizes gonadotropin responsiveness in granulosa cells by suppressing canonical WNT signaling[J]. *FASEB J*, 2016, 30(4):1534-1547.
- [40] 王 鹏, 韩海银, 李文韬, 等. 可变剪接体 WNT4- β 对山羊卵泡颗粒细胞增殖和激素分泌的影响[J]. *畜牧兽医学报*, 2022, 53(10):3480-3489.
- WANG P, HAN H Y, LI W T, et al. Effect of alternative splicing WNT4- β on follicular granulosa cell proliferation and hormone secretion in goats[J]. *Acta Veterinaria et Zootechnica Sinica*, 2022, 53(10):3480-3489. (in Chinese).
- [41] WANG W W, TENG J, HAN X, et al. miR-458b-5p regulates ovarian granulosa cells proliferation through Wnt/ β -catenin signaling pathway by targeting catenin beta-1[J]. *Anim Biosci*, 2021, 34(6):957-966.
- [42] MANABE N, GOTO Y, MATSUDA-MINEHATA F, et al. Regulation mechanism of selective atresia in porcine follicles: regulation of granulosa cell apoptosis during atresia[J]. *J Reprod Dev*, 2004, 50(5):493-514.
- [43] ZHONG L L, LUO Y, ZHOU F, et al. The effects of natural products and bioactive ingredients of traditional Chinese medicine on apoptosis of ovarian granulosa cells[J]. *J Appl Toxicol*, 2023, 43(6):772-788.
- [44] HUANG G M, YAO Q, YE Z F, et al. Gender differential expression of AR/miR-21 signaling axis and its protective effect on renal ischemia-reperfusion injury[J]. *Front Cell Dev Biol*, 2022, 10:861327.
- [45] LIN Y T, LU Z Y, KOKONTIS J, et al. Androgen receptor primes prostate cancer cells to apoptosis through down-regulation of basal p21 expression[J]. *Biochem Biophys Res Commun*, 2013, 430(1):289-293.

(编辑 郭云雁)

《畜牧兽医学报》第六届编辑委员会成员

顾 问 张子仪 刘守仁 吴常信 夏咸柱 刘秀梵

主 任 委 员 陈焕春

副主任委员 阎汉平 汪 明 苏振环 时建忠

编 委 成 员 (按姓名英文字母/汉字笔画排序)

Chris Anderson David Lindsay Graham Gardner W.Bruce Currie
马月辉 王加启 方维焕 宁宜宝 卢德勋 朱士恩 朱兴全 刘湘涛 余锐萍
杜立新 闵于明 陆承平 陈溥言 陈瑶生 李 奎 李庆章 李学伟 李德发
沙玉圣 张 涌 张仲秋 张家骅 沈建忠 杨 宁 罗绪刚 孟庆翔 郑友民
周继勇 姜 平 赵兴绪 赵茹茜 夏国良 涂长春 郭万柱 黄维义 崔治中
童光志 蒋小松 舒鼎铭

名 誉 主 编 陈幼春 蒋金书

主 编 文 杰

副 主 编 张 沅 时建忠 汪 明 杨汉春 于康震 王立贤 任 鹏

编辑部主任 孟 培

主 编 文 杰

广告部主任 程金华 本期责任编辑 范子刚

畜 牧 兽 医 学 报	ACTA VETERINARIA ET ZOOTECHNICA SINICA
(月刊,1956年创刊)	(Monthly, Started in 1956)
2024年 第55卷 第5期	Vol. 55 No. 5 2024
2024年5月23日出版	Published on May 23, 2024
主 管 中国科学技术协会	Supervised by China Association for Science and Technology
主 办 中国畜牧兽医学会	Sponsored by Chinese Association of Animal Science and Veterinary Medicine
编 辑 《畜牧兽医学报》编委会	Edited by Editorial Board of the ACTA VETERINARIA ET ZOOTECHNICA SINICA
地 址 北京海淀区圆明园西路2号	Institute of Animal Science, Chinese Academy of Agricultural Sciences
邮 编 100193	Address No.2 West Road Yuanmingyuan, Haidian Beijing, China. Post Code 100193
电 话 010-62815987 62816996 62893836	Telephone 010-62815987 62816996 62893836
网 址 http://www.xmsyxb.com	Website http://www.xmsyxb.com
电 子 邮 箱 xmsyxb@caas.cn	E-mail xmsyxb@caas.cn
出 版 单 位 《中国畜牧兽医杂志》有限公司	Published by Chinese Journal of Animal Science and Veterinary Medicine Co., Ltd.
印 刷 北京博海升彩色印刷有限公司	Printed by Beijing Bohai Sheng Color Printing Co., Ltd.
国内总发行 中国邮政集团公司北京市报刊发行局	Distributed by Domestic Beijing Post Office, China Post Group Corporation
国外总发行 中国国际图书贸易集团有限公司	Overseas China International Book Trading Corporation, P.O. Box 399
(中国国际书店) 北京399信箱	
订 购 全国各地邮局	
中国标准连续出版物号 ISSN 0366-6964 CN 11-1985/S	

国内定价:50.00元(全年600元) 邮发代号:82-453 国外代号:M446 广告发布登记证号:京海工商广登字20170255号

ISSN 0366-6964





Article

Screening of SNP Loci Related to Leg Length Trait in Leizhou Goats Based on Whole-Genome Resequencing

Jinyang Liu, Shucan Dong, Jianda Lv, Yaokun Li, Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu and Guangbin Liu





Article

Screening of SNP Loci Related to Leg Length Trait in Leizhou Goats Based on Whole-Genome Resequencing

Jinyang Liu, Shucan Dong, Jianda Lv, Yaokun Li , Baoli Sun, Yongqing Guo , Ming Deng, Dewu Liu * and Guangbin Liu *

College of Animal Science, South China Agricultural University, Guangzhou 510642, China; liujinyang@stu.scau.edu.cn (J.L.); dsc15184989294@163.com (S.D.); 18633565621@163.com (J.L.); ykli@scau.edu.cn (Y.L.); baolisun@scau.edu.cn (B.S.); yongqing@scau.edu.cn (Y.G.); dengming@scau.edu.cn (M.D.)

* Correspondence: dwliu@scau.edu.cn (D.L.); gbliu@scau.edu.cn (G.L.)

Abstract: Leizhou goats can be classified into tall and short types based on their size and habits. The tall Leizhou goats are well-suited for grazing management due to their robust physique, while the dwarf types are smaller, grow rapidly, and are more appropriate for feeding management systems. In this study, whole-genome resequencing was conducted to identify genomic variants in 15 Tall-legged (TL) and 15 Short-legged (SL) Leizhou goats, yielding 8,641,229 high-quality SNPs in the Leizhou goat genome. Phylogenetic tree and principal component analyses revealed obvious genetic differentiation between the two groups. *F_{st}* and $\theta\pi$ analyses identified 420 genes in the TL group and 804 genes in the SL group. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses indicated that the phosphatidylinositol signaling system is associated with growth and development. Additionally, Genome-Wide Association Study (GWAS) analysis identified eight genes linked to leg length, including *B4GALT7* and *NR1D1*. Notably, the NC_030818.1 (g.53666634T > C) variant was significantly associated with leg length traits, where the CC genotype was linked to shorter legs and the TT genotype to longer legs. This study identifies candidate genes and molecular markers, serving as a reference point for breeding and genetic improvement efforts in Leizhou goats and other goat breeds.



Citation: Liu, J.; Dong, S.; Lv, J.; Li, Y.; Sun, B.; Guo, Y.; Deng, M.; Liu, D.; Liu, G. Screening of SNP Loci Related to Leg Length Trait in Leizhou Goats Based on Whole-Genome Resequencing. *Int. J. Mol. Sci.* **2024**, *25*, 12450. <https://doi.org/10.3390/ijms252212450>

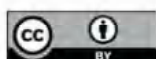
Academic Editor: Brad Freking

Received: 25 October 2024

Revised: 13 November 2024

Accepted: 14 November 2024

Published: 20 November 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: Leizhou goat; leg length trait; whole-genome resequencing; SNPs

1. Introduction

Leizhou goats are native to the Leizhou Peninsula in Guangdong Province, China, and exhibit notable advantages such as strong disease resistance, superior meat quality, rapid growth, and high reproductive rates [1]. Leizhou goats can be categorized into tall-legged and short-legged types based on leg morphology (Figure 1). Compared to the short-legged type, the tall-legged goats have a larger body, smaller abdomen, and less developed udder, resulting in a higher proportion of single-kidding females, making them more suitable for grazing. Conversely, the short-legged type has a smaller body, finer skeletal structure, larger abdomen, and a more developed udder. Although less mobile, they grow faster, have a higher meat yield, and exhibit superior reproductive performance, with a higher incidence of twin births, making them ideal for intensive farming. Identifying and selecting genes related to leg length is crucial for developing optimized indoor-raised Leizhou goat breeds.

Whole-genome resequencing (WGS) has become an important method for exploring breed-specific and selection traits in livestock [2]. WGS can comprehensively and accurately identify SNP variations through high-throughput sequencing of the entire genome. This comprehensively reveals genetic variation in the goat genome and has been used in selection and breeding programs. Traits such as high reproductive performance, good palatability, and high cashmere quality can be directly selected through the published genome maps. Extensive research has identified numerous candidate genes associated with quality traits

in goats. Studies by Wang et al. [3] and Xiong et al. [4] have uncovered genes linked to wool and milk yield traits, reproduction, and immunity, such as *SEMA3D*, *EVPL*, *FGF12*, *SOX5*, *DGAT2*, *GHR*, *ELF5*, and *GLYCAM1*. Gao et al. [5] expanded this list through a resequencing study, highlighting genes like *C2CD3*, *UCP2*, and *TSHR* that influence nervous system functions, growth, and coat color. Lan et al. [6] further contributed to this body of work by identifying the *POU1F1* gene variant associated with litter size, milk yield, and weight gain in goats. Collectively, these findings underscore the genetic diversity that contributes to the phenotypic variation observed across different goat breeds. These studies underscore the potential of whole-genome resequencing in identifying breed-specific variation and supporting the development of new breeds.

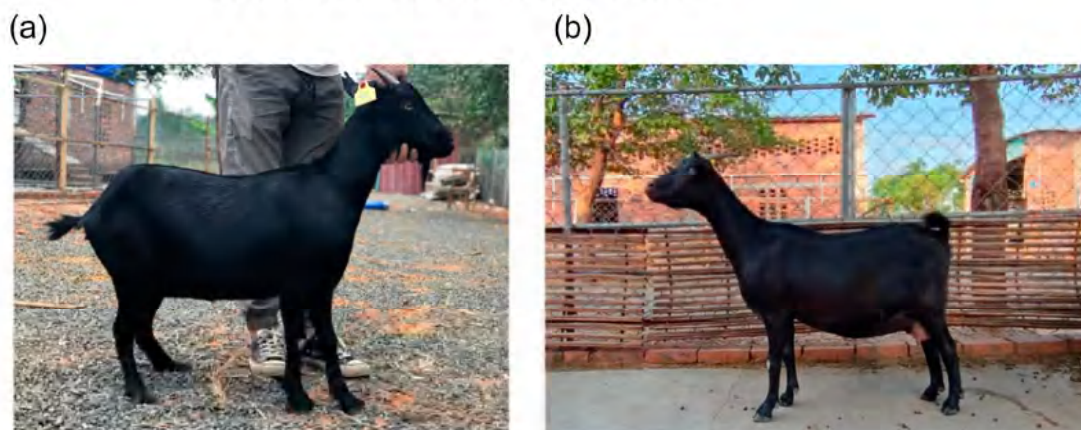


Figure 1. Short-legged (SL) Leizhou goats: (a); tall-legged (TL) Leizhou goats: (b).

Currently, research on growth traits in Leizhou goats remains limited. Existing studies predominantly focus on meat quality and lambing rates in reproductive traits and their associations with genetic polymorphisms. Wang et al. [7] found that the *plectin* (*PLEC*) gene has a regulatory role in muscle development in Leizhou goats and subsequently identified three copy number variants in the *PLEC* gene in 417 Leizhou goats. *PLECCNV-1* was significantly associated with body weight, chest circumference, carcass weight, cross-sectional area of the longissimus dorsi muscle of the back, and shear force in Leizhou goats, and the type of weight gain was highly significantly correlated with the expression of *PLEC* in muscle. Mutations in *POU1F1*, a positive regulator of growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone β -subunit (*TSH β*) in ruminants, were associated with growth traits. At present, the number of studies on the traits of Leizhou goats is still relatively small, and the use of modern molecular technology to screen for growth trait-related genes of Leizhou goats can provide a reference for the subsequent selection and breeding.

2. Results

2.1. Whole-Genome Resequencing and Mutation Detection in Leizhou Goats

2.1.1. Quality Control and Comparison of Whole-Genome Resequencing Data

A total of 855.972 GB of raw sequencing data was generated from 30 Leizhou goat samples, yielding 851.26 GB of clean data after quality control. The results of sequencing statistics are presented in Table 1. The valid data rate for the 30 samples ranged from 98.11% to 99.45%, the Q30 values ranged from 91.24% to 93.34%, and the GC content ranged from 41.73% to 47.05%, with no evidence of significant GC bias. These outcomes confirm the reliability of the Illumina sequencing library construction and data quality. Alignment of the high-quality sequencing data to the reference genome (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/704/415/GCF_001704415.1_ARSL/, accessed on 19 July 2022) was performed using BWA (0.7.8) software. On average, 99.67% of the assembled sequences were successfully mapped to the reference genome, with an average sequencing depth of $7.9 \times$ per sample. The proportion of bases with a depth greater than once covered

94.39% of the reference genome, and the coverage at four times was 83.96% (Table 2). These results indicate that the similarity between the samples and the reference genome meets the requirements for resequencing analysis, providing sufficient depth and coverage for downstream analyses.

Table 1. Summary of sequencing data quality.

Sample	Raw Base (bp)	Clean Base (bp)	Effective Rate (%)	Error Rate (%)	Q20 (%)	Q30 (%)	GC Content (%)
TL1	27,843,832,200	27,649,102,800	99.30	0.03	97.64	93.34	43.72
TL2	30,256,776,900	29,683,815,300	98.11	0.03	96.91	91.86	47.05
TL3	29,662,381,500	29,443,012,200	99.26	0.03	97.37	92.39	43.55
TL4	27,814,504,200	27,618,588,900	99.30	0.03	97.34	92.65	43.62
TL5	28,073,487,000	27,882,138,300	99.32	0.03	97.55	93.10	43.30
TL6	26,578,760,100	26,406,888,300	99.35	0.03	97.44	92.85	43.27
TL7	30,786,686,700	30,546,573,900	99.22	0.03	97.16	92.28	44.21
TL8	34,228,209,600	33,967,403,700	99.24	0.03	97.42	92.84	43.54
TL9	29,552,629,200	29,336,811,600	99.27	0.03	97.53	92.97	43.06
TL10	28,615,713,000	28,427,135,400	99.34	0.03	96.86	91.53	43.52
TL11	30,323,067,600	29,927,976,600	98.70	0.03	97.23	92.77	46.14
TL12	32,162,967,000	31,930,486,500	99.28	0.03	96.74	91.24	43.05
TL13	29,870,390,100	29,705,223,900	99.45	0.03	97.05	91.69	42.67
TL14	30,049,663,200	29,826,423,900	99.26	0.03	97.29	92.34	43.27
TL15	27,483,879,000	27,272,088,600	99.23	0.03	97.34	92.62	42.73
SL1	28,270,083,600	27,854,524,800	98.53	0.03	96.83	91.94	46.79
SL2	30,463,497,900	30,244,026,000	99.28	0.03	97.19	92.12	43.36
SL3	32,409,471,900	32,156,407,800	99.22	0.03	97.48	92.96	43.20
SL4	28,160,716,800	27,989,217,600	99.39	0.03	97.20	92.17	43.16
SL5	27,703,572,000	27,501,890,100	99.27	0.03	97.38	92.67	42.15
SL6	28,712,619,600	28,515,085,500	99.31	0.03	97.44	92.83	41.97
SL7	27,628,634,100	27,444,917,100	99.34	0.03	97.50	92.97	42.48
SL8	27,288,080,700	27,118,727,100	99.38	0.03	97.25	92.40	42.54
SL9	29,785,053,300	29,587,171,500	99.34	0.03	97.26	92.38	42.02
SL10	28,735,499,100	28,576,833,000	99.45	0.03	97.39	92.67	41.86
SL11	26,732,475,300	26,567,756,400	99.38	0.03	97.62	93.23	41.90
SL12	30,159,567,600	29,980,689,900	99.41	0.03	97.34	92.57	41.73
SL13	26,976,734,700	26,800,857,300	99.35	0.03	97.36	92.31	41.89
SL14	26,850,299,400	26,678,291,100	99.36	0.03	97.40	92.72	42.22
SL15	27,713,181,000	27,538,027,500	99.37	0.03	97.49	92.91	42.18

Note: Q20: effective rate: ratio of clean data to raw data after filtering; percentage of bases with a mass value of more than 20 (error rate of less than 1%); Q30: percentage of bases with a mass value of more than 30 (error rate below 0.1%); GC content: the proportion of bases G and C.

Table 2. Sequencing depth and coverage statistics.

Sample	Mapped Reads	Total Reads	Mapping Rate (%)	Average Depth (X)	Coverage_1X	Coverage_4X
TL1	189,132,200	188,528,943	0.9968	8.12	0.9439	0.8355
TL2	189,271,354	188,667,959	0.9968	8.02	0.9452	0.8448
TL3	192,572,802	191,915,407	0.9966	8.01	0.9447	0.8461
TL4	191,883,874	191,247,671	0.9967	7.95	0.9454	0.8431
TL5	178,793,644	178,142,151	0.9964	7.05	0.9440	0.8203
TL6	183,423,434	182,712,676	0.9961	7.51	0.9416	0.8054
TL7	184,470,504	183,775,701	0.9962	7.7	0.9417	0.8101
TL8	189,772,704	189,135,452	0.9966	8.04	0.9455	0.8672
TL9	200,045,044	199,368,412	0.9966	8.4	0.9442	0.8437
TL10	177,627,374	176,935,396	0.9961	7.87	0.9438	0.8305
TL11	188,357,116	187,702,096	0.9965	7.53	0.9418	0.8203
TL12	188,983,800	188,337,891	0.9966	7.84	0.9433	0.8280
TL13	195,918,658	195,318,873	0.9969	8.34	0.9462	0.8524

Table 2. Cont.

Sample	Mapped Reads	Total Reads	Mapping Rate (%)	Average Depth (X)	Coverage_1X	Coverage_4X
TL14	195,665,504	194,946,296	0.9963	8.13	0.9456	0.8527
TL15	178,251,788	177,626,089	0.9965	7.24	0.9426	0.8213
SL1	190,994,270	190,372,600	0.9967	8.04	0.9449	0.8573
SL2	190,854,390	190,252,733	0.9968	8.02	0.9445	0.8404
SL3	184,093,670	183,481,304	0.9967	7.71	0.9448	0.8493
SL4	191,850,262	191,121,591	0.9962	7.98	0.9445	0.8491
SL5	186,145,744	185,600,708	0.9971	7.67	0.9430	0.8339
SL6	190,319,146	189,735,385	0.9969	7.91	0.9436	0.8347
SL7	182,117,432	181,769,071	0.9981	7.36	0.9400	0.7930
SL8	190,220,722	189,555,444	0.9965	8.15	0.9445	0.8611
SL9	183,563,430	182,896,188	0.9964	7.58	0.9444	0.8455
SL10	189,510,436	188,942,424	0.9970	8.10	0.9393	0.8180
SL11	192,508,250	191,930,578	0.9970	8.23	0.9457	0.8538
SL12	191,705,936	191,113,160	0.9969	8.20	0.9445	0.8539
SL13	196,962,274	196,290,642	0.9966	8.44	0.9467	0.8723
SL14	195,311,816	194,163,640	0.9941	8.50	0.9444	0.8348
SL15	194,740,420	193,861,415	0.9955	8.91	0.9466	0.8696

Note: Mapped reads: number of reads mapped to the reference (including single-ended and double-ended matches); total reads: The total number of reads for valid sequencing data; mapping rate: alignment rate, the number of reads aligned to the reference genome divided by the number of reads for valid sequencing data; average depth: average sequencing depth, the total number of bases aligned to the reference genome divided by the genome size; coverage at least 1X: percentage of sites covered by at least one base in the reference genome; coverage at least 4X: the reference genome has at least four bases covering the percentage of sites in the genome.

2.1.2. SNP Variant Detection and Annotation

The distribution of these SNPs across the genome is detailed in Table 3. A total of 20,351,884 original SNP loci were identified, with 8,641,229 high-quality SNP loci retained after quality control screening. SNPs were annotated using ANNOVAR (21 Jun 2013) software, revealing that 5,455,335 SNPs (63.13%) were located in intergenic regions, 2,976,338 SNPs (34.44%) in intronic regions, 120 SNPs (0.0014%) in splice sites, 47,182 SNPs (0.55%) in regions 1 kb downstream of genes, and 1030 SNPs (0.012%) in regions 1 kb upstream. Additionally, 59,878 SNPs (0.69%) were located in exonic regions, with 35,791 (0.41%) being synonymous mutations, 23,807 (0.28%) non-synonymous mutations, and 280 (0.0032%) able to change the termination codon of the gene.

Table 3. SNP detection statistics and annotation results.

Category	Number of SNPs
Upstream	40,870
Exonic	59,878
Stop gain	244
Stop loss	36
Synonymous	35,791
Non-synonymous	23,807
Intronic	2,976,338
Splicing	120
Downstream	47,182
Upstream/Downstream	1030
Intergenic	5,455,335
Total	8,641,229

Note: Total: the total number of SNPs; Upstream: 1 kb region upstream of the gene; Exonic: the variant is located in the exon region; Stop gain: Mutation of the stop codon is obtained in the gene; Stop loss: a mutation that causes a gene to lose a stop codon; Synonymous: synonymous variation; Non-synonymous: non-tautological variation; intronic: the variation is located in the intronic region; Splicing: the variant is located at the splice site (2 bp in the intron close to the exon/intron boundary); Downstream: 1 kb region downstream of the gene; Upstream/Downstream: the 1 kb region upstream of the gene is also 1 kb downstream of another gene; Intergenic: the variant is located in the intergenic region; Total: The sum of the components.

2.2. Population Structure Analysis of Leizhou Goats

In this study, Treebest was used to construct an evolutionary tree for 30 Leizhou goats, and the results are shown in Figure 2a. Most of the TL Leizhou goat groups were clearly genetically differentiated from the SL Leizhou goat groups, but three TL goats were slightly mixed into the SL goat groups. The results of principal component analysis (PCA) showed that the 30 Leizhou goats were able to be divided into two groups (Figure 2b). Structure analysis confirmed a clear separation between the two groups at $K = 2$ (Figure 2c)

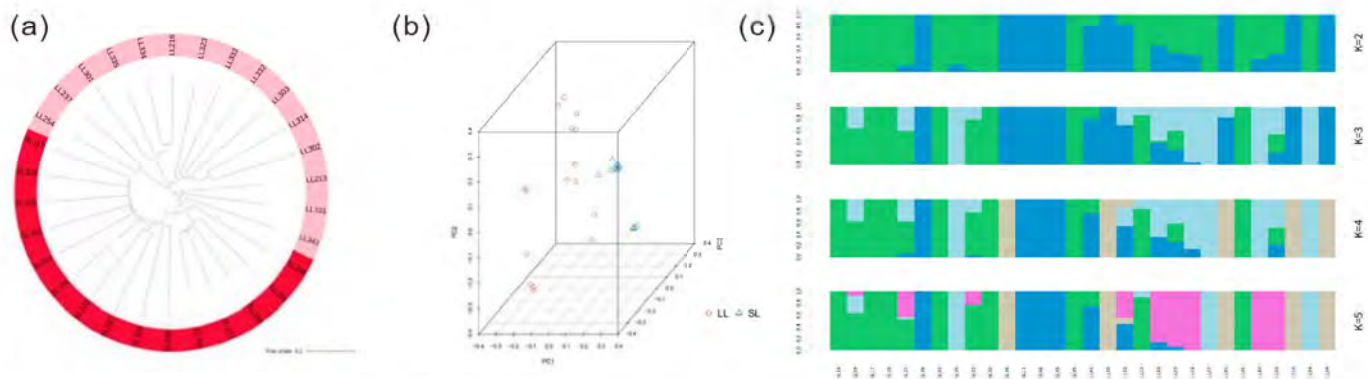


Figure 2. Population structure analysis of Leizhou goats: (a) phylogenetic tree of 30 Leizhou goats; (b) PCA diagram of principal component analysis of 30 Leizhou goats; (c) structure of 30 Leizhou goats.

2.3. Signatures of Selection in Leizhou Goat Populations

2.3.1. Leizhou Goat TL vs. SL Based on F_{st}

F_{st} selection analysis was conducted to identify candidate genes related to leg length traits by comparing genetic differences between TL and SL Leizhou goats (Figure 3a). The results showed that 95.61% of F_{st} values were below 0.05, 4.32% ranged between 0.05 and 0.15, and 0.07% were greater than 0.15, indicating limited genetic differentiation between the two populations (Figure 3b).

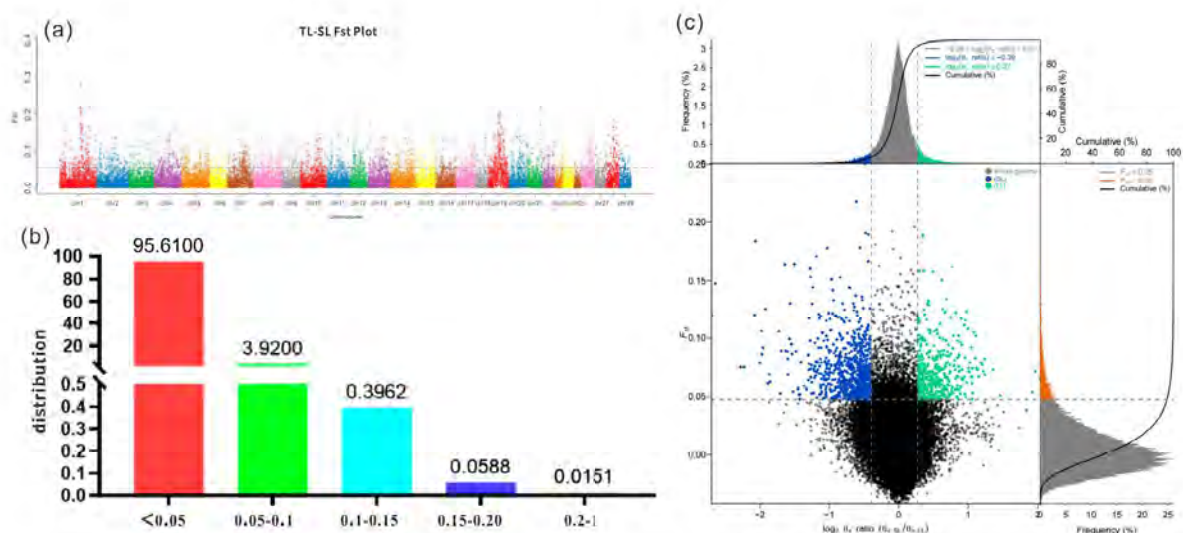


Figure 3. Selection elimination analysis of Leizhou goat populations: (a) autosomal F_{st} distribution of TL vs. SL group in Leizhou goat; (b) F_{st} ratio of Leizhou goat TL vs. SL group; (c) Leizhou goat TL vs. SL group F_{st} and θ_{TL} selection elimination analysis plot (The gray dashed line represents the threshold line for selection signals).

2.3.2. Leizhou Goat TL vs. SL Based on Fst and $\theta\pi$ Selected Regions

In this study, the genomes of Leizhou goats in the TL group and SL group were scanned by Fst and $\theta\pi$ joint analysis, respectively, and the Fst values with window values of both the top 5% and $\theta\pi$ values of the top 5% were selected as the candidate windows for the proposed study in order to explore the candidate genes related to the leg length trait in Leizhou goats. As shown in Figure 3c, a total of 754 selection signal windows were screened in the top 5% selected region of the Fst and $\theta\pi$ joint screening in the SL group, among which the most selection signal windows were found on chromosome 19, with a total of 109, and the least were found on chromosomes 23 and 25, with only one. By annotating these 754 selection signal windows, a total of 804 genes were annotated. Among these genes, *B4GALT7*, *NR1D1*, *PARP2*, *SOST*, *GDF5*, *CERS3*, *EIF2AK2*, and other genes are closely related to biological processes such as chondrogenesis and osteoblast development. Among the top 5% selected regions jointly screened by Fst and $\theta\pi$, a total of 407 selection signal windows were screened in the TL group, among which the selection windows located on chromosome four were the most numerous, with 39, and a total of 420 genes were annotated by annotation of these 407 selection windows. In addition, the selected regions of the two groups had two commonly selected genes, *CFAP221* and *AMBRA1*.

2.3.3. Functional Annotation of Strong Selection Signal Genes in the SL Group

To understand the function of the 804 selected genes in the SL group, GO and KEGG enrichment analyses were performed (Figure 4a). A total of 1591 GO terms were enriched, with 99 entries showing $p < 0.05$. These entries were primarily associated with biological processes such as protein ADP-ribosylation, transcription regulation, and protein phosphorylation. KEGG analysis identified 228 enriched pathways, with the most significant being circadian rhythm, calcium signaling, and glycosaminoglycan biosynthesis pathways. Additionally, key growth pathways such as the MAPK, PI3K-AKT, and WNT signaling pathways were identified (Figure 4b).

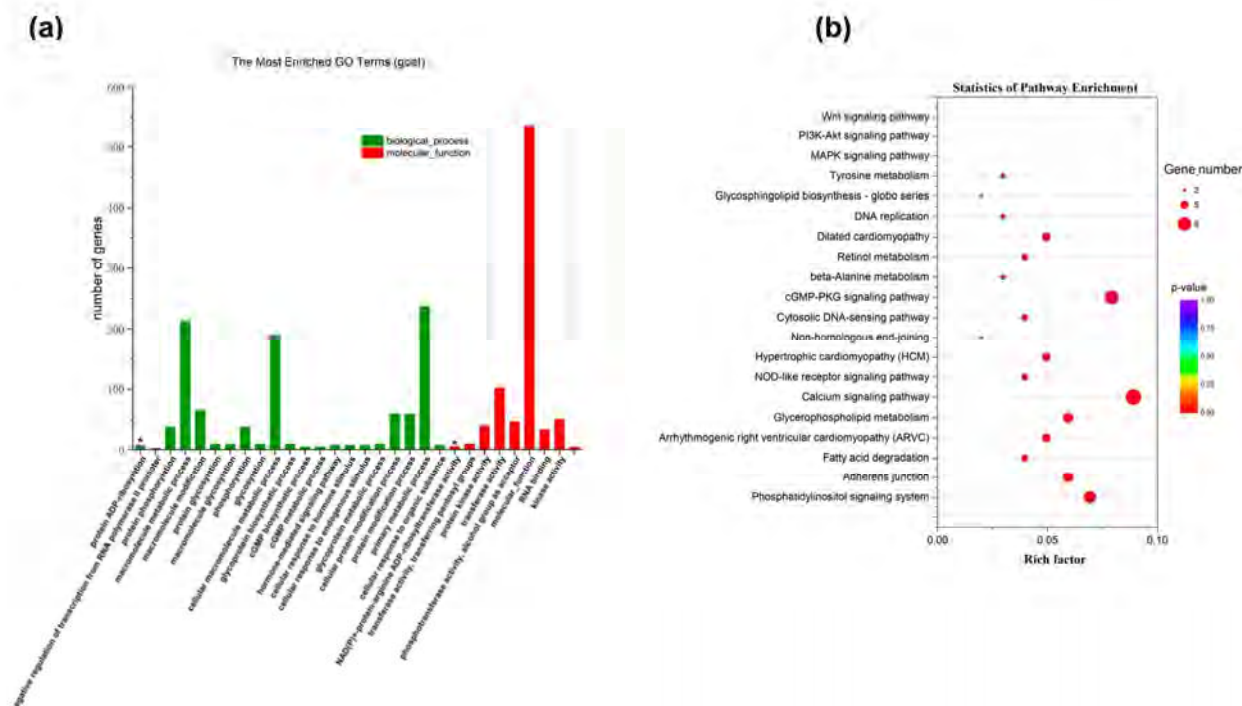


Figure 4. Functional enrichment analysis of selected genes in SL group was performed using GO and KEGG databases: (a) GO enrichment analysis of selected genes for leg length traits in Leizhou goats in SL group; (b) KEGG enrichment analysis of leg length trait of Leizhou goat in SL group (* indicates $p < 0.001$, suggesting a very strong significance).

2.3.4. Functional Annotation of Strong Selection Signal Genes in the TL Group

In the TL group, GO enrichment analysis identified 1338 terms, with 151 showing $p < 0.05$ (Figure 5a). These terms were associated with biological processes like cell adhesion and metabolic processes. KEGG enrichment analysis identified 205 signaling pathways, with the phosphatidylinositol signaling system, calcium signaling pathway, and ECM-receptor interaction among the most significant. These pathways are closely related to animal growth and bone development (Figure 5b).

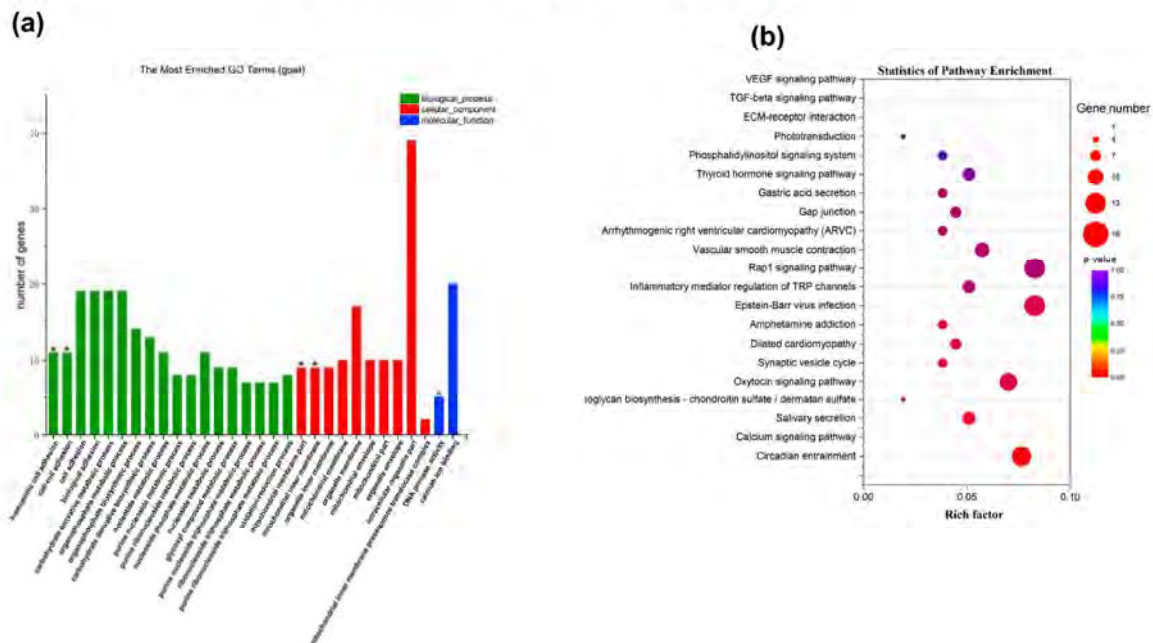


Figure 5. Functional enrichment analysis of selected genes in TL group was performed using GO and KEGG databases: (a) GO enrichment analysis of selected genes for leg length traits of Leizhou goats in TL group; (b) KEGG enrichment analysis of selected genes for leg length traits of Leizhou goats in TL group (* indicates $p < 0.001$, suggesting a very strong significance).

2.4. GWAS Analysis of Leg Length Traits in Leizhou Goats

GWAS identified 11 SNP loci significantly associated with leg length traits (Figure 6). Three loci were located in the intronic regions of the *TIAM1*, *MALRD1*, and *MCPH1* genes, while eight were in intergenic regions. The most significant SNPs were located in the intron of the *MCPH1* gene on chromosome 27.

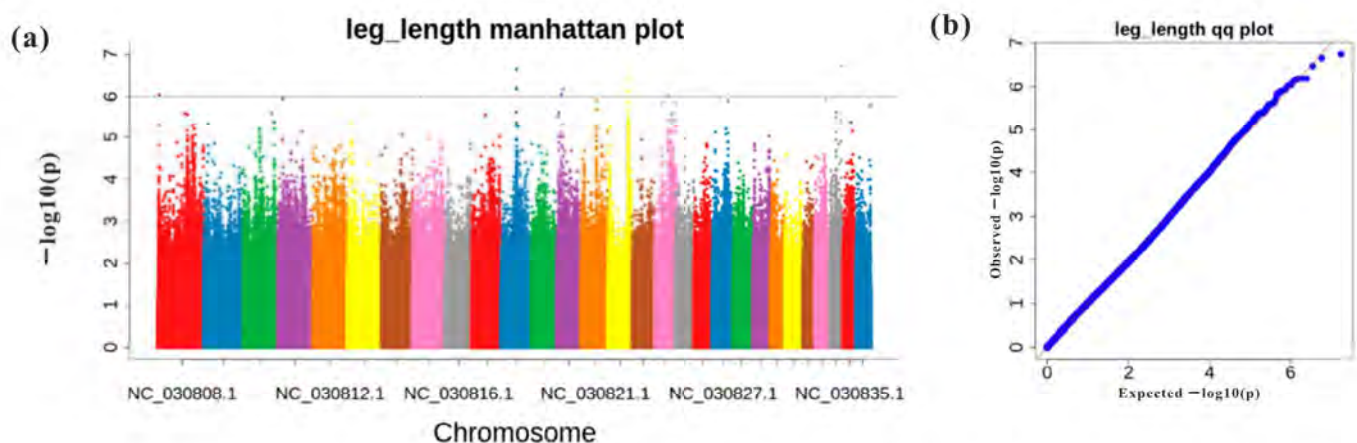


Figure 6. (a): Genome-wide association analysis of leg length in Leizhou goats; (b): Manhattan and Q-Q plots (Color changes in the plot are where chromosomes change).

2.5. Association Analysis of Candidate Gene Polymorphisms and Leg Length Traits

2.5.1. DNA Mixed Pool Detection of Candidate SNP Polymorphisms

Sanger sequencing results showed that mutation sites of NC_030818.1 (g. 53666634 T > C), *SOST* (g. 43291383 G > C), *SHBG* (g. 27088465 A > G), *CAPN12* (g. 49371337 C > T), and *FRMD5* (g. 55009283 C > T) had multiple peak shapes, indicating that these candidate loci might be polymorphic in the population. In contrast, the mutation sites of *NR1D1* (g. 40,040,094 G > C), *NFKB1B* (g. 49514296 C > T), and *PARP2* (g. 76236896 C > T) had a single peak shape, suggesting that they might not be polymorphic in the population (Figure 7a). Therefore, Subsequently, only the NC_030818.1 (g. 53666634 T > C), *SOST* (g. 43291383 G > C), *SHBG* (g. 27088465 A > G), *CAPN12* (g. 49371337 C > T), and *FRMD5* (g. 55009283 C > T) mutation sites were grouped body polymorphism analysis.

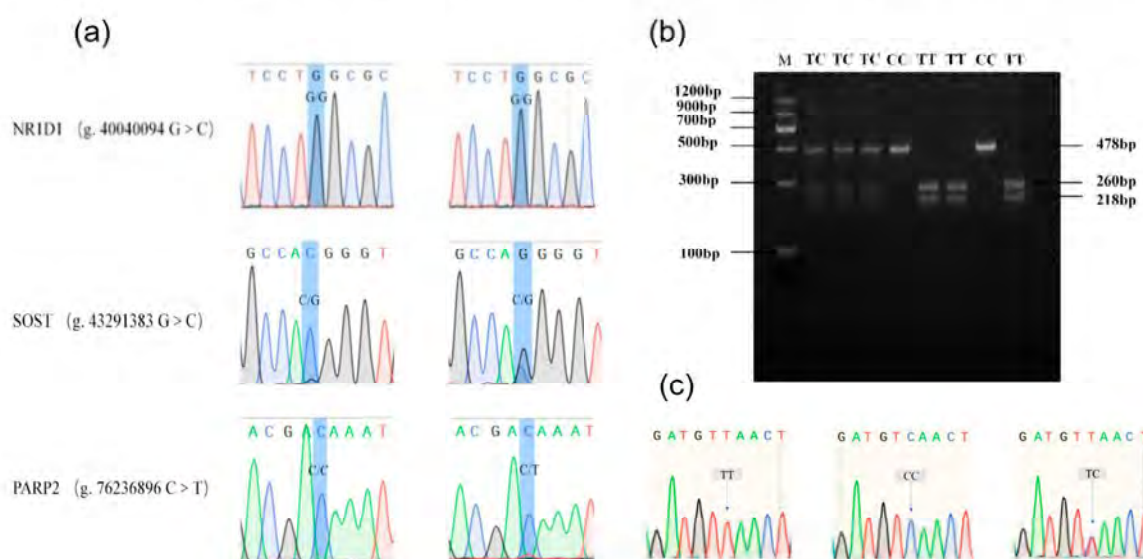


Figure 7. (a) Polymorphisms of candidate SNPs for DNA pooling detection (only some of them are listed); (b) 209 NC_030818.1 53666634 T > C electropherogram after digestion; (c) NC_030818.1 (g. 53666634 T > C) locus.

2.5.2. Candidate Gene SNP Site Genotyping

After conducting an analysis using Snapgene (5.0.5) software, it was found that the mutations in NC_030818.1 (g. 53666634 T > C), *SHBG* (g. 27088465 A > G), *CAPN12* (g. 49371337 C > T), and *FRMD5* (g. 55009283 C > T) were capable of affecting the enzymatically cleaved loci, and the mutations in *SOST* (g. 43291383 G > C) mutations had no effect on the enzymatic locus, so subsequent experiments were performed using the enzymatic method for NC_030818.1 (g. 53666634 T > C), *SHBG* (g. 27088465 A > G), *CAPN12* (g. 49371337 C > T), *FRMD5* (g. 55009283 C > T) genotyping. The amplification products were digested by restriction endonuclease and detected by 2% agarose gel electrophoresis, which showed three genotypes (Figure 7b). Sanger sequencing was performed on these three types of PCR products, and the results showed that the fragment sequences of the three genotypes were consistent with the sequences on NCBI, and the mutation types of the bases were the same as those in the enzyme digestion results (Figure 7c), indicating that the PCR products were amplified accurately and the enzyme digestion sites were selected correctly, which ensured the reliability of the subsequent analysis.

2.5.3. Candidate Gene Polymorphism Analysis

The genetic parameters of the SNP loci of the candidate genes are shown in Table 4, from which it can be seen that the PICs of NC_030818.1 (g. 53666634 T > C) and *SHBG* (g. 27088465 A > G) were between 0.25 and 0.5, which exhibited moderate frequency, while the PICs of *CAPN12* (g. 49371337 C > T) and *FRMD5* (g. 55009283 C > T) all had

polymorphic information content (PIC) less than 0.25, showing lower frequency. The degree of purity and heterozygosity of NC_030818.1 (g. 53666634 T > C) and SHBG (g. 27088465 A > G) were both around 0.5, indicating that these two alleles were more uniformly distributed in the population of Leizhou goat.

Table 4. Polymorphisms of candidate genes and population genetic analysis.

Sites	Gene Frequency		Genotype Frequency			Chi-Square Value (χ^2)	Polymorphic Information Content	Homozygosity	Heterozygosity	Number of Effective Alleles
1	0.29 (C)	0.71 (T)	0.06 (CC)	0.46 (TC)	0.48 (TT)	2.73	0.33	0.59	0.41	1.71
2	0.56 (G)	0.44 (A)	0.09 (AA)	0.21 (GG)	0.70 (AG)	16.49	0.37	0.51	0.49	1.97
3	0.04 (T)	0.96 (C)	0.94 (CC)	0.01 (TT)	0.05 (CT)	2.52	0.07	0.93	0.07	1.08
4	0.95 (C)	0.05 (T)	0.91 (CC)	0.01 (TT)	0.08 (CT)	4.68	0.09	0.9	0.1	1.11

Note: Rank 1 Representative NC_030818.1 (g. 53666634 T > C); Rank 2 Representative SHBG (g. 27088465 A > G); Rank 3 Representative CAPN12 (g. 49371337 C > T); Rank 4 Representative FRMD5 (g. 55009283 C > T).

2.5.4. Association Analysis of NC_030818.1 (g. 53666634 T > C) Gene Polymorphism and Leg Length

SPSS 26.0 software was used to analyze the association between the NC_030818.1 (g. 53666634 T > C) variant locus and the leg length of different genotypes of Leizhou goats, and it can be seen in Table 5 that the mean value of the leg length of the Leizhou goats with the CC genotype was 24.156 cm, the mean value of the leg length of the Leizhou goats with the TC genotype was 28.278 cm, and the mean value of the leg length of the Leizhou goats with the TT genotype was 30.408 cm. The mean value of the leg length of Leizhou goats was 30.408 cm. In terms of leg length of Leizhou goats, the leg length of TT genotype was longer than that of CC genotype by 6.352 cm, with a significant difference ($p < 0.05$). The leg length of TT genotype was longer than that of TC genotype by 2.13 cm, with a significant difference ($p < 0.05$), and the leg length of TC genotype was longer than that of CC genotype by 4.222 cm, with a significant difference ($p < 0.05$).

Table 5. NC_030818.1 53666634T>C) mutation site association analysis with leg length.

Genotype	Frequency	Leg Length/(cm)
CC	16	24.156 ± 1.080 ^a
TC	114	28.278 ± 0.404 ^b
TT	119	30.408 ± 0.396 ^c

Note: Different letters of the shoulder mark of the same column of data indicate significant differences ($p < 0.05$).

3. Discussion

3.1. Characterization of the Distribution of Genetic Variation in the Genomes of TL and SL Groups of Leizhou Goats

In this study, we identified a total of 8,641,229 high-quality SNP sites through whole-genome resequencing of tall and short-legged Leizhou goats. Our analysis revealed that 5,455,335 (63.13%) of these SNPs were intergenic, while 2,976,338 (34.44%) were intronic. These proportions are consistent with previous findings in Hainan black goats by Chen et al. [8], where the majority of SNP variants were also found in non-coding regions. Our results suggest that the large genomic segments occupied by intergenic and intronic regions contribute to the high number of SNPs in these areas. Although intergenic regions do not code for proteins, they can give rise to non-coding RNAs that play significant roles in regulatory processes, as supported by our data and the broader literature.

Our study further indicates that non-coding RNAs, once considered ‘gene noise’ are essential for various biological functions, and their dysregulation can be linked to disease

susceptibility. For example, we observed that certain SNPs in non-coding regions of Leizhou goats are associated with growth performance, which aligns with the reported impact of miR-1666 mutations on chicken growth [9]. This finding underscores the importance of understanding the functional implications of SNPs in non-coding regions for goat breeding programs.

Additionally, our research identified SNPs within exonic regions and the 1 kb flanking sequences of genes. These SNPs are of particular interest because they have the potential to affect protein coding and gene regulation. Our data show that mutations in exons can lead to amino acid changes, protein misfolding, or even embryonic lethality, highlighting the critical role of exon integrity in maintaining phenotypic stability [10]. Moreover, SNPs in the 1 kb upstream region, which is rich in transcription factor binding sites, can modulate gene expression levels, while those in the downstream region can influence transcriptional termination and mRNA processing. These findings are crucial for understanding the molecular basis of phenotypic variation in Leizhou goats and can inform selective breeding strategies.

3.2. Population Genetic Structure Analysis of Leizhou Goats with TL and SL Groups

In this study, we utilized population evolutionary trees, principal component analysis (PCA), and genetic structure analysis to examine the evolutionary relationships between TL and SL Leizhou goats. Our findings suggest a genetic differentiation between these groups, though evidence of genetic mixing was also present. Notably, three individuals from the TL group, identified as TL phenotypically, appeared genetically closer to the SL group in the evolutionary tree. This finding may be attributed to genetic exchange between TL and SL goats during breeding, which is plausible given that both groups share a common farm origin. This observed genetic mixing could be advantageous, as gene flow might help maintain genetic diversity, thereby potentially contributing to the adaptability and resilience of Leizhou goats. Moreover, the minor genetic differentiation observed likely reflects their shared ancestry, which suggests that while selective breeding for distinct leg length may drive differentiation, shared origins and interbreeding constrain it.

3.3. Selected Genes and Functional Analysis of TL and SL Groups in Leizhou Goats

In this study, the TL and SL groups of Leizhou goats were jointly analyzed using F_{st} and θ_{π} for population selection elimination, and a total of 1161 selected regions were screened in the top 5%, and a total of 1222 selected genes were obtained from the gene annotation of these selected windows, of which 420 genes were screened in the TL group, and 804 genes were screened in the SL group. The common genes screened in both groups were *CFAP221* and *AMBRA1*. The *CFAP221* gene encodes a protein involved in the regulation of cilia and flagellum assembly, stability, and motility. It has been shown that the *CFAP221* gene regulates the assembly and motility of cilia and flagella. Mutations in this gene have been associated with primary ciliary dyskinesia, abnormal spermatogenesis, and embryonic death in mice models [11]. Studies on *CFAP221* in goats have not yet been reported. The protein encoded by the *AMBRA1* (*AutophagyAndBeclin1Regulator1*) gene is a key regulator in autophagy, which is highly conserved in vertebrates and has a wide range of biological functions, including metabolism, cell death, and cell division [12]. Its mutation has been implicated in nervous system disorders and carcinogenesis, as well as skeletal muscle maintenance and autism in human and mouse studies [13]. Therefore, mutations in the *AMBRA1* gene may affect autophagic activity in the animal organism and thus participate in the regulation of animal life activities, but whether it can be used as a molecular marker gene for tall and short feet in Leizhou goats still needs further research and exploration.

In the top 5% of selection signals for the SL group, several genes closely associated with biological processes, such as cartilage and osteoblast development, were annotated, including *B4GALT7*, *NR1D1*, *PARP2*, *SOST*, *GDF5*, *CERS3*, and *EIF2AK2*. Animal growth and development are closely linked to skeletal growth. Mammalian bone development primarily occurs through intramembranous and endochondral ossification, with endochon-

dral ossification being the predominant form in limb formation [14]. The *B4GALT7* gene encodes a protein that belongs to the β -1,4-galactosyltransferase family, which is specific for the donor substrate UDP-galactose [15]. Numerous studies indicate that mutations in the *B4GALT7* gene can lead to abnormal skeletal development, resulting in conditions such as short stature and dwarfism [16]. Furthermore, mutations in *B4GALT7* have been linked to dwarfism in Frisian horses, with the specific mutation *B4GALT7*(g. 4535550 C > T) leading to a significant decrease in *B4GALT7* expression and resulting in shortened limbs [17]. In zebrafish, the knockdown of *B4GALT7* results in the loss or severe deformation of craniofacial cartilage and bone structures [18]. Thus, *B4GALT7* plays a key role in early cartilage, bone, and muscle development, and its mutations may influence skeletal development in Leizhou goats, affecting leg length traits.

NR1D1, a member of the nuclear receptor family, regulates target genes associated with various physiological processes, including autophagy, immunity, inflammation, metabolism, and multi-organ aging [19]. The *SOST* gene encodes sclerostin, primarily expressed in osteocytes, and several GWAS findings indicate that SNP sites at the *SOST* locus are significantly associated with bone mineral density and fracture susceptibility [20]. In humans, mutations in the *SOST* gene are strongly linked to osteoporosis, particularly in postmenopausal women [21]. *GDF5*, a member of the BMP family, is highly expressed in the surface layer of articular cartilage. The *GDF5* gene is a key risk locus for osteoarthritis, with *GDF5*-deficient mice exhibiting abnormal joint development, underscoring its critical role in joint development and homeostasis [22]. Numerous studies have found that *GDF5* mutations correlate with susceptibility to human osteoarthritis [23], and the TT genotype of *GDF5* SNP rs143383 increases susceptibility to knee arthritis in northern Mexico [24]. Furthermore, molecular marker association analysis in cattle has linked SNPs in the *GDF5* gene to domestic cattle size traits, indicating that the *GDF5* SNP may serve as a valuable genetic marker for breeding [25]. These findings suggest that mutations in these genes may influence leg length traits in Leizhou goats by affecting skeletal development.

Additionally, several genes associated with bone development were identified in the TL group, including *SP1* and *KIF7*. The protein encoded by *SP1* is a zinc finger transcription factor that regulates various physiological processes, including cell growth, apoptosis, differentiation, and immune response, by binding to GC-rich promoter regions. *SP1* has been shown to regulate the expression of the *bone morphogenetic protein 2* (*BMP2*) gene [26], which is crucial for osteoblast differentiation. Conditional knockout of *BMP2* in mice results in reduced bone mass and skeletal defects [27]. Therefore, SNPs in *SP1* may influence *BMP2* protein expression, thereby regulating osteoblast differentiation during the growth and development of Leizhou goats. Moreover, *SP1* can bind to the *PPAR α* promoter region, affecting its activity and thus regulating bone metabolism [28]. Polymorphisms in *SP1* binding sites within the *COL1A1* gene are significantly associated with clinical phenotypes, including collagen disease and osteogenesis imperfecta (OI), with mutations at the rs1800012 site linked to bone mineral density in women aged 45 years and older [29]. Proper regulation of chondrocyte proliferation and differentiation is essential for normal skeletal growth. During endochondral bone development, signaling pathways regulate growth plate chondrocyte differentiation, and disruptions can lead to skeletal dysplasia and short stature [30]. The Hedgehog (Hh) signaling pathway plays a vital role in bone growth regulation. *KIF7*, a kinesin motor protein, is involved in Hh signaling, and *KIF7*-null embryos exhibit mild ectopic activation of the Hh pathway. *KIF7* limits the inhibitory effect of *SUFU*, promoting Hh signal transduction in growth plate chondrocytes [31]. Although this study has identified many candidate genes associated with leg length traits in Leizhou goats, it remains to be determined whether these genes are critical within the population. Nevertheless, the candidate genes identified here provide valuable references for breeding tall and short Leizhou goats in practical applications.

Functional enrichment analysis of the selected genes revealed associations with various signaling pathways related to bone development, including glycosaminoglycan biosynthesis (cartilage sulfate/skin sulfate), the WNT signaling pathway, and the Hedgehog

signaling pathway. Chondroitin sulfate, a major component of bone and connective tissues, promotes chondrocyte proliferation and differentiation, regulating skeletal growth and repair processes. Furthermore, chondroitin sulfate influences the synthesis and degradation of the extracellular matrix, maintaining bone tissue health and facilitating normal bone formation and function. During endochondral ossification, Ihh production by chondrohypertrophic chondrocytes signals perichondrial cells to induce osteoblast differentiation. In endochondral bone, the Hedgehog signaling pathway initiates osteoblast differentiation by inducing WNT ligand expression. These signaling molecules ultimately regulate osteocyte proliferation and differentiation through transcription factor expression [32]. WNT proteins are key regulators of differentiation and activity in both mouse and human osteoblasts. Activation of WNT signaling by a single allele of *Sfrp1*, *SOST*, or *Dkk1* increases osteoblast numbers and activity, while inhibition of WNT ligand secretion suppresses bone formation in mice [33]. β -Catenin, a core protein in the WNT signaling pathway, collaborates with TCF1 to directly stimulate Runx2 transcription, regulating skeletogenesis [34]. The WNT signaling pathway also activates PKCD and mTORC1, promoting protein synthesis, bone formation, and osteoblast differentiation [35]. Thus, genes subject to selection in the dwarf population may regulate glycosaminoglycan biosynthesis, the cartilage sulfate/skin sulfate pathway, WNT signaling, and the Hedgehog signaling pathway.

Additionally, GO and KEGG functional enrichment analyses of selected genes indicated involvement in the Notch signaling pathway, vascular endothelial growth factor (VEGF) signaling pathway, and TGF- β signaling pathway, all of which are critical for animal growth and skeletal development. The Notch signaling pathway regulates the differentiation and function of osteoblasts and osteoclasts, playing a key role in skeletal development, chondrogenesis, osteogenesis, and osteoclastogenesis [36]. Notch1 inhibits osteoblast formation by suppressing Runt-associated transcription factor 2 and cytoplasmic β -catenin, while Notch2 induces osteoclast differentiation, increasing bone resorption and decreasing bone mass [37]. The Notch signaling pathway is also associated with the growth, differentiation, and apoptosis of bone marrow stem cells (BMSCs), promoting the formation of immature osteoblasts while inhibiting the maturation of terminal osteoblasts. Angiogenesis is essential for bone production during skeletal development, with HIF-1 α and the HIF-responsive gene VEGF being key factors linking osteogenesis and angiogenesis [38]. VEGFA promotes the maturation of bone marrow osteoclasts in vitro and induces the differentiation of bone marrow osteoclasts into mature osteoclasts, thus playing a role in skeletal development. The TGF- β signaling pathway is crucial for multiple cellular life activities and essential for bone formation during mammalian growth. It interacts with other signaling pathways, including MAPK, WNT, Hedgehog, Notch, and FGF pathways, to regulate skeletal development. Notably, knockdown of the *TGFBR2* gene within the TGF- β signaling pathway results in abnormal bone development, including shortened limbs and defects in long bone and mandibular formation. Therefore, mutations in the selected genes may affect Notch signaling, VEGF signaling, TGF- β signaling, and other pathways, ultimately influencing the development of goat bones and affecting leg length traits.

3.4. GWAS Association Analysis of Leg Length Traits in Leizhou Goats

In this study, the association analysis of leg length data from 30 Leizhou goats identified 11 SNP loci significantly associated with leg length traits, of which three were annotated to the *TIAM1*, *MALRD1*, and *MCPH1* genes. The *TIAM1* gene encodes proteins that play important roles in cell signaling and migration. *TIAM1* is a guanylate nucleotide exchange factor that primarily mediates the activation of the Rac small G protein. Rac, a member of the Rho family of GTPases, plays a crucial role in cytoskeletal remodeling, cell migration, cell adhesion, and other processes [39]. By promoting Rac activation, *TIAM1* regulates cytoskeletal dynamics, thereby influencing cell polarity, adhesion, and migratory capacity [40]. This enables *TIAM1* to play an important role in tumor metastasis and invasion processes. Additionally, *TIAM1* is involved in neuronal formation and the regulation of synaptic plasticity, both of which are crucial for the development and function of the

nervous system [41]. In pigs, several studies have identified the *TIAM1* SNP locus as a potential marker for economically important traits, with significant correlations found between the *TIAM1* gene SNP and residual feeding as well as litter size in Chinese native pigs [42]. However, no studies have reported on this gene in goats.

The *MALRD1* gene encodes a protein containing multiple MAM (meprin-A5 protein tyrosine phosphatase mu) and LDLRA2 (low-density lipoprotein receptor A2) domains, which regulate FGF19 production in human intestinal cell lines, thereby modulating bile acid levels, inhibiting gluconeogenesis and lipogenesis, and promoting glycogen synthesis [43]. Within-population GWAS and cross-population meta-analyses of two different strains of Large White pigs indicated that mutations in the *MALRD1* gene were significantly associated with gestation length [44]. Moreover, a genome-wide association study in white Australians with type 2 diabetes revealed that mutations in *MALRD1* (rs12267418) were significantly associated with diabetic macular edema.

The *MCPH1* gene encodes a DNA damage response protein involved in maintaining the inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1) and plays a crucial role in G2/M checkpoint arrest [45]. The *MCPH1* gene encodes a DNA damage response protein involved in maintaining the inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1) and plays a crucial role in G2/M checkpoint arrest [46]. Numerous studies have demonstrated that mutations in the *MCPH1* gene are associated with type 1 primary microcephaly and precocious chromosome condensation syndrome [47]. Additionally, the *MCPH1* gene acts as a repressor of hTERT, mediating the DNA damage response and maintaining chromosomal integrity, and its downregulated expression is linked to the occurrence of cervical cancer [48].

4. Materials and Methods

4.1. Sample Collection and DNA Extraction

One hundred and two ewes of similar ages and body conditions and with consistent feeding and management conditions, good physical condition, and no disease record were selected from Leizhou Goat Farm in Zhanjiang City, Guangdong Province. After body measurements, 10 mL of jugular vein blood was collected from Leizhou goat ewes using disposable vacuum blood collection tubes containing EDTA-K2 as an anticoagulant. Following collection, the tubes were gently inverted to ensure thorough mixing of the blood with the anticoagulant. The samples were then immediately transferred to liquid nitrogen for preservation and transportation. Upon arrival at the laboratory, they were stored at -80°C . Blood samples from 15 Leizhou goats (Table A1) with the longest leg lengths and 15 with the shortest leg lengths were sent to Beijing Novogene Co., Ltd. (Beijing, China), for whole-genome resequencing. Genomic DNA extraction and quality testing were also performed by Beijing Novogene Co., Ltd. The DNA quality assessment methods included (1) agarose gel electrophoresis to check for contamination by proteins, RNA, or signs of degradation; (2) nanodrop spectrophotometry to measure the OD260/280 ratio and assess protein or RNA contamination; and (3) Qubit fluorometry for precise DNA concentration quantification. Only DNA samples with OD260/280 ratios between 1.8 and 2.0, and DNA concentrations of 1.5 μg or higher, were selected for library construction and sequencing.

4.2. DNA Library Construction and Quality Testing

Qualified DNA samples were randomly fragmented into 350 bp lengths using a Covaris ultrasonic crusher. The fragmented DNA underwent end repair, polyA tailing, adapter ligation, purification, and PCR amplification to complete library preparation. The NEBNext[®] Ultra[™] II DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) was used for library construction. Following this, the product was quantified using Qubit 3.0 and diluted to a concentration of 1 ng/ μL . The Agilent 5300 and Q-PCR were then employed to accurately measure the effective concentration of the library, ensuring it exceeded 2 nM. Finally, the library was subjected to PE150 sequencing using the Illumina platform, with a sequencing depth of $10\times$.

4.3. Sequencing Data Quality Control and Mapping

Raw image data files obtained from the Illumina platform were converted into raw sequenced reads, known as “raw data”, using CASAVA (1.8.2) BaseCalling analysis. Since the raw data cannot be directly analyzed, it underwent a series of filtering processes before being used for further analysis. The filtering criteria included (1) removing read pairs with adapter sequences, (2) discarding reads where the proportion of ‘N’ content exceeded 10%, and (3) excluding reads where more than 50% of the bases had a quality score of $Q \leq 5$. After applying these filtering conditions, “clean data” were obtained, and all subsequent analyses were conducted on these clean reads.

Paired-end reads were mapped to the goat reference genome using the BWA software (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/704/415/GCF_001704415.1_ARS1/, accessed on 19 July 2022), with the command “bwa mem -t 4 -k 32 -M”. After mapping, potential PCR duplicates were removed using the “rmdup” command in SAMTOOLS, retaining only the read pairs with the highest mapping quality.

4.4. SNP Detection and Annotation

Sequencing data were aligned for SNP calling at the population scale using the Bayesian approach in SAMTOOLS, then genotype likelihoods were calculated based on the number of reads for each individual at each genomic position, and allele frequencies in the samples were calculated using the Bayesian approach. Individual SNPs were identified using SAMTOOLS (Dp4-miss0.1-maf0.05). Then, in order to retain high-quality SNPs to exclude SNP identification errors due to mismapping or INDELS, a filtering process was required for the raw SNPs, with the filtering conditions that (1) the quality value of SNPs was ≥ 20 , and (2) the number of read supports of SNPs was ≥ 4 . The filtered high-quality SNPs were annotated using ANNOVAR based on the goat DNA reference genome. According to the genome annotation, SNPs were categorized into exonic regions (overlapping with coding exons), intronic regions (overlapping with introns), splice sites (within 2 bp of splice junctions), upstream and downstream regions (within a 1-kb region upstream and downstream of the transcriptional start site), and intergenic regions. SNPs on coding exons were further categorized into synonymous SNPs (which do not cause amino acid changes) and non-synonymous SNPs (which cause amino acid changes), as well as SNPs that make based on the gain or loss of a stop codon.

4.5. Population Genetic Structure Analysis

In this study, the neighbor-joining method was employed to construct the phylogenetic tree. After SNP detection, the individual SNPs obtained were used to calculate the genetic distance between populations. The p -distance between two individuals i and j was calculated using Equation (1):

$$D_{ij} = \frac{1}{L} \sum_{i=1}^L d_{ij}^{(1)} \quad (1)$$

where L is the length of the region with high-quality SNPs. If the allele at position 1 is A/C, the genetic distance function is defined as:

$$d_{ij}^{(1)} = \begin{cases} 0, & \text{If the genotypes of two individuals are AA and AA} \\ 0.5, & \text{If the genotypes of two individuals are AA and AC} \\ 0.5, & \text{If the genotypes of two individuals are AC and AC} \\ 1, & \text{If the genotypes of two individuals are AA and CC} \end{cases} \quad (2)$$

The distance matrix was computed using Treebest-1.9.2 software, and a phylogenetic tree was constructed using the neighbor-joining method. Bootstrap values were obtained after performing 1000 iterations.

In this study, principal component analysis (PCA) was performed as follows: the SNPs at position k of individual i , are denoted by $[0-2]$, which is zero if individual i is purely homozygous for the reference allele, one if heterozygous, and two if individual i is purely

homozygous for the non-reference allele. m is a matrix containing the $n \times S$ matrix of standard genotypes:

$$d'_{ik} = (d_{ik} - E(d_k)) / \sqrt{E(d_k) \times (1 - E(d_k)/2)/2} \quad (3)$$

In Equation (3), $E(d_k)$ is the mean of d_k , and the covariance $n \times n$ matrix of individual samples is calculated by $X = MMT/S$. The PCA analysis was performed using GCTA (1.24.2) software, and finally, the values of the first three PCs were taken for plotting. In this study, PLINK [49] was used for population structure analysis, first creating a PLINK input file and then using ADMIXTURE (1.23) software [50] to construct population genetic structure and population lineage information.

(PLINK (https://s3.amazonaws.com/plink1-assets/1.07/plink1_linux_x86_64.zip), accessed on 13 November 2024).

4.6. Population Selection Elimination Analysis

In this study, the whole-genome of the Leizhou goat TL group and SL group was scanned with the sliding window method, and three methods, F_{st} , $\theta\pi$, and F_{st} and $\theta\pi$, were used to detect the selection signal of the Leizhou goats. The window size was first selected based on the density of SNPs on the genome, and it was found that the number of SNPs on the window tended to stabilize from 100 kb through analysis, so the window length for selective elimination analysis was selected to be 100 kb.

Population genetic differentiation index F_{st} is an index used to measure the degree of genetic differences between different populations, which plays an important role in understanding the genetic structure, kinship, and genetic flow between different populations. The closer it is to one, the larger the genetic differences between populations, the higher the degree of differentiation, and the more distant the kinship. In this study, *vcftools_v0.1.14* software was used to calculate the F_{st} value for each window, assessing the genetic structure of the tall- and short-legged Leizhou goat populations.

Nucleotide polymorphism $\theta\pi$ is often used in animal genetic breeding to assess genetic diversity, QTL localization, kinship analysis, and population structure analysis, and is a positive guide for selecting parents, improving quantitative traits, and avoiding inbreeding and selection bias. The study used *vcftools_v0.1.14* software [51] to calculate the $\theta\pi$ values of different subgroups of Leizhou goats with 100 kb for each window and 50% overlap as the step size. $\theta\pi$ values that deviate more from one represent a higher degree of being subjected to selection.

As an effective method for detecting selection elimination regions, the joint screening of F_{st} and $\theta\pi$ can jointly screen for stronger selection signals, which in turn facilitates the screening of candidate genes for target traits. In this study, the top 5% of windows for both F_{st} and $\theta\pi$ were intersected to identify common selection regions. These regions were then annotated to identify candidate genes potentially associated with leg length traits in Leizhou goats.

4.7. Gene Functional Enrichment Analysis

In this study, we first mapped the genes annotated to the F_{st} and $\theta\pi$ selected regions into the GO database by mapping them to the F_{st} and $\theta\pi$ selected regions, then we calculated how many genes were mapped to each entry, and finally, we calculated the p -value values of the mapped entries using hypergeometric test analysis. The KEGG pathway enrichment analysis was performed on the genes annotated to the F_{st} and $\theta\pi$ selected regions using the KOBAS website to analyze the potential biological functions of the annotated genes in the selected regions [52].

4.8. Genome-Wide Association Analysis

In this study, data from 30 Leizhou goats were obtained based on whole-genome resequencing and analyzed using GWAS using GEMMA (7 March 2010) [53], a genome-

wide efficient mixed-model association software package. For MLM analysis, Equation (4) was used:

$$y = X\alpha + Z\beta + W\mu + e \quad (4)$$

In Equation (4), y represents the phenotype, X represents the genotype, S is the structure matrix, and Z is the indicator matrix of SNP. α is the estimated parameter of fixed effect, β is the effect of SNP, W is the indicator matrix of random effect, μ is the predicted random individual, and e is the random residuals. Statistical analysis was performed using the GEMMA package. A significant (0.05/ N) p -value threshold was set to control for genome-wide type 1 error rates (N is the number of SNP involved in the analysis: 8,631,706).

4.9. Candidate SNP Gene Polymorphisms and Their Leg Length Trait Association Analysis

Sanger sequencing and enzymatic digestion methods were employed to examine the candidate SNP loci in an expanded goat population. The experimental procedure encompassed DNA extraction, primer design, PCR amplification, enzymatic digestion of PCR products, and subsequent Sanger sequencing.

(1) Blood genomic DNA extraction

DNA was extracted from the blood of 250 Leizhou goats with recorded body size measurements. Blood genomic DNA was extracted from the collected samples using the universal DNA extraction kit of Meiji Bio, and the operation steps were as follows: (1) 20 μ L of proteinase K was added to the centrifuge tube in advance, then 200 μ L of anticoagulated blood was added, vortexed, and shaken; (2) 200 μ L of buffer was added after the above steps and vortexed and shaken, and then incubated at 70 °C for 10 min; (3) after the above steps, 200 μ L of anhydrous ethanol was added, and vortexed and shaken; (4) the above reaction solution was transferred to the HiPureDNAMiniColumn1 filtration column, and centrifuged at 10,000 $\times g$ for 1 min; (5) 500 μ L of ethanol-diluted BufferGW1 was added to the filtration column and centrifuged at 10,000 $\times g$ for 1 min; (6) 650 μ L of ethanol-diluted BufferGW2 was added and centrifuged at 10,000 $\times g$ for 1 min; (7) the filtrate was discarded and centrifugation was continued at 10,000 $\times g$ for 3 min; (8) 50 μ L of BufferAE as added at 70 °C to the filtration column, left at room temperature for 3 min, and then centrifuged at 10,000 $\times g$ for 1 min; and (9) an ultra-micro spectrophotometer was used to perform concentration and OD260/280 detection, and DNA samples were randomly drawn for agarose gel electrophoresis to analyze the DNA integrity (Figure 7a).

(2) Primer design

Primers for the SNP regions were designed using the NCBI Pick primer tool. The primer sequences are listed in Table 6.

Table 6. Primer sequences.

Sites	Chromosome	Gene ID	Upstream Primers (5'-3')	Downstream Primers (5'-3')
g. 53666634 T > C	NC_030818.1	102191807	TCCCTCCCCCAAATGTGATG	TCATCTTGTGGGAGCCGATT
g. 40040094 G > C	NC_030826.1	102176826	CCATTGCTGTGGGCTGGT	AGGCCCTGAACAGTTTACGC
g. 76236896 C > T	NC_030817.1	102179855	AGTGCCATTAGGACCAGCAAG	ATACGGACCTGGTTGGGGTTA
g. 43291383 G > C	NC_030826.1	102185686	GGGATGATTTCCTGGGCATC	TGGCACTATGCAGCTCTCTC
g. 49514296 C > T	NC_030825.1	102181421	TCGTAGTGGCTGGTAAACACA	GAATCACTGCTGCCCAAGGT
g. 27088465 A > G	NC_030826.1	102191733	GCCCACAGCAAGCAAATGAC	CCCTGGCTCAAAACCACCAT
g. 49371337 C > T	NC_030825.1	102178102	GTCCTTGTTCCCGTGAGTGT	GCCCAGCTCATCTGCATCTT
g. 55009283 C > T	NC_030828.1	102186636	ACCACAGGCTTTTCTGGAGG	GAGAGTCAGAGACAAGCGGG

(3) PCR amplification

Primers were designed based on 600 bp regions upstream and downstream of the SNP mutation sites using NCBI resources. PCR amplification of Leizhou goat genomic DNA was performed, and the PCR products were analyzed via agarose gel electrophoresis (Figure 7b). PCR amplification of the extracted DNA was performed using the two \times SuperTaq PCR

StarMix from Kangrun Bio (Guangzhou, China). The reaction conditions and program are shown in Tables 7 and 8.

Table 7. PCR reaction system.

Component	Volume
Two × SuperTaq PCR StarMix	25 µL
Forward primers (10 µM)	2.5 µL
Reverse primers (10 µM)	5.5 µL
DNA	50 ng
Sterile, enzyme-free water	Complement to 50 µL

Table 8. PCR reaction procedure.

Process	Temperature	Time	Number of Cycles
Pre-denaturation	95 °C	2 min	/
Denaturation	95 °C	15 s	35 circulate
Anneal	50–72 °C	15 s	
Extend	72 °C	15 s/kb	
Terminal extension	72 °C	5 min	/

(4) PCR product digestion and Sanger sequencing

The PCR products were digested with the appropriate restriction enzyme based on the SNP mutation site, and genotypes were determined from the digestion products. For the fragments with no suitable endonuclease at the SNP mutation site, the products were genotyped by Sanger sequencing, which was carried out by Sangon Biotech (Shanghai, China).

(5) Select SNP loci

SNP loci with strong correlations between the selective sweep analysis and GWAS were selected, and then the Leizhou goat population was expanded for association analysis. The selected SNP loci included NC_030818.1 (g. 53666634 T > C), *NR1D1* (g. 40040094 G > C), *PARP2* (g. 76236896 C > T), *SOST* (g. 43291383 G > C), *NFKB1B* (g. 49514296 C > T), *SHBG* (g. 27088465 A > G), *CAPN12* (g. 49371337 C > T), and *FRMD5* (g. 55009283 C > T) (Table A2).

(6) DNA mixed pool detection of candidate SNP polymorphisms

DNA samples of 30 Leizhou goats were randomly selected to make DNA mixing pools, and a total of three DNA mixing pools were made. Using the DNA mixed pool as a template, PCR amplification was carried out using specific primers, and the amplified products were sequenced by Sanger sequencing.

(7) Candidate gene SNP site genotyping

Using goat DNA as a template, specific primers were used to amplify the fragments where the mutation sites of the candidate genes were located, after which genotyping was carried out by enzymatic digestion.

4.10. Data Processing and Analysis

The leg length data of Leizhou goats were calculated as the difference between body height and chest depth. Statistical analysis was performed using the General Linear Model (GLM) in SPSS 26.0. The analysis utilized a constructed unit point effect model to assess the relationship between molecular marker loci and leg length in Leizhou goats. Multiple comparisons between means were conducted using LSD and Dunnett's T3 methods to evaluate the effect of genotype on leg length. Results are expressed as "mean ± standard deviation" (Mean ± SD). The linear model used for statistical analysis is represented by Equation (5):

$$y_{jkl} = \mu + G_k + e_{jkl} \quad (5)$$

where y_{jkl} is the leg length value of the Leizhou goat, μ is the population mean, G_k is the fixed effect of the k th genotype, and e_{jkl} is the random residual effect.

5. Conclusions

- (1) A total of 8,641,229 high-quality SNPs were identified in 30 Leizhou goats using whole-genome resequencing. Eight candidate genes that might be related to leg length traits in Leizhou goats were screened using selective elimination analysis, including *B4GALT7*, *NR1D1*, *PARP2*, *SOST*, *GDF5*, *EIF2AK2*, *SP1*, and *KIF7*.
- (2) NC_030818.1 (g. 53666634 T > C) and *SHBG* (g. 27088465 A > G) showed intermediate allele frequency in the Leizhou goat population. In addition, the NC_030818.1 (g. 53666634 T > C) variant was associated with leg length traits, with shorter leg lengths in the CC type and longer leg lengths in the TT type.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms252212450/s1>.

Author Contributions: Conceptualization, D.L. and G.L.; methodology, B.S.; software, Y.L.; validation, J.L. (Jinyang Liu), S.D. and J.L. (Jianda Lv); formal analysis, J.L. (Jinyang Liu) and S.D.; investigation, Y.G.; resources, D.L.; data curation, M.D.; writing—original draft preparation, J.L. (Jinyang Liu) and S.D.; writing—review and editing, J.L. (Jinyang Liu) and S.D.; visualization, J.L. (Jinyang Liu) and S.D.; supervision, G.L.; project administration, D.L. and G.L.; funding acquisition, D.L.; co-first authors, J.L. (Jinyang Liu) and S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Project of Agriculture and Rural Department of Guangdong Province, grant number 2024-XPY-00-008.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of South China Agricultural University (license number: SYXK-2018-0123) for studies involving animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials Figure S1. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Body size information of 30 candidate Leizhou goats.

Sample	Body Height/cm	Chest Depth/cm	Leg Lenth/cm
TL1	62.0	34.0	30.5
TL2	63.0	33.5	31.0
TL3	65.0	32.0	32.0
TL4	58.5	34.5	29.0
TL5	60.5	31.5	30.0
TL6	63.5	34.0	31.5
TL7	61.8	30.5	30.8
TL8	62.8	33.5	31.3
TL9	60.0	33.5	30.0
TL10	61.5	33.8	30.8
TL11	58.5	33.3	29.5
TL12	62.0	32.0	31.5
TL13	59.0	31.8	30.5
TL14	58.0	30.3	30.5
TL15	63.0	28.5	33.5

Table A1. Cont.

Sample	Body Height/cm	Chest Depth/cm	Leg Lenth/cm
SL1	55.5	31.5	21.5
SL2	57.0	32.0	23.5
SL3	54.5	33.0	22.5
SL4	59.0	29.5	24.5
SL5	54.0	30.5	22.5
SL6	59.5	32.0	25.5
SL7	53.5	31.0	23.0
SL8	59.0	31.5	25.5
SL9	59.0	30.0	25.5
SL10	60.0	30.8	26.3
SL11	59.3	29.0	26.0
SL12	57.5	30.5	25.5
SL13	57.5	28.5	25.8
SL14	55.0	27.5	24.8
SL15	52.0	29.5	23.5

Table A2. Candidate SNP locus gene information.

Gene	Chromosome Number	Chromosomal Location	Reference Genomic Bases	Mutant Base
NC_030818.1	NC_030808.1	2469037	A	G
NR1D1	NC_030826.1	40040094	G	C
PARP2	NC_030817.1	76236896	C	T
SOST	NC_030826.1	43291383	G	C
NFKBIB	NC_030825.1	49514296	C	T
SHBG	NC_030826.1	27088465	A	G
CAPN12	NC_030825.1	49371761	C	T
FRMD5	NC_030828.1	55009283	C	T

References

- Liu, H.; Peng, W.; Mao, K.; Yang, Y.; Wu, Q.; Wang, K.; Zeng, M.; Han, X.; Han, J.; Zhou, H. The Changes in Fecal Bacterial Communities in Goats Offered Rumen-Protected Fat. *Microorganisms* **2024**, *12*, 822. [\[CrossRef\]](#) [\[PubMed\]](#)
- Quinlan, A.R.; Hall, I.M. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **2010**, *26*, 841–842. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, F.H.; Gong, G.; Yan, X.C.; Zhang, L.T.; Zhang, F.T.; Liu, H.F.; Lv, Q.; Wang, R.J.; Zhang, Y.J.; Wang, Z.X.; et al. Genome-wide association study of fleece traits in Inner Mongolia Cashmere goats. *Anim. Genet.* **2021**, *52*, 375–379. [\[CrossRef\]](#)
- Xiong, J.; Bao, J.; Hu, W.; Shang, M.; Zhang, L. Whole-genome resequencing reveals genetic diversity and selection characteristics of dairy goat. *Front. Genet.* **2023**, *13*, 1044017. [\[CrossRef\]](#) [\[PubMed\]](#)
- Gao, J.; Lyu, Y.; Zhang, D.; Reddi, K.K.; Sun, F.; Yi, J.; Liu, C.; Li, H.; Yao, H.; Dai, J.; et al. Genomic Characteristics and Selection Signatures in Indigenous Chongming White Goat (*Capra hircus*). *Front. Genet.* **2020**, *11*, 901. [\[CrossRef\]](#)
- Lan, X.Y.; Pan, C.Y.; Chen, H.; Lei, C.Z.; Hua, L.S.; Yang, X.B.; Qiu, G.Y.; Zhang, R.F.; Lun, Y.Z. Ddel Polymorphism in Coding Region of Goat POU1F1 Gene and Its Association with Production Traits. *Asian-Australasian J. Anim. Sci.* **2007**, *20*, 1342–1348. [\[CrossRef\]](#)
- Wang, K.; Zhang, Y.; Han, X.; Wu, Q.; Liu, H.; Han, J.; Zhou, H. Effects of Copy Number Variations in the Plectin (PLEC) Gene on the Growth Traits and Meat Quality of Leizhou Black Goats. *Animals* **2023**, *13*, 3651. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chen, Q.; Chai, Y.; Zhang, W.; Cheng, Y.; Zhang, Z.; An, Q.; Chen, S.; Man, C.; Du, L.; Zhang, W. Whole-genome se-quencing reveals the genomic characteristics and selection signatures of hainan black goat. *Genes* **2022**, *13*, 1539. [\[CrossRef\]](#)
- Zhou, J.; Meng, C.; Li, Y.; Fu, Y.; Long, W.; Huang, H.; Liu, Y.; Lyu, P.; Xiao, S. MiRNA-423 rs6505162 and miRNA-6811 rs2292879 SNP associated with lung cancer in Hainan, China. *Biosci. Rep.* **2023**, *43*, BSR20231152. [\[CrossRef\]](#)
- Shastri, B.S. SNPs: Impact on gene function and phenotype. In *Single Nucleotide Polymorphisms: Methods and Protocols*; Humana Press: Totowa, NJ, USA, 2009; pp. 3–22. [\[CrossRef\]](#)
- McKenzie, C.W.; Preston, C.C.; Finn, R.; Eyster, K.M.; Faustino, R.S.; Lee, L. Strain-specific differences in brain gene expression in a hydrocephalic mouse model with motile cilia dysfunction. *Sci. Rep.* **2018**, *8*, 13370. [\[CrossRef\]](#)
- McKenzie, C.W.; Lee, L. Genetic interaction between central pair apparatus genes CFAP221, CFAP54, and SPEF2 in mouse models of primary ciliary dyskinesia. *Sci. Rep.* **2020**, *10*, 12337. [\[CrossRef\]](#)

13. Cianfanelli, V.; De Zio, D.; Di Bartolomeo, S.; Nazio, F.; Strappazzon, F.; Cecconi, F. Ambra1 at a glance. *J. Cell Sci.* **2015**, *128*, 2003–2008. [\[CrossRef\]](#)
14. Crespi, B.; Read, S.; Ly, A.; Hurd, P. AMBRA1, Autophagy, and the Extreme Male Brain Theory of Autism. *Autism Res. Treat.* **2019**, *2019*, 1968580. [\[CrossRef\]](#)
15. Mitjans, M.; Begemann, M.; Ju, A.; Dere, E.; Wüstefeld, L.; Hofer, S.; Hassouna, I.; Balkenhol, J.; Oliveira, B.; van der Auwera, S.; et al. Sexual dimorphism of AMBRA1-related autistic features in human and mouse. *Transl. Psychiatry* **2017**, *7*, e1247. [\[CrossRef\]](#)
16. Qin, Y.-Q.; Liu, S.-Y.; Lv, M.-L.; Sun, W.-L. Ambra1 in cancer: Implications for clinical oncology. *Apoptosis* **2022**, *27*, 720–729. [\[CrossRef\]](#)
17. Gambarotto, L.; Metti, S.; Chrisam, M.; Cerqua, C.; Sabatelli, P.; Armani, A.; Zanon, C.; Spizzotin, M.; Castagnaro, S.; Strappazzon, F.; et al. Ambra1 deficiency impairs mitophagy in skeletal muscle. *J. Cachex-Sarcopenia Muscle* **2022**, *13*, 2211–2224. [\[CrossRef\]](#)
18. Zhang, C. Transcriptional regulation of bone formation by the osteoblast-specific transcription factor Osx. *J. Orthop. Surg. Res.* **2010**, *5*, 37. [\[CrossRef\]](#)
19. Liu, C.; Jia, Y.; Zhao, X.; Wang, Z.; Zhu, X.; Zhang, C.; Li, X.; Zhao, X.; Gong, T.; Zhao, H.; et al. Targeting B4GALT7 suppresses the proliferation, migration and invasion of hepatocellular carcinoma through the Cdc2/CyclinB1 and miR-338-3p/MMP2 pathway. *PeerJ* **2023**, *11*, e16450. [\[CrossRef\]](#)
20. Boegheim, I.J.; Leegwater, P.A.; van Lith, H.A.; Back, W. Current insights into the molecular genetic basis of dwarfism in livestock. *Veter- J.* **2017**, *224*, 64–75. [\[CrossRef\]](#)
21. Leegwater, P.A.; Vos-Loohuis, M.; Ducro, B.J.; Boegheim, I.J.; van Steenbeek, F.G.; Nijman, I.J.; Monroe, G.R.; Bastiaansen, J.W.M.; Dibbitts, B.W.; van de Goor, L.H.; et al. Dwarfism with joint laxity in Friesian horses is associated with a splice site mutation in B4GALT7. *BMC Genom.* **2016**, *17*, 839. [\[CrossRef\]](#)
22. Delbaere, S.; Van Damme, T.; Syx, D.; Symoens, S.; Coucke, P.; Willaert, A.; Malfait, F. Hypomorphic zebrafish models mimic the musculoskeletal phenotype of β 4GalT7-deficient Ehlers-Danlos syndrome. *Matrix Biol.* **2020**, *89*, 59–75. [\[CrossRef\]](#)
23. Zhang-Sun, Z.-Y.; Xu, X.-Z.; Escames, G.; Lei, W.-R.; Zhao, L.; Zhou, Y.-Z.; Tian, Y.; Ren, Y.-N.; Acuña-Castroviejo, D.; Yang, Y. Targeting NR1D1 in organ injury: Challenges and prospects. *Mil. Med. Res.* **2023**, *10*, 62. [\[CrossRef\]](#)
24. Ye, W.; Wang, Y.; Mei, B.; Hou, S.; Liu, X.; Wu, G.; Qin, L.; Zhao, K.; Huang, Q. Computational and functional characterization of four SNPs in the SOST locus associated with osteoporosis. *Bone* **2018**, *108*, 132–144. [\[CrossRef\]](#)
25. He, J.; Zhang, H.; Wang, C.; Zhang, Z.; Yue, H.; Hu, W.; Gu, J.; Fu, W.; Hu, Y.; Li, M.; et al. Associations of Serum Sclerostin and Polymorphisms in the SOST Gene With Bone Mineral Density and Markers of Bone Metabolism in Postmenopausal Chinese Women. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E665–E673. [\[CrossRef\]](#)
26. Styrkarsdottir, U.; Thorleifsson, G.; Gudjonsson, S.A.; Sigurdsson, A.; Center, J.R.; Lee, S.H.; Nguyen, T.V.; Kwok, T.C.; Lee, J.S.; Ho, S.C.; et al. Sequence variants in the PTCH1 gene associate with spine bone mineral density and osteoporotic fractures. *Nat. Commun.* **2016**, *7*, 10129. [\[CrossRef\]](#)
27. Murakami, T.; Ruengsinpinya, L.; Takahata, Y.; Nakaminami, Y.; Hata, K.; Nishimura, R. HOXA10 promotes Gdf5 expression in articular chondrocytes. *Sci. Rep.* **2023**, *13*, 22778. [\[CrossRef\]](#)
28. Zhang, R.; Yao, J.; Xu, P.; Ji, B.; Luck, J.V.; Chin, B.; Lu, S.; Kelsoe, J.R.; Ma, J. A comprehensive meta-analysis of association between genetic variants of GDF5 and osteoarthritis of the knee, hip and hand. *Inflamm. Res.* **2015**, *64*, 405–414. [\[CrossRef\]](#)
29. García-Alvarado, F.; Rosales-González, M.; Arellano-Pérez-Vertti, D.; Espino-Silva, P.; Meza-Velazquez, M.; Ruiz-Flores, P. Association Between the SNP rs143383+104T/C in the GDF5 Gene and the Risk of Knee Osteoarthritis in a Population from Northern Mexico-A Case-Control Study. *Genet. Test. Mol. Biomarkers* **2018**, *22*, 503–506. [\[CrossRef\]](#)
30. Liu, Y.F.; Zan, L.S.; Li, K.; Zhao, S.P.; Xin, Y.P.; Lin, Q.; Tian, W.Q.; Wang, Z.W. A novel polymorphism of GDF5 gene and its association with body measurement traits in Bos taurus and Bos indicus breeds. *Mol. Biol. Rep.* **2009**, *37*, 429–434. [\[CrossRef\]](#)
31. Xu, J.; Rogers, M.B. Modulation of Bone Morphogenetic Protein (BMP) 2 gene expression by Sp1 transcription factors. *Gene* **2007**, *392*, 221–229. [\[CrossRef\]](#)
32. Bandyopadhyay, A.; Tsuji, K.; Cox, K.; Harfe, B.D.; Rosen, V.; Tabin, C.J. Genetic Analysis of the Roles of BMP2, BMP4, and BMP7 in Limb Patterning and Skeletogenesis. *PLoS Genet.* **2006**, *2*, e216. [\[CrossRef\]](#)
33. Qian, Z.; Zhang, Y.; Kang, X.; Li, H.; Zhang, Y.; Jin, X.; Gao, X.; Xu, M.; Ma, Z.; Zhao, L.; et al. Postnatal Conditional Deletion of Bmal1 in Osteoblasts Enhances Trabecular Bone Formation Via Increased BMP2 Signals. *J. Bone Miner. Res.* **2020**, *35*, 1481–1493. [\[CrossRef\]](#)
34. Duttonhoefer, F.; Biswas, S.K.; Igwe, J.C.; Sauerbier, S.; Bierhaus, A. Sp1-dependent regulation of PPAR γ in bone metabolism. *Int. J. Oral Maxillofac. Implant.* **2014**, *29*, e107–e116. [\[CrossRef\]](#)
35. Dehghan, M.; Pourahmad-Jaktaji, R. Sp1 binding site polymorphism of a collagen gene (rs 1800012) in women aged 45 and over and its association with bone density. *Turk. J. Med. Sci.* **2015**, *45*, 644–650. [\[CrossRef\]](#)
36. Karsenty, G.; Kronenberg, H.M.; Settembre, C. Genetic Control of Bone Formation. *Annu. Rev. Cell Dev. Biol.* **2009**, *25*, 629–648. [\[CrossRef\]](#)
37. Hsu, S.-H.C.; Zhang, X.; Yu, C.; Li, Z.J.; Wunder, J.S.; Hui, C.-C.; Alman, B.A. Kif7 promotes hedgehog signaling in growth plate chondrocytes by restricting the inhibitory function of Sufu. *Development* **2011**, *138*, 3791–3801. [\[CrossRef\]](#)
38. Kobayashi, T.; Kronenberg, H.M. Overview of Skeletal Development. *Methods Mol. Biol.* **2021**, *2230*, 3–16. [\[CrossRef\]](#)
39. Maruyama, T.; Jiang, M.; Hsu, W. Gpr177, a novel locus for bone mineral density and osteoporosis, regulates osteogenesis and chondrogenesis in skeletal development. *J. Bone Miner. Res.* **2012**, *28*, 1150–1159. [\[CrossRef\]](#)

40. Morvan, F.; Boulukos, K.; Clément-Lacroix, P.; Roman, S.R.; Suc-Royer, I.; Vayssière, B.; Ammann, P.; Martin, P.; Pinho, S.; Pognonec, P.; et al. Deletion of a Single Allele of the *Dkk1* Gene Leads to an Increase in Bone Formation and Bone Mass. *J. Bone Miner. Res.* **2006**, *21*, 934–945. [[CrossRef](#)]
41. Gaur, T.; Lengner, C.J.; Hovhannisyan, H.; Bhat, R.A.; Bodine, P.V.N.; Komm, B.S.; Javed, A.; van Wijnen, A.J.; Stein, J.L.; Stein, G.S.; et al. Canonical WNT Signaling Promotes Osteogenesis by Directly Stimulating Runx2 Gene Expression. *J. Biol. Chem.* **2005**, *280*, 33132–33140. [[CrossRef](#)]
42. Karner, C.M.; Long, F. Wnt signaling and cellular metabolism in osteoblasts. *Cell. Mol. Life Sci.* **2016**, *74*, 1649–1657. [[CrossRef](#)]
43. Ballhause, T.M.; Jiang, S.; Baranowsky, A.; Brandt, S.; Mertens, P.R.; Frosch, K.-H.; Yorgan, T.; Keller, J. Relevance of Notch Signaling for Bone Metabolism and Regeneration. *Int. J. Mol. Sci.* **2021**, *22*, 1325. [[CrossRef](#)]
44. Gao, Y.; Fu, Z.; Guan, J.; Liu, X.; Zhang, Q. The role of Notch signaling pathway in metabolic bone diseases. *Biochem. Pharmacol.* **2022**, *207*, 115377. [[CrossRef](#)]
45. Guo, Q.; Yang, J.; Chen, Y.; Jin, X.; Li, Z.; Wen, X.; Xia, Q.; Wang, Y. Salidroside improves angiogenesis-osteogenesis coupling by regulating the HIF-1 α /VEGF signalling pathway in the bone environment. *Eur. J. Pharmacol.* **2020**, *884*, 173394. [[CrossRef](#)]
46. Hsu, G.C.-Y.; Wang, Y.; Lu, A.Z.; Gomez-Salazar, M.A.; Xu, J.; Li, D.; Meyers, C.; Negri, S.; Wangsiricharoen, S.; Broderick, K.P.; et al. TIAM1 acts as an actin organization regulator to control adipose tissue-derived pericyte cell fate. *J. Clin. Investig.* **2023**, *8*, e159141. [[CrossRef](#)]
47. Mertens, A.E.; Roovers, R.C.; Collard, J.G. Regulation of Tiam1–Rac signalling. *FEBS Lett.* **2003**, *546*, 11–16. [[CrossRef](#)]
48. Ru, Q.; Lu, Y.; Bin Saifullah, A.; Blanco, F.A.; Yao, C.; Cata, J.P.; Li, D.-P.; Tolias, K.F.; Li, L. TIAM1-mediated synaptic plasticity underlies comorbid depression-like and ketamine antidepressant-like actions in chronic pain. *J. Clin. Investig.* **2022**, *132*, e158545. [[CrossRef](#)]
49. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.R.; Bender, D.; Maller, J.; Sklar, P.; de Bakker, P.I.W.; Daly, M.J.; et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am. J. Hum. Genet.* **2007**, *81*, 559–575. [[CrossRef](#)]
50. Alexander, D.H.; Novembre, J.; Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **2009**, *19*, 1655–1664. [[CrossRef](#)]
51. Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; DePristo, M.A.; Handsaker, R.E.; Lunter, G.; Marth, G.T.; Sherry, S.T.; et al. The variant call format and VCFtools. *Bioinformatics* **2011**, *27*, 2156–2158. [[CrossRef](#)]
52. Bu, D.; Luo, H.; Huo, P.; Wang, Z.; Zhang, S.; He, Z.; Wu, Y.; Zhao, L.; Liu, J.; Guo, J.; et al. KOBAS-i: Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res.* **2021**, *49*, W317–W325. [[CrossRef](#)] [[PubMed](#)]
53. Zhou, X.; Stephens, M. Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.* **2012**, *44*, 821–824. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.



Article

Selection and Regulatory Network Analysis of Differential CircRNAs in the Hypothalamus of Goats with High and Low Reproductive Capacity

Shuaixiang Mao, Cuiying Wu, Guanghang Feng, Yaokun Li, Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu and Guangbin Liu

Special Issue

Molecular Genetics and Breeding Mechanisms in Domestic Animals: 2nd Edition

Edited by
Prof. Dr. Guiping Zhao





Article

Selection and Regulatory Network Analysis of Differential CircRNAs in the Hypothalamus of Goats with High and Low Reproductive Capacity

Shuaixiang Mao, Cuiying Wu, Guanghang Feng, Yaokun Li , Baoli Sun, Yongqing Guo , Ming Deng, Dewu Liu * and Guangbin Liu *

College of Animal Science, South China Agricultural University, Guangzhou 510642, China

* Correspondence: dwliu@scau.edu.cn (D.L.); gbliu@scau.edu.cn (G.L.)

Abstract: The objectives of this investigation were to identify differentially expressed circular RNAs (circRNAs) in the hypothalamus of goats with high and low prolificacy and construct a circRNA-miRNA regulatory network to uncover key potential circRNAs that influence goat prolificacy. Transcriptome analysis was performed on hypothalamus samples from low-prolificacy ($n = 5$) and high-prolificacy ($n = 6$) Chuanzhong black goats to identify circRNAs that influence prolificacy in these goats. Differential expression analysis identified a total of 205 differentially expressed circRNAs, comprising 100 upregulated and 105 downregulated circRNAs in the high-prolificacy group compared with the low-prolificacy group. Enrichment analysis of these differentially expressed circRNAs indicated significant enrichment in Gene Ontology terms associated with mammalian oogenesis, negative regulation of neurotransmitter secretion, reproductive developmental processes, hormone-mediated signaling pathways, and negative regulation of hormone secretion. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis highlighted significant enrichment in the oxytocin signaling pathway, GnRH signaling pathway, and hormone-mediated oocyte maturation. The hypothalamus of low- and high-prolificacy goats contains circular RNAs (circRNAs), including chirc_063269, chirc_097731, chirc_017440, chirc_049641, chirc_008429, chirc_145057, chirc_030156, chirc_109497, chirc_030156, chirc_176754, and chirc_193363. Chuanzhong black goats have the potential to influence prolificacy by modulating the release of serum hormones from the hypothalamus. A circRNA-miRNA regulatory network was constructed, which determined that miR-135a, miR-188-3p, miR-101-3p, and miR-128-3p may interact with differentially expressed circRNAs, thereby regulating reproductive capacity through the hypothalamic-pituitary-gonadal axis. The results of this study enhance our knowledge of the molecular mechanisms that regulate prolificacy in Chuanzhong black goats at the hypothalamic level.

Keywords: Chuanzhong black goat; hypothalamus; prolificacy; circRNA



Citation: Mao, S.; Wu, C.; Feng, G.; Li, Y.; Sun, B.; Guo, Y.; Deng, M.; Liu, D.; Liu, G. Selection and Regulatory Network Analysis of Differential CircRNAs in the Hypothalamus of Goats with High and Low Reproductive Capacity. *Int. J. Mol. Sci.* **2024**, *25*, 10479. <https://doi.org/10.3390/ijms251910479>

Academic Editor: Brad Freking

Received: 21 August 2024

Revised: 26 September 2024

Accepted: 27 September 2024

Published: 28 September 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Goats possess unique advantages in environmental adaptability and functional diversity. Goat farming is considered a relatively low-investment venture compared with farming other livestock due to their body size, productivity levels, dietary preferences, and production costs, all of which are favorable for human use [1]. Goats do not compete with humans for food resources and can provide abundant meat, milk, fur, and other products, which is why they have become one of the most widely distributed livestock species globally [2]. The Chuanzhong black goat, a local breed found in central shallow hilly areas and adapted to high-altitude climates, exhibits rapid growth, excellent meat quality, strong adaptability, and resilience to coarse forage [3]. Kid production is a crucial reproductive trait in goats, directly impacting their economic viability. Therefore, breeding goat breeds with high reproductive rates is essential for developing the goat industry [4,5].

The Chuanzhong black goat population includes groups with both high and low reproductive rates. Utilizing high-throughput sequencing techniques to identify genes associated with kid production traits in Chuanzhong goats can provide valuable insights into the genetic factors influencing goat prolificacy.

The ovary is a reproductive organ in female animals. In the estrous cycle of goats, the number of mature oocytes released by the ovary is a crucial factor influencing goat kid production. Ovarian function is regulated by the hypothalamus and the pituitary gland [6]. The hypothalamus primarily regulates the ovary primarily by secreting gonadotropin-releasing hormone (GnRH), which controls the secretion and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. These hormones can bind to receptors in the ovary to regulate the ovulation process [7]. Studies have shown that goats exhibit a surge in LH prior to ovulation [8], indicating that the hypothalamus plays a central role in the reproductive control process in goats. The synthesis and secretion of GnRH in the hypothalamus may be influenced by non-coding RNA. However, research on the molecular mechanisms through which the hypothalamus affects goat prolificacy is relatively limited.

Circular RNA (circRNA) is a biologically active nucleic acid molecule that, unlike mRNA, lacks a polyadenylated tail [9,10]. Current research has identified six primary functions of circRNA [11]. The first function is its ability to localize the synthesis site and the host gene, thereby upregulating exons or truncating transcripts [12,13]. The second function of circRNA is its capacity to bind to U1 small nuclear ribonucleoprotein, interact with PolIII, and enhance the expression of the parent gene [14]. The third function is that it acts as a miRNA sponge, competitively binding miRNA [15]. The fourth function is its ability to interact with proteins [16]. The fifth function is that if circular RNA contains an internal ribosome entry site (IRES), then it can recruit ribosomes and undergo translation [17]. The sixth function is that circRNA containing N6-methyladenosine (m6A) can be recognized by YTHDF3, thereby triggering the translation process [18]. Whole transcriptome analysis can identify both coding and non-coding RNA, quantify the heterogeneity of gene expression across tissues and organs [19], and enhance our understanding of the regulatory relationships between genes [20]. Therefore, utilizing transcriptome sequencing technology to investigate genes associated with reproduction in the hypothalamus is crucial for comprehending the molecular mechanisms through which the hypothalamus regulates animal reproduction.

This study employs transcriptome sequencing technology to investigate the hypothalamus of low- and high-prolificacy Chuanzhong black goats, with the aim of identifying differential circRNAs that may influence the reproductive performance of this breed. The objective is to provide insights into the molecular mechanisms regulating reproduction in Chuanzhong black goats and establish a theoretical foundation for the prolificacy traits of this breed.

2. Results

2.1. Quality Detection of RNA-Seq Sequencing Data

Before further analysis, quality control checks were performed on the raw data obtained from sequencing the hypothalamus samples of the low-prolificacy group (CZ_L) and the high-prolificacy group (CZ_H). Eleven independent cDNA libraries were constructed from the hypothalamic tissue RNA of both the CZ_L and CZ_H groups. A total of 1,142,854,448 raw sequence reads were generated from the 11 sequencing libraries, of which 1,134,036,480 high-quality reads passed quality control and were used for subsequent analysis. The base identification rate for each sample ranged from 92.04% to 93.48%, with more than 99.9% of bases identified correctly. The alignment rates of high-quality reads to the reference genome were above 84.98%. Among these high-quality reads, 1.45% to 2.38% aligned to multiple locations, while 97.62% to 98.55% aligned to unique positions. These data indicate high sequencing quality, fully meeting the requirements for subsequent

analysis, as illustrated in Table 1 [21]. The correlation analysis of circRNA expression among the samples is depicted in Figure 1A.

Table 1. Overview of RNA sequencing of the hypothalamus in Chuangzhong black goats.

Sample	Raw Reads	Clean Reads	Clean Reads Rate /%	Multiple Mapped Rate /%	Uniquely Mapped Rate /%	Q30 /%
CZ_L1	105,651,828	104,878,170	99.26	2.38	97.62	93.33
CZ_L2	106,461,850	105,641,916	99.22	2.32	97.68	93.01
CZ_L3	103,419,368	102,762,390	99.36	1.94	98.06	93.48
CZ_L4	103,316,372	102,518,058	99.22	2.13	97.87	93.09
CZ_L5	101,666,550	100,924,246	99.26	1.45	98.55	92.51
CZ_H1	106,945,128	106,155,188	99.26	2.04	97.96	92.73
CZ_H2	103,019,774	102,205,262	99.20	1.79	98.21	92.50
CZ_H3	106,627,266	105,676,988	99.10	1.78	98.22	92.04
CZ_H4	102,279,224	101,431,548	99.17	1.63	98.37	92.29
CZ_H5	102,088,070	101,272,006	99.20	1.65	98.35	92.37
CZ_H6	101,379,018	100,570,708	99.20	2.13	97.87	92.43

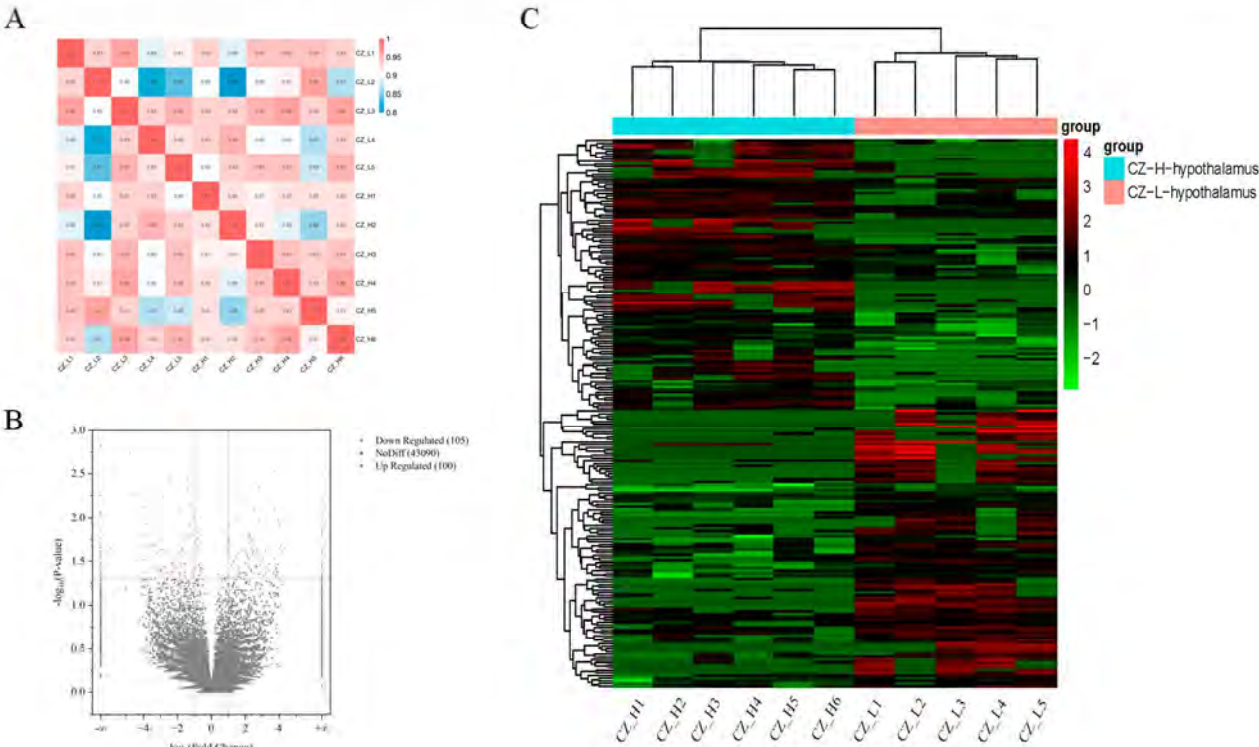


Figure 1. Correlation analysis and differential circRNA profile of hypothalamic samples in Chuangzhong black goats with different reproductive capacities. (A) Correlation analysis heatmap of circRNA expression across different samples. (B) Volcano plot of differentially expressed circRNAs. (C) Heatmap of clustering analysis for differentially expressed circRNAs.

2.2. CircRNA Differential Expression Analysis

The hypothalamus, a crucial component of the hypothalamus-pituitary-ovary axis in goats, may influence the reproductive capacity of Chuangzhong goats through the differential expression of genes or transcripts. On the basis of the criteria of $|\log_2 \text{Fold Change}| > 1$ and a $p\text{-value} < 0.05$, we identified differentially expressed circular RNAs (DEcircRNAs) between the low-prolificacy and high-prolificacy groups. With the use of hypothalamic samples from the low-prolificacy group as a control, the volcano plot revealed a total of 43,295 circular RNAs detected in both the low- and high-prolificacy groups. Among these,

205 circRNAs exhibited differential expression, with 100 circRNAs upregulated and 105 circRNAs downregulated (Figure 1B, Supplementary Table S1). Clustering analysis indicated that the gene expression levels and patterns of the six samples from the high-proliferacy group were similar, while the five samples from the low-proliferacy group also displayed comparable patterns (Figure 1C).

2.3. Functional Enrichment Analysis of Host Genes with Differentially Expressed circRNAs

In our investigation of the functional roles of differentially expressed circRNAs in the hypothalamus of Chuanzhong black goats with varying reproductive capacities, we identified the host genes associated with these circRNAs (Figure 2, Supplementary Table S4) and performed Gene Ontology (GO) functional enrichment analysis on these host genes. Our analysis revealed that the host genes of differentially expressed circRNAs were enriched in 3257 GO terms. A differential significance enrichment analysis of the results identified 486 significantly enriched GO terms ($p < 0.05$), which included 338 biological processes (BP), 59 cellular components (CC), and 89 molecular functions (MF). The four most enriched biological processes were cellular processes, regulation of biological processes, biological regulation, and metabolic processes. The two most enriched cellular components were protein-containing complexes and cellular anatomical entities. The four most enriched molecular functions were binding, catalytic activity, molecular function regulation, and ATP-dependent activity (Figure 3A,B, Supplementary Table S2).

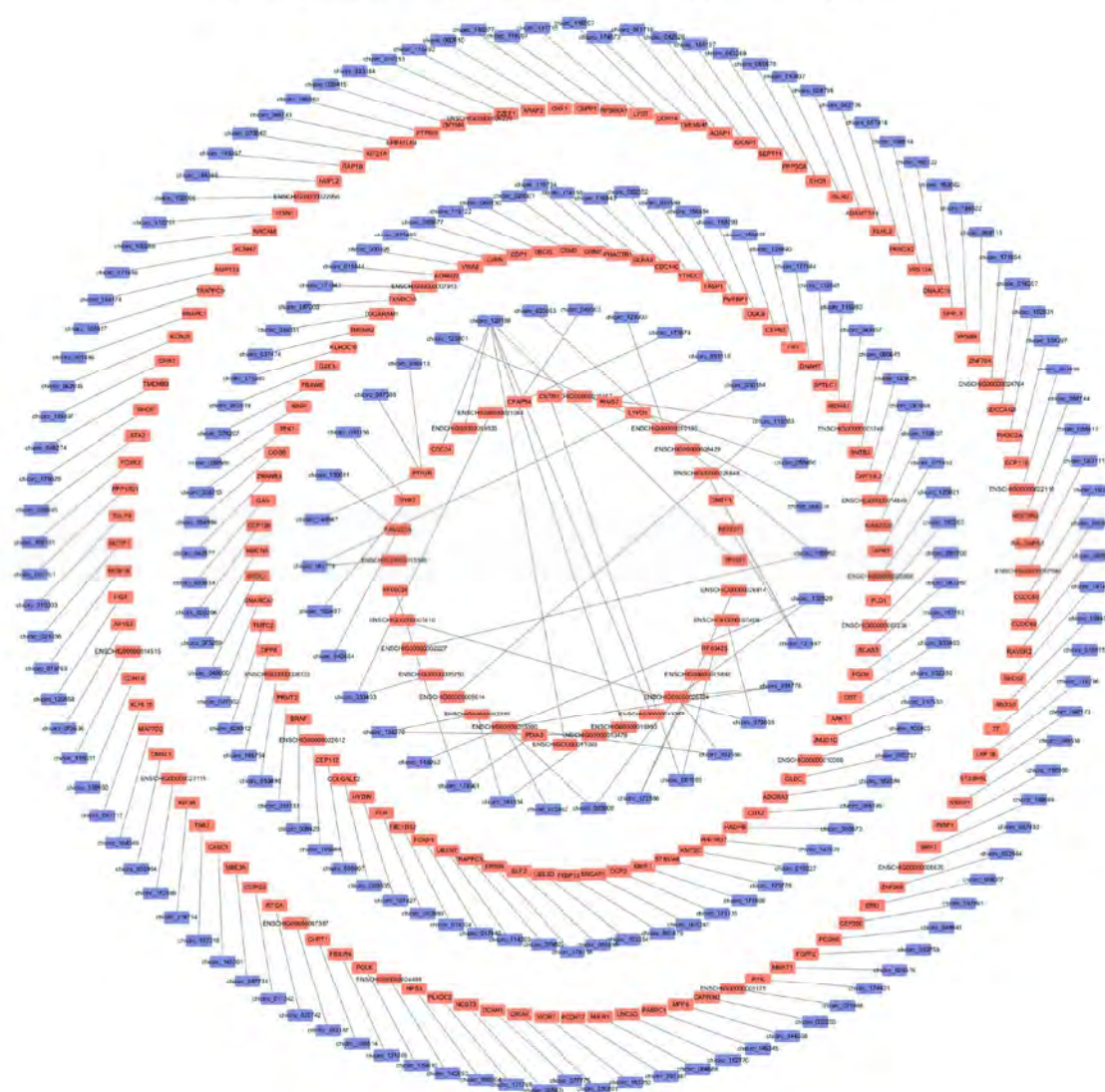


Figure 2. Host gene network map of differentially expressed circRNAs.

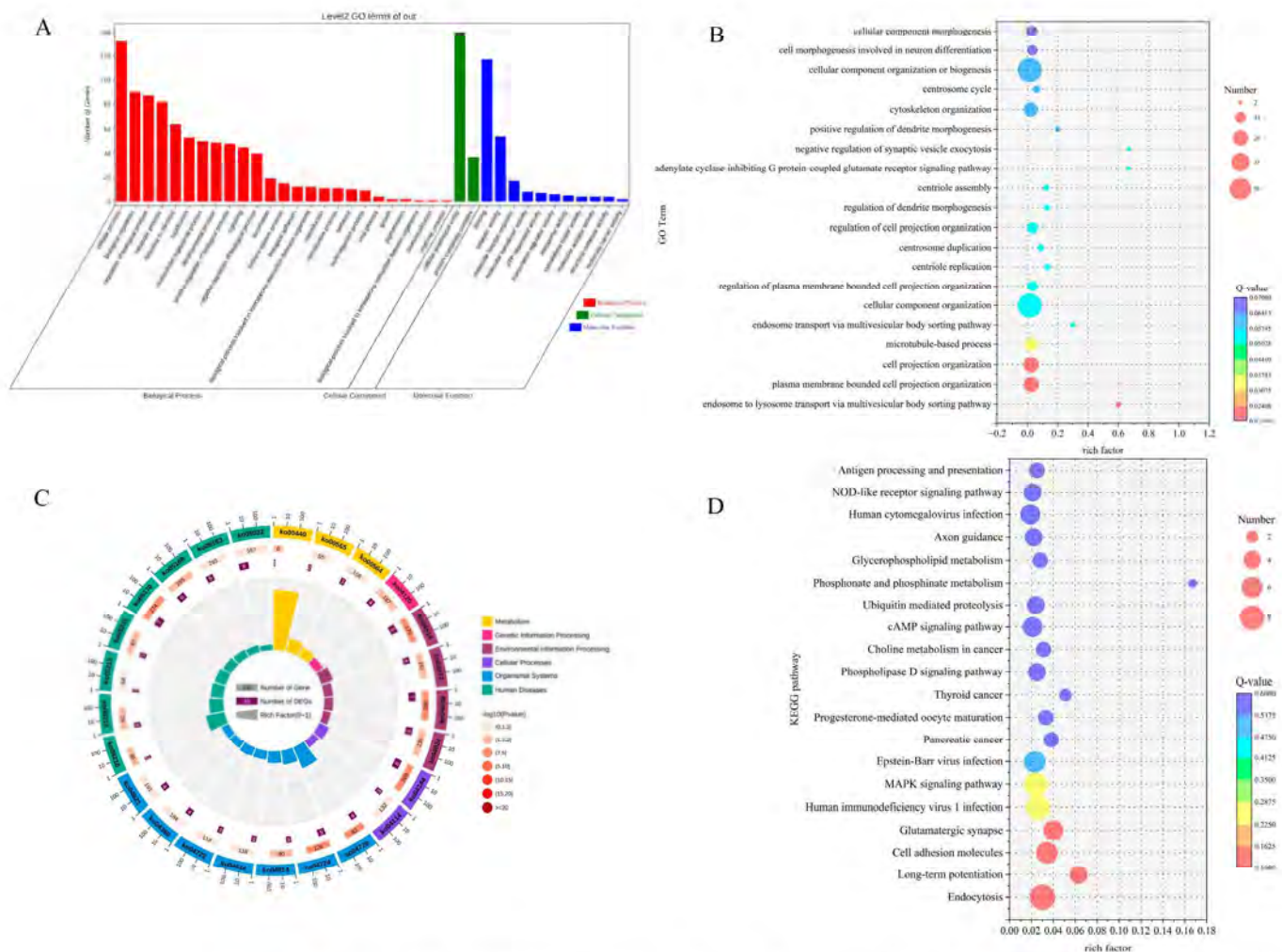


Figure 3. GO and KEGG analysis of differentially expressed circRNA host genes. **(A)** GO annotation involves categorizing genes based on BP, CC, and MFs. **(B)** Bubble plot of GO enrichment for target genes of differentially expressed circRNAs. **(C)** Circular plot of KEGG pathway enrichment for target genes of differentially expressed circRNAs. **(D)** Bubble plot illustrating the KEGG pathway enrichment for target genes of differentially expressed circRNAs.

For the host genes of differentially expressed circRNAs, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed, identifying enrichment in 183 pathways, of which 15 were significantly enriched ($p < 0.05$). These pathways include progesterone-mediated oocyte maturation, the MAPK signaling pathway, the cAMP signaling pathway, and the phospholipase D signaling pathway (Figure 3C,D, Supplementary Table S3).

2.4. Analysis of the Regulatory Network of Differential circRNAs and miRNAs

CircRNAs can adsorb miRNAs, thereby inhibiting their functions and serving as important molecular sponges for miRNAs. Utilizing prediction results from miRanda and TargetScan, we identified 205 differentially expressed circRNAs in the hypothalamus of Chuanzhong black goats with varying reproductive abilities, which collectively target 167 miRNAs (Supplementary Table S5). On the basis of the p -values, we selected the top 15 differentially expressed circRNAs to construct a regulatory network of circRNAs and miRNAs (Figure 4). The results indicate that these top 15 differentially expressed circRNAs collectively target 164 miRNAs, among which miR-135a, miR-188-3p, miR-101-3p, and miR-128-3p may interact with the differentially expressed circRNAs.

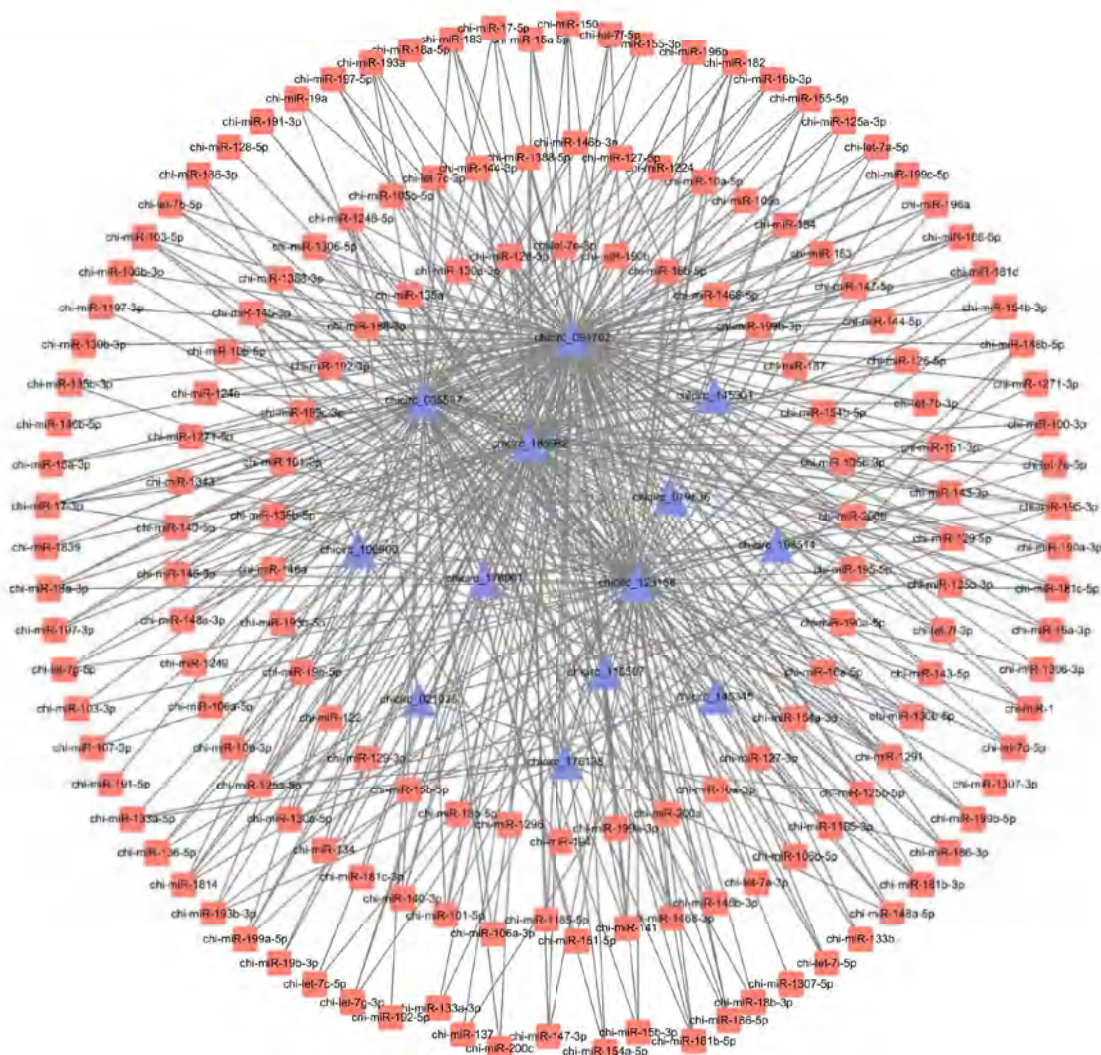


Figure 4. Regulatory network diagram of differentially expressed circRNAs and miRNAs.

2.5. Prediction of circRNA Coding Potential

CircRNAs can undergo translation through primary mechanisms: the presence of IRES and the existence of m6A residues. According to predictions from the IRES finder, 150 circRNAs rely on internal ribosome entry sites, of which 26 circRNAs have a score exceeding 0.8. Additionally, predictions from the sequence-based RNA adenosine methylation site predictor (SRAMP) indicate that 192 circRNAs initiate translation dependent on m6A residues, with 78 circRNAs achieving a score greater than 0.7, which meets the criteria for very high confidence. In this study, the open reading frame (ORF) finder predicted 198 circRNAs containing ORFs among differentially expressed circRNAs. Intersecting these two translation mechanisms shows that 141 circRNAs may potentially utilize both mechanisms simultaneously (Figure 5).

2.6. Real-Time Fluorescence Quantitative PCR Validation

RT-qPCR validation analysis was conducted on six differentially expressed circRNAs to validate the RNA-seq results (Figure 6A). The results indicate differences in expression levels between the two methods. Sanger sequencing confirmed the nucleotide sequences of the circRNA circularization splice sites. The findings demonstrated that the nucleotide sequences of these splice sites were consistent between Sanger sequencing and transcriptome sequencing (Figure 6B). The trends were aligned, suggesting the reliability of the sequencing results.

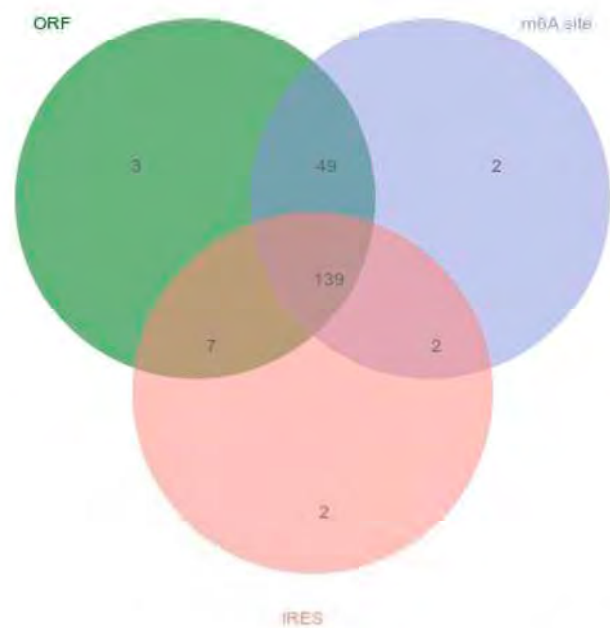


Figure 5. Venn diagram of circRNA coding potential predictions by ORF, SRAMP, and IRES Finder.

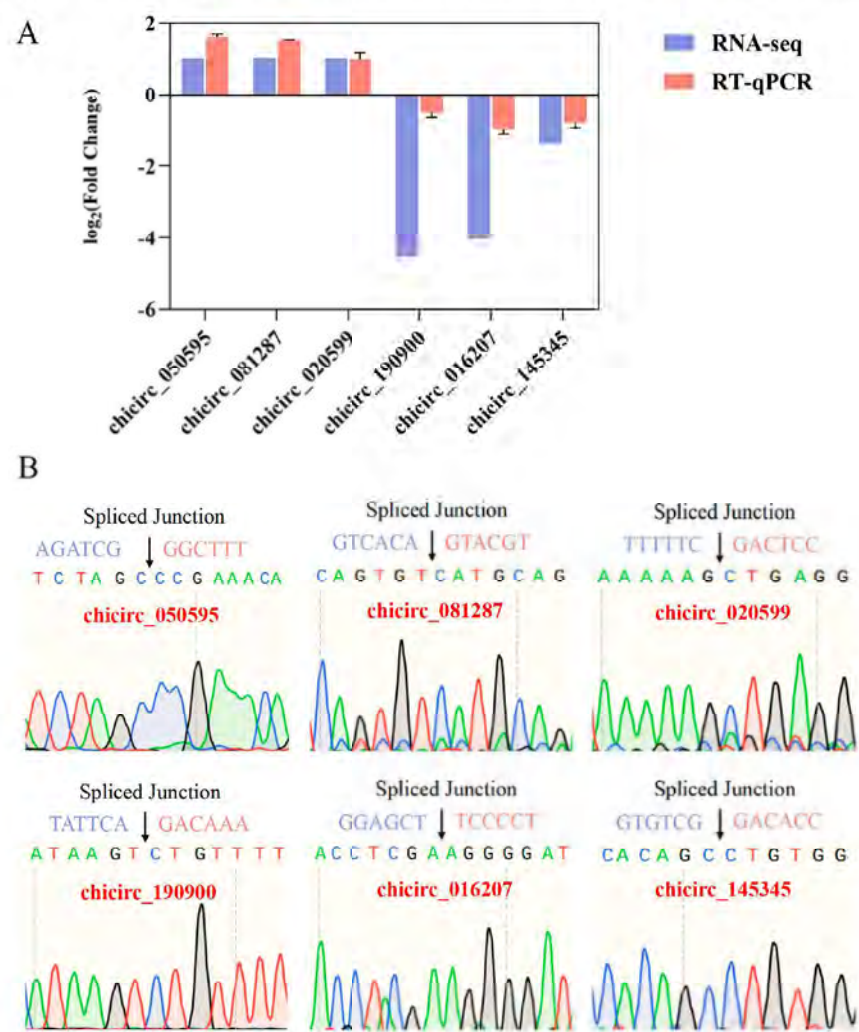


Figure 6. Validation of differentially expressed circRNA. (A) RT-qPCR validation results of differentially expressed circRNAs and (B) sequencing results of circularization splice sites of differentially expressed circRNAs.

3. Discussion

Through high-throughput sequencing of the hypothalamus RNA in Chuanzhong black goats with low- and high-reproductive capacities, 43,295 circRNAs were identified, including 205 differentially expressed circRNAs. GO and KEGG enrichment analyses of the host genes associated with these differentially expressed circRNAs revealed significant enrichment in processes related to gonadal development, hormone secretion, steroid hormone-mediated signaling pathways, GnRH signaling pathway, oxytocin signaling pathway, and progesterone-mediated oocyte maturation. CircRNAs are a distinct class of non-coding RNA characterized by their covalently closed loop structure, which is formed through back-splicing of precursor mRNA [22]. CircRNAs exhibit high stability and are evolutionarily conserved across species because of their circular nature [23,24]. They demonstrate tissue-specific expression patterns and perform unique cellular functions [25], suggesting their potential as biomarkers for assessing the reproductive capacity of goats.

In this study, GO enrichment analysis of the host genes of 205 differentially expressed circRNAs revealed enrichment of PPP3CA, UBE3A, FOXP1, PCSK5, BRAF, and RAP1B in GO terms associated with reproductive pathways. The protein phosphatase 3 catalytic subunit alpha (PPP3CA) has been closely linked to precocious puberty [26]. A whole-genome association analysis in Dazu black goats demonstrated that PPP3CA influences estrogen signaling and oocyte meiosis [27]. Furthermore, a 20 bp insertion-deletion polymorphism in the PPP3CA gene was significantly associated with litter size in Shaanbei cashmere goats [28,29]. The circRNA-mRNA regulatory network indicates that chicirc_063269 originates from PPP3CA. Prader-Willi syndrome (PWS) is characterized by growth hormone deficiency and hypogonadism, possibly due to abnormalities in the hypothalamic-pituitary-gonadal (HPG) axis [30]. Loss of UBE3A may lead to PWS [31], as UBE3A has been shown to interact with the ubiquitin-conjugating enzyme UBC7 to enhance progesterone receptor transactivation and promote estrogen receptor degradation [32,33]. Therefore, UBE3A may play a role in regulating litter size in goats, as indicated by the circRNA-mRNA regulatory network with chicirc_097731 as its host gene. Single-cell sequencing results revealed enrichment of FOXP2 in the pituitary gonadotroph cluster. Its loss disrupts gonadal development and affects the regulation of *lhb* and *Fshb* expression [34]. FOXP1, FOXP2, and FOXP4 can form oligomeric complexes in the brains of zebra finches [35]. FOXP1 can influence overall neural function; thus, it may affect goat reproduction by regulating the expression of *lhb* and *Fshb*, as suggested by the circRNA-mRNA regulatory network with chicirc_017440 as its host gene. When preovulatory follicles cultured in vitro were treated with LH, an increase in the mRNA and protein levels of PCSK5 was observed. PCSK5, a subtype of PCSK5, is involved in gonadotropin regulation in the ovary and contributes significantly to ovulation by processing Pro-TGF β and matrix metalloproteinases [36]. PCSK5 has been shown to facilitate follicular development in rats [37]. Therefore, PCSK5 may play a role in goat reproduction, as indicated by the circRNA-mRNA regulatory network with chicirc_049641 as its host gene. BRAF mRNA transcripts are localized in the central nervous system, where BRAF plays a crucial role in the development of the hypothalamic-pituitary axis in both mice and humans [38]. CREB1 can bind to BRAF to increase its expression and regulate cell proliferation [39]. BRAF is also enriched in corpus luteum-mediated oocyte maturation. Hence, BRAF may regulate goat reproduction, as indicated by the circRNA-mRNA regulatory network with chicirc_008429 as its host gene. Immunohistochemistry of cells shows that GnRH can also increase the protein levels of RAP1B [40]. RAP1B, a novel gene rapidly induced by GnRH, is a candidate gene involved in regulating gonadotropin secretion in rats [41]. According to the circRNA-mRNA regulatory network, the host gene of chicirc_145057 is RAP1B.

KEGG enrichment analysis of the host genes associated with the 205 differentially expressed circRNAs found that KIF3A, PLD1, PPP3CA, and RYR2 were significantly enriched in the GnRH signaling pathway, oxytocin signaling pathway, and progesterone-mediated oocyte maturation signaling pathway. Cilia on GnRH neurons may play a regulatory role in kisspeptin signaling [42] and can be eliminated through the conditional disruption of

KIF3A [43]. Therefore, KIF3A may influence kisspeptin signaling by affecting cilia, as suggested by the circRNA-mRNA regulatory network, with *chicirc_030156* and *chicirc_176754* serving as host genes for KIF3A. PLD1 is known to regulate the secretion of LH and FSH in the GnRH signaling pathway [44]. Additionally, PLD1 can promote spindle assembly and migration by regulating autophagy in mouse oocytes during meiosis [45], indicating its involvement in GnRH secretion, which in turn affects LH and FSH secretion. The circRNA-mRNA regulatory network identifies *chicirc_193363* as the host gene for PLD1. RYR2 plays a critical role in regulating insulin secretion and maintaining glucose homeostasis [46]. High glucose concentrations can cause irreversible damage to GnRH neurons in vitro, potentially leading to dysfunction in GnRH secretion [47]. Consequently, RYR2 may regulate GnRH secretion by modulating hypothalamic glucose concentrations [48]. This finding is supported by the circRNA-mRNA regulatory network, which identifies *chicirc_030156* and *chicirc_109497* as host genes for RYR2. We speculate that circRNAs play significant biological roles in regulating goat reproduction.

Through their interaction with miRNA response elements, circRNAs can indirectly enhance the transcription of their target mRNAs, thereby functioning as molecular sponges for miRNAs [49]. Notably, miR-135a, miR-188-3p, miR-101-3p, and miR-128-3p have the potential to interact with circRNAs that are produced in various ways. FSH and LH regulate the expression of the NPPC gene during oocyte meiosis by altering the protein levels of TGBR2, TGBR1, and SMAD in ovarian granulosa cells [50]. Decreased levels of the NPPC gene lead to a reduction in the synthesis of the downstream protein CNP, which, in turn, inhibits the generation of cGMP in cumulus cells. This inhibition consequently alleviates the suppressive effects on cAMP in the oocyte and triggers the resumption of meiosis [51]. Therefore, circular RNAs such as *chicirc_091702*, *chicirc_185982*, and *chicirc_145345* may regulate the expression of miR-135a, which subsequently affects the production of FSH and LH and ultimately influences the meiotic process. The adsorption of miR-188-3p by circular RNAs in dental pulp stem cells leads to a decrease in Beclin-1-induced autophagy and an increase in RUNX1 expression [52,53]. The RUNX1 protein is essential for ovarian function and ovulation in rats [54]. Thus, circRNAs *chicirc_091702*, *chicirc_185982*, and *chicirc_116507* may regulate goat fertility by modulating RUNX1 expression through the regulation of miR-188-3p. MicroRNA-101-3p controls the production of ZEB1, which stimulates the process of epithelial-mesenchymal transition [55]. By modulating GnRH promoter activity in the hypothalamus, ZEB1 can influence the estrous cycle in adult mice [56]. Hence, *chicirc_091702* may impact the reproductive capacity of Chuanzhong black goats by regulating ZEB1 through the adsorption of miR-101-3p. MicroRNA-128-3p is crucial for the formation and functioning of the central nervous system [57]. Research has shown that it can affect the progression of glioma by regulating the signaling pathways of GREM1 and BMP [58]. Moreover, the expression of GREM1 is associated with fertility [59]; infertile individuals exhibit lower levels of GREM1 expression, while increased GREM1 expression correlates with elevated FSH production [60]. Thus, circular RNAs such as *chicirc_185982*, *chicirc_123158*, and *chicirc_091702* may influence the reproductive capacity of Chuanzhong black goats by regulating miR-128-3p and the coordination between the brain, pituitary, and gonadal systems.

4. Materials and Methods

4.1. Test Animals and Sample Collection

Eleven healthy female Chuanzhong black goats, aged between 3.5 and 4.5 years, were maintained under consistent management conditions and had each given birth to three or more litters. The goats were divided into two groups: a high-prolificacy group ($n = 6$) and a low-prolificacy group ($n = 5$). The high-prolificacy group comprised goats that produced two or more offspring per litter, while the low-prolificacy group included goats that produced only one offspring per litter (Table 2). Following synchronized estrus, the 11 selected Chuanzhong black goats were slaughtered. The hypothalamus was collected

and stored in cryogenic vials, rapidly frozen in liquid nitrogen, and subsequently stored long term at -80°C .

Table 2. Chuanzhong black goat lambing record.

Serial Number	Date of First Delivery	Number of Lambs in the First Litter	Date of Second Delivery	Number of Lambs in the Second Litter	Date of Third Delivery	Number of Lambs in the Third Litter	Reproductive Condition
CZ_L1	10 November 2016	1	17 July 2017	1	12 April 2018	1	low fecundity
CZ_L2	14 November 2016	1	16 July 2017	1	20 April 2018	1	low fecundity
CZ_L3	28 November 2016	1	9 August 2017	1	11 May 2018	1	low fecundity
CZ_L4	1 October 2016	1	16 September 2017	1	27 April 2018	1	low fecundity
CZ_L5	19 December 2016	1	7 September 2017	1	2 June 2018	1	low fecundity
CZ_H1	3 January 2017	2	31 August 2017	3	22 March 2018	2	high fecundity
CZ_H2	5 September 2016	2	24 February 2017	2	21 December 2017	3	high fecundity
CZ_H3	16 July 2016	2	14 February 2017	2	15 January 2018	2	high fecundity
CZ_H4	11 November 2016	3	28 July 2017	2	22 February 2018	2	high fecundity
CZ_H5	20 November 2016	2	30 June 2017	2	10 February 2018	3	high fecundity
CZ_H6	18 November 2016	2	24 July 2017	3	19 February 2018	2	high fecundity

4.2. RNA Extraction, cDNA Library Preparation, and Sequencing

According to the manufacturer's instructions, total RNA was extracted from the samples using TRIzol reagent (Thermo Fisher, Shanghai, China). Ribosomal RNA was subsequently removed from the total RNA using the Ribo-Zero rRNA Removal Kit (Illumina, Inc., San Diego, CA, USA). The integrity and quantity of the RNA were assessed using the Agilent 2100 Bioanalyzer with the RNA 6000 Nano LabChip Kit (Agilent, Santa Clara, Santa Clara, CA, USA). Gel electrophoresis with 1% agarose was performed to confirm RNA integrity and the absence of genomic DNA contamination. Samples that met the quality criteria were sent to PacBio Bioinformatics (Shanghai, China) for sequencing on the Illumina HiSeq 2500 platform. For library construction, 1 μL of total RNA was utilized, and the RNA was fragmented into 200–300 bp segments. The first-strand cDNA was synthesized using random hexamers and reverse transcriptase, followed by synthesizing the second-strand cDNA. The NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA) was employed for 150 bp paired-end sequencing in accordance with the manufacturer's instructions.

4.3. Quality Assessment of Original Sequencing Data and Assembly of Transcripts

After the raw image data from the HiSeq platform were converted into FASTQ format, Cutadapt (version 1.16) was employed to control the quality of the sequence data, which involved removing adapters and low-quality reads. The filtered clean reads were then aligned to the *Capra hircus* reference genome (Genebuild by Ensembl, Genome: ARS1) using TopHat2. (version 2.1.1) Subsequently, the alignment files (in BAM format) were processed with StringTie software (version 1.3.3) to map reads to the genome and quantify transcript expression levels for each sample, as well as circRNA expression in terms of TPM (transcripts per million).

4.4. Screening of circRNA

To filter out lowly expressed single-exon transcripts from the transcript assembly results, we selected transcripts that contained at least two exons and had a length of ≥ 200 bp. Using Cuffcompare software (version 2.2.1), we identified transcripts that overlapped with annotated exonic regions in the database. After aligning the data to the reference genome, we identified circRNAs from the unmapped reads. The anchored sequences of each sample were aligned to the reference genome, and the alignment results of all samples were merged to identify circRNAs using the find_circ tool. Subsequently, we filtered highly reliable circRNAs on the basis of the following criteria: (1) retaining circRNAs with only one clear breakpoint; (2) ensuring that the overlap of the aligned positions on the genome for the anchored sequences at both ends of each read does not exceed 2 bp; (3) allowing only

2 bp mismatches; (4) ensuring that the number of unique reads is greater than two and is supported by more than half of the samples; (5) ensuring that the alignment score of at least one of the anchored sequences of each read to the genome is higher than 35; and (6) ensuring that the length of circRNA is less than 100 kb. The expression level of circRNAs was estimated in terms of TPM.

4.5. Differential Expression Analysis and Enrichment Analysis

Using the DESeq package in the R programming language (version 1.22.2), we conducted a differential expression analysis on the gene expression levels of the two groups. Genes with an absolute log₂ fold change greater than and a significant *p*-value of less than 0.05 were identified as differentially expressed genes. The TopGO package (version 2.38.1) was utilized for GO enrichment analysis, employing the hypergeometric distribution method to identify significantly enriched GO terms (with a significance threshold set at *p* < 0.05). Additionally, KEGG pathway enrichment analysis was performed using the ClusterProfiler software (version 2.8.1), with significant enrichment defined as *p* < 0.05. On the basis of the results of the GO and KEGG enrichment analyses and their biological significance, target genes were selected for further investigation.

4.6. Prediction of miRNAs Targeted by circRNAs

CircRNA can adsorb miRNA, thereby inhibiting its function. The target genes of miRNAs were predicted using the miRanda (version 3.3a) and TargetScan software (version 7.2). The intersection of the predictions from both software was utilized to minimize false positive results during the prediction process. The thresholds for screening candidate miRNAs were established as TargetScan_Score ≥ 50 and miRanda_Energy < −10.

4.7. Prediction of circRNA Translation Potential

The ORF finder was used to identify the presence of ORFs (ORF finder; <https://www.ncbi.nlm.nih.gov/orffinder/>, accessed on 15 April 2024), SRAMP was used to predict the presence of m6A modification sites (<http://www.cuilab.cn/sramp/>, accessed on 15 April 2024), and the IRES finder was used to predict the presence of IRES (IRES finder; <https://github.com/xiaofengsong/IRESfinder>, accessed on 15 April 2024).

4.8. Real-Time Quantitative PCR Verification and DNA Sequencing Validation of RNA-Seq

According to the sequencing results, six circRNAs were selected for expression validation. GAPDH was utilized as the internal reference gene for circRNA analysis. Reverse transcription was conducted using the Takara reverse transcription kit (Takara, Kusatsu, Shiga, Japan), and gene expression levels were quantified using the 2× Ultra SYBR Green qPCR Mix (Life ABI, Astin, USA) fluorescence quantitative reagent kit. The RT-qPCR cycling parameters were pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 5 s, and annealing/extension at 60 °C for 20 s. Each experiment included three biological replicates. The relative expression levels of the target genes were analyzed using the 2^{−ΔΔCt} method. Sanger sequencing was performed on the qPCR products to verify the nucleotide sequence of the circRNA circularization splice site. The primer sequences are listed in Table S6.

5. Conclusions

Through high-throughput sequencing technology, we investigated the circRNA profiles of the hypothalamus in Chuanzhong black goats with varying reproductive capabilities. Our differential expression analysis revealed 205 significantly differentially expressed circRNAs. Subsequent GO and KEGG enrichment analyses of the host genes associated with these differentially expressed circRNAs, along with the construction of circRNA-miRNA regulatory networks, led to the identification of several key molecules: chicirc_063269, chicirc_097731, chicirc_017440, chicirc_049641, chicirc_008429, chicirc_145057, chicirc_030156, chicirc_109497, chicirc_030156, chicirc_176754, chicirc_193363, miR-135a, miR-188-3p, miR-

101-3p, and miR-128-3p. These molecules may influence GnRH secretion and the HPG axis, thereby influencing the reproductive capabilities of Chuanzhong black goats. These findings provide a valuable reference for studying the molecular mechanisms that regulate reproduction in Chuanzhong black goats and establish a theoretical foundation for enhancing their prolificacy.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms251910479/s1>.

Author Contributions: Sample collection: S.M. and C.W. Data curation and writing—original draft: S.M. Methodology: G.F. and C.W. Project administration: G.L. and D.L. Software: G.L. and B.S. Supervision: Y.L. and Y.G. Validation: M.D. and G.F. Writing review and editing: G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Science and Technology Program from Forestry Administration of Guangdong Province (2023KJCX004) and Guangdong Provincial Department of Agriculture and Rural Affairs Project (2022-XPY-00-009).

Institutional Review Board Statement: The animal studies were approved by Ethics Committees of the Laboratory Animal Center of South China Agricultural University (permit number: SYXK-2014-0136). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA016713) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>, accessed on 27 May 2024.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Paim, T.D.; Faria, D.A.; Hay, E.H.; McManus, C.; Lanari, M.R.; Esquivel, L.C.; Cascante, M.I.; Alfaro, E.J.; Mendez, A.; Faco, O.; et al. New World Goat Populations Are a Genetically Diverse Reservoir for Future Use. *Sci. Rep.* **2019**, *9*, 1476. [CrossRef] [PubMed]
2. Yao, Z.; Zhang, S.; Wang, X.; Guo, Y.; Xin, X.; Zhang, Z.; Xu, Z.; Wang, E.; Jiang, Y.; Huang, Y. Genetic Diversity and Signatures of Selection in BoHuai Goat Revealed by Whole-Genome Sequencing. *BMC Genom.* **2023**, *24*, 116. [CrossRef]
3. Li, L.; Zhang, J.; Zhu, J.; Gu, S.; Sun, Q.; Zhou, G.; Fu, C.; Li, Q.; Chen, L.; Li, D.; et al. Genetic Diversity of Nine Populations of the Black Goat (*Capra Hircus*) in Sichuan, PR China. *Zool. Sci.* **2006**, *23*, 229–234. [CrossRef]
4. Kang, Z.; Bai, Y.; Lan, X.; Zhao, H. Goat AKAP12: Indel Mutation Detection, Association Analysis With Litter Size and Alternative Splicing Variant Expression. *Front. Genet.* **2021**, *12*, 648256. [CrossRef]
5. Wang, Z.; Pan, Y.; He, L.; Song, X.; Chen, H.; Pan, C.; Qu, L.; Zhu, H.; Lan, X. Multiple Morphological Abnormalities of the Sperm Flagella (MMAF)-Associated Genes: The Relationships between Genetic Variation and Litter Size in Goats. *Gene* **2020**, *753*, 144778. [CrossRef] [PubMed]
6. Zhao, Z.Q.; Wang, L.J.; Sun, X.W.; Zhang, J.J.; Zhao, Y.J.; Na, R.S.; Zhang, J.H. Transcriptome Analysis of the *Capra Hircus* Ovary. *PLoS ONE* **2015**, *10*, e0121586. [CrossRef] [PubMed]
7. Duffy, D.M.; Ko, C.; Jo, M.; Brannstrom, M.; Curry, T.E. Ovulation: Parallels With Inflammatory Processes. *Endocr. Rev.* **2019**, *40*, 369–416. [CrossRef]
8. Saleh, M.; Holtz, W. LH Pattern and Ovarian Response in Ovsynch-Synchronized Superovulated Goats Induced to Ovulate with GnRH or hCG. *Theriogenology* **2022**, *185*, 61–69. [CrossRef]
9. Chen, L.; Wang, C.; Sun, H.; Wang, J.; Liang, Y.; Wang, Y.; Wong, G. The Bioinformatics Toolbox for circRNA Discovery and Analysis. *Brief. Bioinform.* **2020**, *22*, 1706–1728. [CrossRef]
10. Li, X.; Yang, L.; Chen, L.-L. The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol. Cell* **2018**, *71*, 428–442. [CrossRef]
11. Zhou, W.-Y.; Cai, Z.-R.; Liu, J.; Wang, D.-S.; Ju, H.-Q.; Xu, R.-H. Circular RNA: Metabolism, Functions and Interactions with Proteins. *Mol. Cancer* **2020**, *19*, 172. [CrossRef] [PubMed]

12. Conn, V.M.; Hugouvieux, V.; Nayak, A.; Conos, S.A.; Capovilla, G.; Cildir, G.; Jourdain, A.; Tergaonkar, V.; Schmid, M.; Zubieta, C.; et al. A circRNA from SEPALLATA3 Regulates Splicing of Its Cognate mRNA through R-Loop Formation. *Nat. Plants* **2017**, *3*, 17053. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Xu, X.; Zhang, J.; Tian, Y.; Gao, Y.; Dong, X.; Chen, W.; Yuan, X.; Yin, W.; Xu, J.; Chen, K.; et al. CircRNA Inhibits DNA Damage Repair by Interacting with Host Gene. *Mol. Cancer* **2020**, *19*, 128. [\[CrossRef\]](#)
14. Li, Z.; Huang, C.; Bao, C.; Chen, L.; Lin, M.; Wang, X.; Zhong, G.; Yu, B.; Hu, W.; Dai, L.; et al. Exon-Intron Circular RNAs Regulate Transcription in the Nucleus. *Nat. Struct. Mol. Biol.* **2015**, *22*, 256–264. [\[CrossRef\]](#)
15. Mohanapriya, R.; Akshaya, R.L.; Selvamurugan, N. A Regulatory Role of circRNA-miRNA-mRNA Network in Osteoblast Differentiation. *Biochimie* **2022**, *193*, 137–147. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Zang, J.; Lu, D.; Xu, A. The Interaction of circRNAs and RNA Binding Proteins: An Important Part of circRNA Maintenance and Function. *J. Neurosci. Res.* **2020**, *98*, 87–97. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Zhang, L.; Hou, C.; Chen, C.; Guo, Y.; Yuan, W.; Yin, D.; Liu, J.; Sun, Z. The Role of N6-Methyladenosine (m6A) Modification in the Regulation of circRNAs. *Mol. Cancer* **2020**, *19*, 105. [\[CrossRef\]](#)
18. Di Timoteo, G.; Dattilo, D.; Centrón-Broco, A.; Colantoni, A.; Guarnacci, M.; Rossi, E.; Incarnato, D.; Oliviero, S.; Fatica, A.; Morlando, M.; et al. Modulation of circRNA Metabolism by m6A Modification. *Cell Rep.* **2020**, *31*, 107641. [\[CrossRef\]](#)
19. Jiang, Z.; Zhou, X.; Li, R.; Michal, J.J.; Zhang, S.; Dodson, M.V.; Zhang, Z.; Harland, R.M. Whole Transcriptome Analysis with Sequencing: Methods, Challenges and Potential Solutions. *Cell. Mol. Life Sci.* **2015**, *72*, 3425–3439. [\[CrossRef\]](#)
20. Byrne, A.; Cole, C.; Volden, R.; Vollmers, C. Realizing the Potential of Full-Length Transcriptome Sequencing. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2019**, *374*, 20190097. [\[CrossRef\]](#)
21. Hou, B.; Mao, M.; Dong, S.; Deng, M.; Sun, B.; Guo, Y.; Li, Y.; Liu, D.; Liu, G. Transcriptome Analysis Reveals mRNAs and Long Non-Coding RNAs Associated with Fecundity in the Hypothalamus of High-and Low-Fecundity Goat. *Front. Vet. Sci.* **2023**, *10*, 1145594. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Xiong, J.; Zhang, H.; Wang, Y.; Cheng, Y.; Luo, J.; Chen, T.; Xi, Q.; Sun, J.; Zhang, Y. Rno_circ_0001004 Acts as a miR-709 Molecular Sponge to Regulate the Growth Hormone Synthesis and Cell Proliferation. *IJMS* **2022**, *23*, 1413. [\[CrossRef\]](#)
23. Yang, B.; Wang, Y.-W.; Zhang, K. Interactions between circRNA and Protein in Breast Cancer. *Gene* **2024**, *895*, 148019. [\[CrossRef\]](#)
24. Zhang, H.; Hu, B.; Xiong, J.; Chen, T.; Xi, Q.; Luo, J.; Jiang, Q.; Sun, J.; Zhang, Y. Genomewide Analysis of Circular RNA in Pituitaries of Normal and Heat-Stressed Sows. *BMC Genom.* **2019**, *20*, 1013. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Dong, J.; Zeng, Z.; Huang, Y.; Chen, C.; Cheng, Z.; Zhu, Q. Challenges and Opportunities for circRNA Identification and Delivery. *Crit. Rev. Biochem. Mol. Biol.* **2023**, *58*, 19–35. [\[CrossRef\]](#)
26. Dias, M.M.; Souza, F.R.P.; Takada, L.; Feitosa, F.L.B.; Costa, R.B.; Diaz, I.D.P.S.; Cardoso, D.F.; Tonussi, R.L.; Baldi, F.; Albuquerque, L.G.; et al. Study of Lipid Metabolism-Related Genes as Candidate Genes of Sexual Precocity in Nellore Cattle. *Genet. Mol. Res.* **2015**, *14*, 234–243. [\[CrossRef\]](#)
27. Zhao, Y.J.; Huang, Y.-F. Selection Signatures of Litter Size in Dazu Black Goats Based on a Whole Genome Sequencing Mixed Pools Strategy. *Mol. Biol. Rep.* **2019**, *46*, 5517–5523. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Bai, Y.; Li, J.; Zhu, H.; Liu, J.; Dong, S.; Li, L.; Qu, L.; Chen, H.; Song, X.; Lan, X. Deletion Mutation within the Goat PPP3CA Gene Identified by GWAS Significantly Affects Litter Size. *Reprod. Fertil. Dev.* **2021**, *33*, 476–483. [\[CrossRef\]](#)
29. Bai, Y.; Zhang, T.; Liu, N.; Wang, C.; Guo, Z.; Pan, C.; Zhu, H.; Lan, X. Investigation of Copy Number Variations (CNVs) of the Goat PPP3CA Gene and Their Effect on Litter Size and Semen Quality. *Animals* **2022**, *12*, 445. [\[CrossRef\]](#)
30. Pulix, M.; Plagge, A. Imprinted Genes and Hypothalamic Function. In *Developmental Neuroendocrinology*; Wray, S., Blackshaw, S., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 265–294. ISBN 978-3-030-40002-6.
31. Napolitano, L.; Barone, B.; Morra, S.; Celentano, G.; La Rocca, R.; Capece, M.; Morgera, V.; Turco, C.; Caputo, V.F.; Spena, G.; et al. Hypogonadism in Patients with Prader Willi Syndrome: A Narrative Review. *Int. J. Mol. Sci.* **2021**, *22*, 1993. [\[CrossRef\]](#)
32. Davies, W.; Lynn, P.M.Y.; Relkovic, D.; Wilkinson, L.S. Imprinted Genes and Neuroendocrine Function. *Front. Neuroendocrinol.* **2008**, *29*, 413–427. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Li, L.; Li, Z.; Howley, P.M.; Sacks, D.B. E6AP and Calmodulin Reciprocally Regulate Estrogen Receptor Stability. *J. Biol. Chem.* **2006**, *281*, 1978–1985. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Wang, M.; Luo, J.; Dai, L.; Feng, M.; Cao, X.; Zhang, J.; Wan, Y.; Yang, X.; Wang, Y. Foxp2 Deficiency Impairs Reproduction by Modulating the Hypothalamic–Pituitary–Gonadal Axis in Zebrafish. *Biol. Reprod.* **2024**, *110*, 908–923. [\[CrossRef\]](#)
35. Co, M.; Anderson, A.G.; Konopka, G. FOXP Transcription Factors in Vertebrate Brain Development, Function, and Disorders. *WIREs Dev. Biol.* **2020**, *9*, e375. [\[CrossRef\]](#)
36. Bae, J.-A.; Park, H.-J.; Seo, Y.-M.; Roh, J.; Hsueh, A.J.W.; Chun, S.-Y. Hormonal Regulation of Proprotein Convertase Subtilisin/Kexin Type 5 Expression during Ovarian Follicle Development in the Rat. *Mol. Cell. Endocrinol.* **2008**, *289*, 29–37. [\[CrossRef\]](#)
37. Chen, S.-Y.; Schenkel, F.S.; Melo, A.L.P.; Oliveira, H.R.; Pedrosa, V.B.; Araujo, A.C.; Melka, M.G.; Brito, L.F. Identifying Pleiotropic Variants and Candidate Genes for Fertility and Reproduction Traits in Holstein Cattle via Association Studies Based on Imputed Whole-Genome Sequence Genotypes. *BMC Genom.* **2022**, *23*, 331. [\[CrossRef\]](#)
38. Munkley, J.; Lafferty, N.P.; Kalna, G.; Robson, C.N.; Leung, H.Y.; Rajan, P.; Elliott, D.J. Androgen-Regulation of the Protein Tyrosine Phosphatase PTPRR Activates ERK1/2 Signalling in Prostate Cancer Cells. *BMC Cancer* **2015**, *15*, 9. [\[CrossRef\]](#) [\[PubMed\]](#)

39. Xia, Q.; Li, Q.; Gan, S.; Guo, X.; Zhang, X.; Zhang, J.; Chu, M. Exploring the Roles of Fecundity-Related Long Non-Coding RNAs and mRNAs in the Adrenal Glands of Small-Tailed Han Sheep. *BMC Genet.* **2020**, *21*, 39. [\[CrossRef\]](#)
40. Yuen, T.; Choi, S.G.; Pincas, H.; Waring, D.W.; Sealfon, S.C.; Turgeon, J.L. Optimized Amplification and Single-Cell Analysis Identify GnRH-Mediated Activation of Rap1b in Primary Rat Gonadotropes. *Mol. Cell Endocrinol.* **2012**, *350*, 10–19. [\[CrossRef\]](#)
41. Hosseinzadeh, S.; Masoudi, A.A. Investigating the Expression of Fertility-Regulating LncRNAs in Multiparous and Uniparous Shal Ewe's Ovaries. *Genome* **2024**, *67*, 78–89. [\[CrossRef\]](#)
42. Stubbs, T.; Mykityn, K. Primary Cilia and Gonadotropin-Releasing Hormone Neurons. *Curr. Opin. Endocr. Metab. Res.* **2024**, *34*, 100504. [\[CrossRef\]](#)
43. Fan, R.; Lai, K.-O. Understanding How Kinesin Motor Proteins Regulate Postsynaptic Function in Neuron. *FEBS J.* **2022**, *289*, 2128–2144. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Li, J.; Shi, B.; Wang, C.; Shao, C.; Liu, X.; Zhang, D. Comprehensive CircRNA Profiling and Selection of Key CircRNAs Reveal the Potential Regulatory Roles of CircRNAs throughout Ovarian Development and Maturation in Cynoglossus Semilaevis. *Biology* **2021**, *10*, 830. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Zhang, J.; Tian, Y.; Xu, X.; Wang, B.; Huang, Z.; Song, K.; Lou, S.; Kang, J.; Zhang, N.; Li, J.; et al. PLD1 Promotes Spindle Assembly and Migration through Regulating Autophagy in Mouse Oocyte Meiosis. *Autophagy* **2024**, *20*, 1616–1638. [\[CrossRef\]](#)
46. Santulli, G.; Pagano, G.; Sardu, C.; Xie, W.; Reiken, S.; D'Ascia, S.L.; Cannone, M.; Marziliano, N.; Trimarco, B.; Guise, T.A.; et al. Calcium Release Channel RyR2 Regulates Insulin Release and Glucose Homeostasis. *J. Clin. Investig.* **2015**, *125*, 1968–1978. [\[CrossRef\]](#)
47. Pal, L.; Chu, H.-P.; Shu, J.; Topalli, I.; Santoro, N.; Karkanias, G. In Vitro Evidence of Glucose-Induced Toxicity in GnRH Secreting Neurons: High Glucose Concentrations Influence GnRH Secretion, Impair Cell Viability, and Induce Apoptosis in the GT1-1 Neuronal Cell Line. *Fertil. Steril.* **2007**, *88*, 1143–1149. [\[CrossRef\]](#)
48. Dong, S.; Hou, B.; Yang, C.; Li, Y.; Sun, B.; Guo, Y.; Deng, M.; Liu, D.; Liu, G. Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats. *Genes* **2023**, *14*, 444. [\[CrossRef\]](#)
49. Tong, K.-L.; Tan, K.-E.; Lim, Y.-Y.; Tien, X.-Y.; Wong, P.-F. CircRNA-miRNA Interactions in Atherogenesis. *Mol. Cell Biochem.* **2022**, *477*, 2703–2733. [\[CrossRef\]](#)
50. Wang, L.; Chen, Y.; Wu, S.; Tang, J.; Chen, G.; Li, F. miR-135a Suppresses Granulosa Cell Growth by Targeting Tgfb1 and Cnd2 during Folliculogenesis in Mice. *Cells* **2021**, *10*, 2104. [\[CrossRef\]](#)
51. Yang, J.; Zhang, Y.; Xu, X.; Li, J.; Yuan, F.; Bo, S.; Qiao, J.; Xia, G.; Su, Y.; Zhang, M. Transforming Growth Factor- β Is Involved in Maintaining Oocyte Meiotic Arrest by Promoting Natriuretic Peptide Type C Expression in Mouse Granulosa Cells. *Cell Death Dis.* **2019**, *10*, 558. [\[CrossRef\]](#)
52. Wang, M.; Wu, J.; Wu, P.; Li, Y. Emerging Roles of Circular RNAs in Stem Cells. *Genes. Dis.* **2023**, *10*, 1920–1936. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Ji, F.; Zhu, L.; Pan, J.; Shen, Z.; Yang, Z.; Wang, J.; Bai, X.; Lin, Y.; Tao, J. Hsa_circ_0026827 Promotes Osteoblast Differentiation of Human Dental Pulp Stem Cells Through the Beclin1 and RUNX1 Signaling Pathways by Sponging miR-188-3p. *Front. Cell Dev. Biol.* **2020**, *8*, 470. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Liu, J.; Park, E.-S.; Jo, M. Runt-Related Transcription Factor 1 Regulates Luteinized Hormone-Induced Prostaglandin-Endoperoxide Synthase 2 Expression in Rat Perioovulatory Granulosa Cells. *Endocrinology* **2009**, *150*, 3291–3300. [\[CrossRef\]](#)
55. Liang, H.; Yu, T.; Han, Y.; Jiang, H.; Wang, C.; You, T.; Zhao, X.; Shan, H.; Yang, R.; Yang, L.; et al. LncRNA PTAR Promotes EMT and Invasion-Metastasis in Serous Ovarian Cancer by Competitively Binding miR-101-3p to Regulate ZEB1 Expression. *Mol. Cancer* **2018**, *17*, 119. [\[CrossRef\]](#)
56. Messina, A.; Prevot, V. Hypothalamic microRNAs Flip the Switch for Fertility. *Oncotarget* **2017**, *8*, 8993–8994. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Kiel, K.; Król, S.K.; Bronisz, A.; Godlewski, J. MiR-128-3p—A Gray Eminence of the Human Central Nervous System. *Mol. Ther. Nucleic Acids* **2024**, *35*, 102141. [\[CrossRef\]](#)
58. Fu, C.; Li, D.; Zhang, X.; Liu, N.; Chi, G.; Jin, X. LncRNA PVT1 Facilitates Tumorigenesis and Progression of Glioma via Regulation of MiR-128-3p/GREM1 Axis and BMP Signaling Pathway. *Neurotherapeutics* **2018**, *15*, 1139–1157. [\[CrossRef\]](#)
59. López Navarro, E.; Ortega, F.J.; Francisco-Busquets, E.; Sabater-Masdeu, M.; Álvarez-Castaño, E.; Ricart, W.; Fernández-Real, J.M. Thyroid Hormone Receptors Are Differentially Expressed in Granulosa and Cervical Cells of Infertile Women. *Thyroid* **2016**, *26*, 466–473. [\[CrossRef\]](#)
60. Schang, G.; Ongaro, L.; Brûlé, E.; Zhou, X.; Wang, Y.; Boehm, U.; Ruf-Zamojski, F.; Zamojski, M.; Mendelev, N.; Seenarine, N.; et al. Transcription Factor GATA2 May Potentiate Follicle-Stimulating Hormone Production in Mice via Induction of the BMP Antagonist Gremlin in Gonadotrope Cells. *J. Biol. Chem.* **2022**, *298*, 102072. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.



Article

miR-128-3p Regulates Follicular Granulosa Cell Proliferation and Apoptosis by Targeting the Growth Hormone Secretagogue Receptor

Shucan Dong, Shengwei Jiang, Biwei Hou, Yaokun Li, Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu and Guangbin Liu





Article

miR-128-3p Regulates Follicular Granulosa Cell Proliferation and Apoptosis by Targeting the Growth Hormone Secretagogue Receptor

Shucan Dong, Shengwei Jiang, Biwei Hou, Yaokun Li , Baoli Sun, Yongqing Guo , Ming Deng, Dewu Liu * and Guangbin Liu *

Guangdong Laboratory for Lingnan Modern Agriculture, College of Animal Science, South China Agricultural University, Guangzhou 510642, China; scdong@stu.scau.edu.cn (S.D.); jsw1678693227@126.com (S.J.); 20213139038@stu.scau.edu.cn (B.H.); ykli@scau.edu.cn (Y.L.); baolisun@scau.edu.cn (B.S.); yongqing@scau.edu.cn (Y.G.); dengming@scau.edu.cn (M.D.)

* Correspondence: dwliu@scau.edu.cn (D.L.); gbliu@scau.edu.cn (G.L.)

Abstract: The proliferation and apoptosis of granulosa cells (GCs) affect follicle development and reproductive disorders, with microRNAs playing a crucial regulatory role. Previous studies have shown the differential expression of miR-128-3p at different stages of goat follicle development, which suggests its potential regulatory role in follicle development. In this study, through the Cell Counting Kit-8 assay, the EDU assay, flow cytometry, quantitative real-time polymerase chain reaction, Western blot, and the dual-luciferase reporter assay, we used immortal human ovarian granulosa tumor cell line (KGN) cells as materials to investigate the effects of miR-128-3p and its predicted target gene growth hormone secretagogue receptor (GHSR) on GC proliferation and apoptosis. The results show that overexpression of miR-128-3p inhibited the proliferation of KGN cells, promoted cell apoptosis, and suppressed the expression of proliferating cell nuclear antigen (PCNA) and B-cell lymphoma-2 (BCL2) while promoting that of Bcl-2 associated X protein (BAX). The dual-luciferase reporter assay revealed that miR-128-3p bound to the 3' untranslated region sequence of GHSR, which resulted in the inhibited expression of GHSR protein. Investigation of the effects of GHSR on GC proliferation and apoptosis revealed that GHSR overexpression promoted the expression of PCNA and BCL2, enhanced GC proliferation, and inhibited cell apoptosis, whereas the opposite effects were observed when GHSR expression was inhibited. In addition, miR-128-3p and GHSR can influence the expression of extracellular signal-regulated kinase 1/2 protein. In conclusion, miR-128-3p inhibits KGN cell proliferation and promotes cell apoptosis by downregulating the expression of the GHSR gene.

Keywords: miR-128-3p; granulosa cells; proliferation; apoptosis; follicular development



Citation: Dong, S.; Jiang, S.; Hou, B.; Li, Y.; Sun, B.; Guo, Y.; Deng, M.; Liu, D.; Liu, G. miR-128-3p Regulates Follicular Granulosa Cell Proliferation and Apoptosis by Targeting the Growth Hormone Secretagogue Receptor. *Int. J. Mol. Sci.* **2024**, *25*, 2720. <https://doi.org/10.3390/ijms25052720>

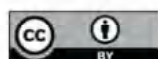
Academic Editor: Jan Tesarik

Received: 10 January 2024

Revised: 19 February 2024

Accepted: 23 February 2024

Published: 27 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Follicular development is related to animal reproduction and has piqued considerable research interest. Ovarian follicles comprise theca cells (TCs), granulosa cells (GCs), and oocytes and are widely considered a functional unit of the ovary for reproductive function [1]. The proliferation, apoptosis, and differentiation of GCs affect the fate of follicles [2,3]. GCs occupy the middle layer between TCs and oocytes, and they can influence the quality of oocytes by signaling and exchanging substances with them through gap junctions and cooperating with TCs to produce steroid hormones [4,5]. GCs can also influence the growth of surrounding follicles through autocrine and paracrine secretion [6]. Mammals possess large pools of primitive follicle reserves; however, the ovulation efficiency of follicles is inefficient; less than 1% of follicles can ovulate during follicle development, and the rest of the follicles remain in atresia [7]. Apoptosis of GCs is an important cause of

follicular atresia [2,8,9]. Apoptosis of GCs is regulated by various factors, including gene expression, endocrine hormones, and epigenetic modifications [10–12].

Past studies have extensively explored gene expression during follicle development and revealed important signaling pathways that regulate follicle development [13]. Non-coding RNAs, as important regulators of gene expression, show a close relation to the life activities of plants and animals [14]. However, exploration of the regulation of gene expression by these factors remains incomplete. MicroRNAs (miRNAs) have been widely recognized as regulatory factors of gene expression because they can modulate the stability and translation of messenger RNA (mRNA) and thereby influence gene protein expressions [15,16]. MiRNAs are essential for the reproduction of female animals [17]. They can regulate the synthesis of ovarian steroid hormones and participate in the proliferation, differentiation, and apoptosis of follicular cells [18–20]. miR-128-3p refers to a conserved RNA sequence that can regulate the proliferation, apoptosis, and disease occurrence among various tissues and cells [21–23]. miR-128-3p is expressed in ovarian follicles, with low and high expressions in small and large follicles, respectively [24]. Furthermore, compared with that in young women with normal ovarian reserves, the expression of miR-128-3p is considerably upregulated in young women with diminished ovarian reserves [25]. These findings suggest the possible important role of miR-128-3p in the process of follicular development.

Follicle development refers to a dynamic process involving the continuously changing expressions of many genes to regulate follicle growth. Previous transcriptomic studies have revealed that compared with that in small follicles, the mRNA expression level of the *growth hormone secretagogue receptor (GHSR)* gene is substantially decreased in large follicles [24]. This condition suggests that *GHSR* may play a regulatory role in the process of follicle development. The *GHSR* gene is widely expressed in various animal tissues and participates in the regulation of food intake, energy metabolism, cardiovascular function, cell proliferation, and reproduction [26–30]. Ghrelin is the endogenous ligand for *GHSR* and plays a role in the reproductive processes of animals [31]. The administration of ghrelin in vivo can cause changes in the morphology and structure of rat ovaries, which result in ovarian atrophy and induce cell apoptosis [32]. Ghrelin also increases phosphor extracellular signal-regulated kinase (ERK) 1/2 levels and cell proliferation in cultured GCs in a *GHSR1a*-dependent manner and increases the expressions of estradiol and aromatase activity in GCs [31,33]. These studies demonstrated the crucial role of *GHSR* in follicle development. Investigation of the regulation of *GHSR* expression is crucial for a comprehensive understanding of follicular development. However, there is currently no research reporting which genes regulate the expression of the *GHSR* gene during follicle development. miRNAs can regulate gene expression by targeting the 3' untranslated region (3'UTR) of mRNA. So, it is speculated that the expression of the *GHSR* gene is regulated by miRNA. Exploration of the miRNAs that regulate the *GHSR* gene will contribute to revealing its regulatory network and its role in follicular development.

Investigating the genes that influence granulosa cell proliferation and apoptosis is of significant importance in regulating follicular development and addressing follicular diseases. Therefore, this study focuses on exploring the relationship between miR-128-3p and the *GHSR* gene, as well as their expression's impact on granulosa cell proliferation and apoptosis, aiming to provide potential molecular targets for regulating follicular development.

2. Results

2.1. Prediction of miR-128-3p Target Genes and Functional Enrichment Analysis of Its Target Genes

RNA-sequencing of large and small follicles of Leizhou goat showed the differential expression of miR-128-3p in large and small follicles, with a significantly higher differential expression in large follicles than in small follicles [24]. Quantitative real-time polymerase chain reaction (qRT-PCR) results indicate that the expression patterns of miR-128-3p and *GHSR* gene in goat ovarian follicles are consistent with the sequencing findings reported

by Feng et al. (Figure 1A). Using the Miranda database, we predicted 1191 target genes of miR-128-3p. The classic mechanism of miRNA suggests that miRNAs inhibit the stability or translation efficiency of target gene mRNAs by binding to their 3'UTR through seed sequence complementarity. On this basis, we performed Venn analysis on predicted target genes and considerably downregulated mRNAs in large follicles of Leizhou goats based on sequencing data. A total of 179 common genes were identified (Figure 1B). Functional enrichment analysis of these 179 genes was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) to explore the possible functions of miR-128-3p in follicular development. KEGG results show the significant enrichment of these target genes in the endocrine system, signal transduction pathways, the nervous system, and the digestive system. These genes particularly exhibit enrichment in the endocrine system and signal transduction pathways, including melanogenesis, insulin secretion, thyroid hormone synthesis, ovarian steroidogenesis, the cGMP-PKG signaling pathway, the WNT signaling pathway, the cAMP signaling pathway, etc. (Figure 1C). GO enrichment analysis revealed the most significant biological process, cellular component, and molecular function terms, namely, embryonic placenta development, the cell junction, and chloride channel activity, respectively. In addition, these target genes are significantly enriched in cell proliferation, cell migration, mitogen-activated protein kinase cascade, and ERK1 and ERK2 cascades (Figure 1D). Therefore, miR-128-3p possibly plays an important role in animal follicle development and may be involved in the regulation of cell proliferation and apoptosis.

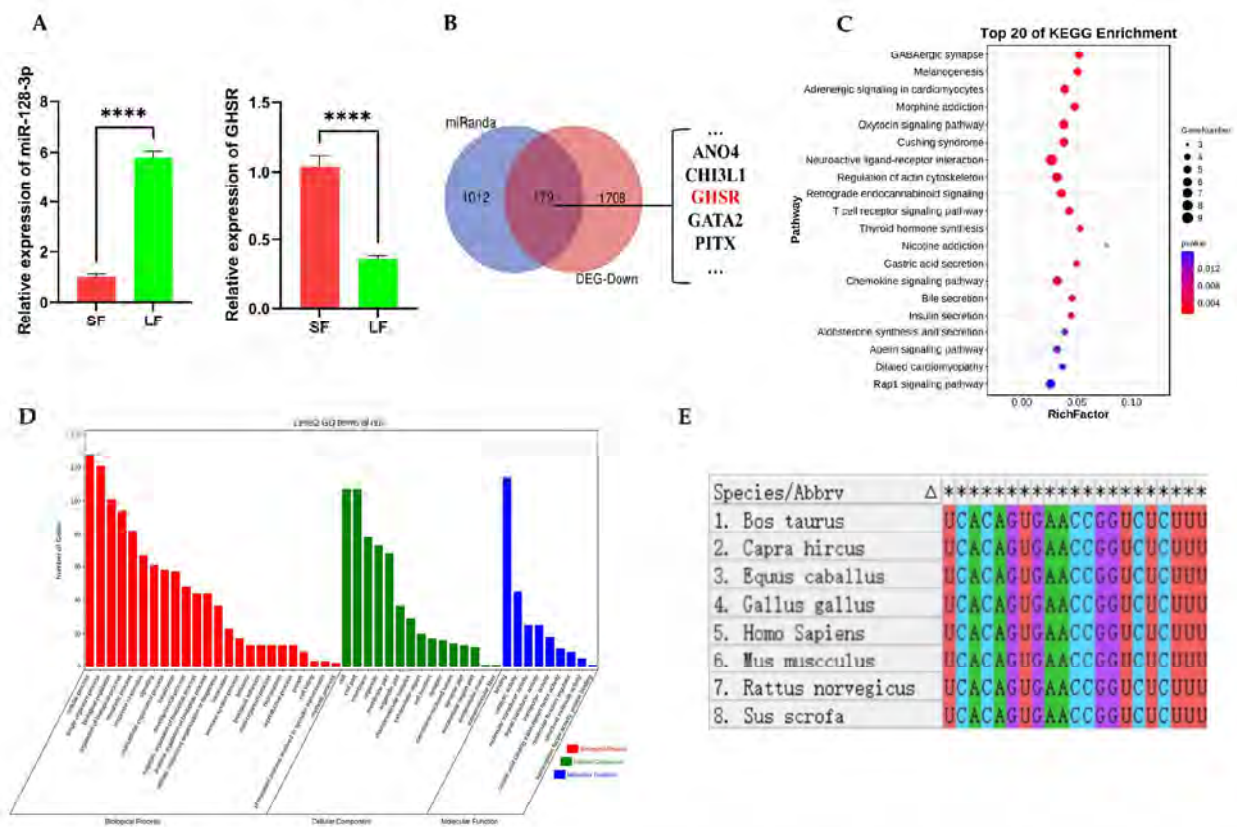


Figure 1. Functional enrichment analysis of predicted target genes for miR-128-3p. (A) Relative expression levels of miR-128-3p and GHSR genes in small and large follicles validated via qRT-PCR. (B) Venn diagram analysis of predicted target genes and downregulated differentially expressed genes in large follicles. (C) Enrichment analysis of KEGG pathways for predicted target genes. (D) Enrichment analysis of GO functions for predicted target genes. (E) Conservation analysis of miR-128-3p sequences in eight species, “*” represents the consistent ribonucleic acid (RNA) base sequence in the column. Data are presented as mean ± standard deviation (SD); **** $p < 0.0001$.

MEGA 7.0 software was used to conduct sequence similarity analysis of miR-128-3p sequences in eight common mammalian species, including humans, goats, pigs, horses, cows, chickens, mice, and rats. The results reveal that the miR-128-3p sequences are completely identical across these mammalian species (Figure 1E), which suggests a high level of conservation and possible conserved functions of miR-128-3p in mammals.

2.2. MiR-128-3p Is Involved in GC Apoptosis and Proliferation

To investigate the effect of miR-128-3p on the proliferation and apoptosis of GCs, we conducted transfection experiments using exogenous miR-128-3p mimics and inhibitors in immortal human ovarian granulosa tumor cell line (KGN) cells. The expression levels of miR-128-3p increased and decreased upon transfection with mimics and inhibitors, respectively (Figure 2A). In addition, Cell Counting Kit-8 (CCK8) analysis revealed the considerably reduced viability of KGN cells at 48 and 72 h post-transfection with miR-128-3p mimics. Conversely, the proliferation viability of cells substantially increased at 48 h following transfection with miR-128-3p inhibitors. However, no significant difference was observed at 24 and 72 h (Figure 2B). Similar to the CCK-8 results, the EDU assay indicated that the proliferation of KGN cells was inhibited by miR-128-3p at 48 h (Figure 2C). The effect of miR-128-3p on apoptosis was examined via flow cytometry. Flow cytometry results show that compared with that in the control group, the apoptosis rate was increased in the miR-128-3p overexpression group and decreased in the suppression group (Figure 2D). In addition, miR-128-3p mimics remarkably downregulated the expression level of the antiapoptotic *B-cell lymphoma-2* (*BCL2*) gene and upregulated the mRNA expression level of the proapoptotic *Bcl-2 associated X protein* (*BAX*) gene and *BAX/BCL2* ratio in KGN cells (Figure 2E). Western blot results revealed that overexpression of miR-128-3p greatly increased the protein level of BAX and suppressed the expression of proliferating cell nuclear antigen (PCNA) and BCL2 protein in KGN cells (Figure 2F). On the contrary, inhibition of miR-128-3p expression resulted in decreased protein expression of BAX and increased the protein level of PCNA in KGN cells, but that of BCL2 protein was unaffected (Figure 2G). In general, these results suggest that miR-128-3p may inhibit the proliferation of KGN cells and promote their apoptosis.

2.3. GHSR Is a Target Gene of miR-128-3p

Venn diagram analysis of target genes predicted by miRNA and differentially expressed genes in large and small follicles of Leizhou goat predicted GHSR and other genes as miR-128-3p target genes. RNAhybrid database analysis showed that the miR-128-3p seed sequence can bind to the 3'UTR region of GHSR (Figure 3A). To determine whether miR-128-3p targets the GHSR gene, we constructed luciferase reporter plasmids containing the GHSR-predicted binding site or mutation and cotransfected them with miR-128-3p mimics into 293Ts. Luciferase activity was measured 48 h after transfection. miR-128-3p significantly reduced the luciferase activity of the reporter containing the GHSR-predicted binding site fragment, and no change in the reporter activity was observed when using the GHSR-predicted binding site fragment mutant. These findings indicate that *GHSR* is a direct target of miR-128-3p (Figure 3B). Furthermore, miR-128-3p mimics significantly suppressed GHSR protein expression but did not affect changes in *GHSR* mRNA levels (Figure 3C,D). Overall, these results imply that miR-128-3p directly targets GHSR and inhibits its protein expression.

2.4. GHSR Is Involved in GC Apoptosis and Proliferation

To determine the function of GHSR in the proliferation and apoptosis of KGN cells, we overexpressed and knocked down GHSR in KGN cells using an overexpression vector and small interfering RNA (siRNA) and observed the remarkably increased and decreased transcript and protein levels of GHSR, respectively (Figure 4A,B). CCK8 and EDU assay showed that overexpression or knockdown of GHSR significantly promoted and inhibited cell proliferation, respectively (Figure 4C,D). Flow cytometric results show that overexpres-

sion of GHSR greatly inhibited KGN cell apoptosis, whereas the opposite was true for its inhibited expression (Figure 4E). At the mRNA level, GHSR overexpression significantly decreased *BAX* expression and increased that of *BCL2*. After inhibition of the expression of GHSR, the expression of the *BAX* gene increased, but the difference was nonsignificant. In addition, the expression level of *BCL2* showed a significant reduction (Figure 4F). Western blot results revealed that the overexpression of GHSR can increase the PCNA and BCL2 protein levels but did not affect the BAX protein expression. After inhibition of the expression of GHSR, BAX protein expression increased significantly, and the protein level of PCNA and BCL2 notably decreased (Figure 4G,H). All these results suggest that GHSR can promote GC proliferation and inhibit apoptosis.

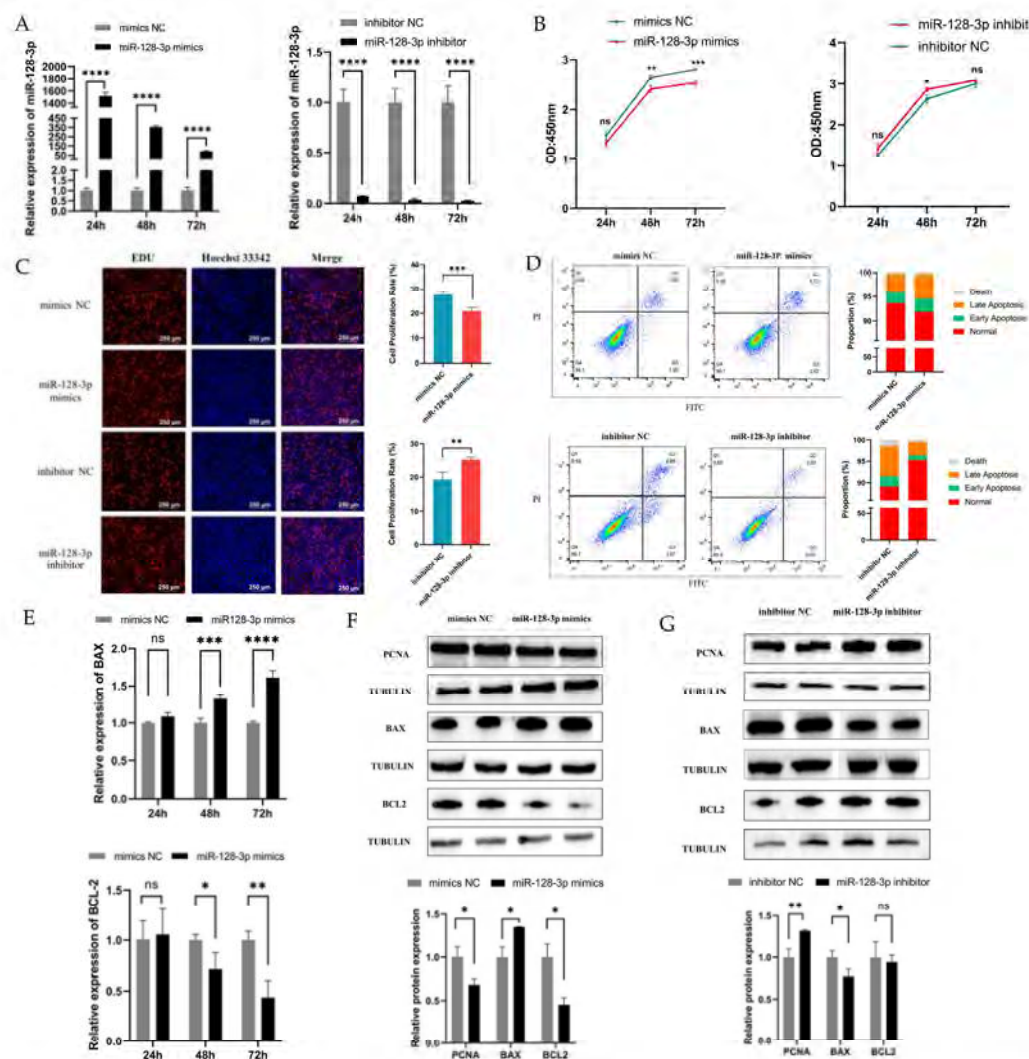


Figure 2. Regulation of GC proliferation and apoptosis by miR-128-3p. (A) Relative expression levels of miR-128-3p after transfection of miR-128-3p mimics and inhibitors into GCs detected by qRT-PCR. (B,C) Effect of miR-128-3p mimics and inhibitor on GC proliferation shown by CCK-8 and EDU assay. (D) Apoptosis rate of GCs measured via flow cytometry. (E) Relative mRNA expression levels of *BAX* and *BCL2* genes. (F,G) Effects of miR-128-3p on PCNA, *BAX*, and *BCL2* protein expressions in GCs via Western blot, respectively. Data are presented as mean \pm SD; ns, no significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

2.5. Cotransfection of GHSR and miR-128-3p Affects Apoptosis in GCs

To investigate the effect of the interaction between miR-128-3p and GHSR on the proliferation and apoptosis of KGN cells, we conducted transfection experiments using pcDNA3.1-GHSR or pcDNA3.1 plasmids in combination with miR-128-3p mimics. Our

findings demonstrate significantly higher cell proliferation in the cotransfected group with pcDNA3.1-GHSR and miR-128-3p than in the cotransfected group with pcDNA3.1 and miR-128-3p (Figure 5A). Notably, the overexpression of GHSR along with miR-128-3p transfection significantly reduced the mRNA expression level of *BAX* and the ratio of *BAX/BCL2*, but no difference was detected in the protein expression levels between *BAX* and *BCL2* (Figure 5B,C). These results suggest that the GHSR gene can attenuate the effects of miR-128-3p by promoting apoptosis and inhibiting the proliferation of GCs.

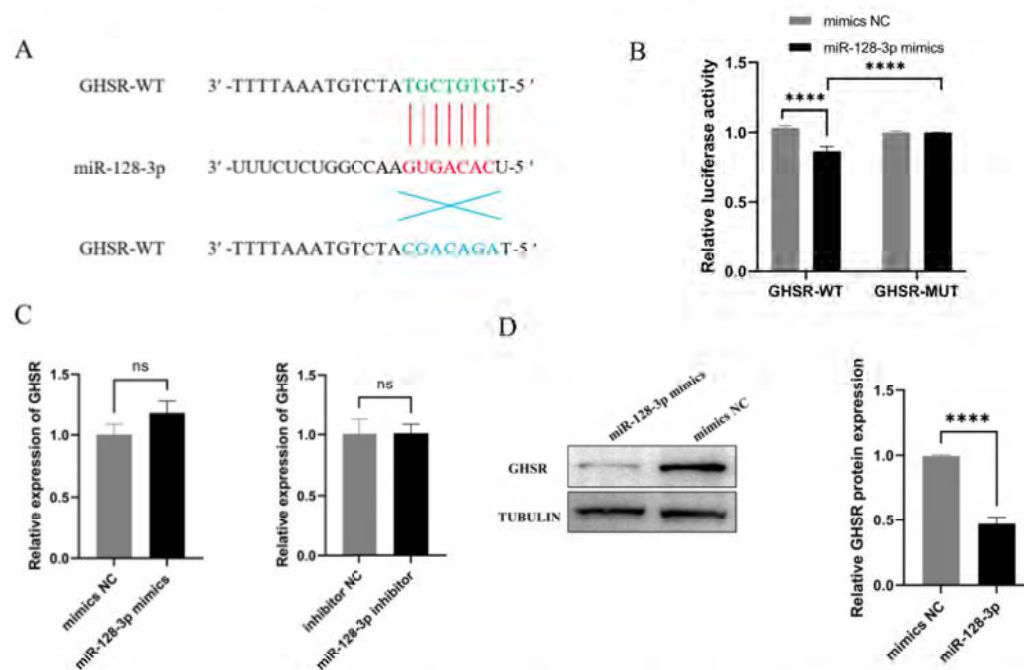


Figure 3. Analysis of the targeting relationship between miR-128-3p and its predicted target gene *GHSR*. (A) Schematic representation of the interaction between miR-128-3p and wild-type (green) and mutant (blue) 3'UTR regions of *GHSR*, with the miR-128-3p seed sequence highlighted in red. (B) Dual-luciferase reporter assay of the regulatory relationship between miR-128-3p and predicted binding sites on *GHSR*. (C) Effect of miR-128-3p mimics and inhibitor on *GHSR* gene mRNA levels. (D) Effects of miR-128-3p overexpression on *GHSR* protein expression. Data are presented as mean \pm SD; ns, no significance; **** $p < 0.0001$.

2.6. miR-128-3p and GHSR Regulate ERK1/2 Protein Expression

ERK1/2 refers to important intracellular signaling molecules that regulate various cellular physiological and pathological processes and play a crucial role in the regulation of cell proliferation and apoptosis. To verify the effects of miR-128-3p and GHSR on the ERK pathway, we first used Western blot analysis to examine the effects of miR-128-3p overexpression or inhibition on the protein expressions of ERK1/2 in KGN cells. The results show that compared with the mimics NC group, the overexpression of miR-128-3p significantly upregulated the protein expression levels of ERK1/2, whereas the miR-128-3p inhibitor showed no effect (Figures 6A and 6B, respectively). We then examined the effect of GHSR overexpression on the protein expression of ERK in KGN cells and observed that the protein expression levels of ERK1/2 decreased substantially, and the inhibition of GHSR expression had no effect on ERK1/2 protein expression (Figure 6A,B). These data suggest the possible involvement of miR-128-3p and GHSR in the regulation of the ERK pathway.

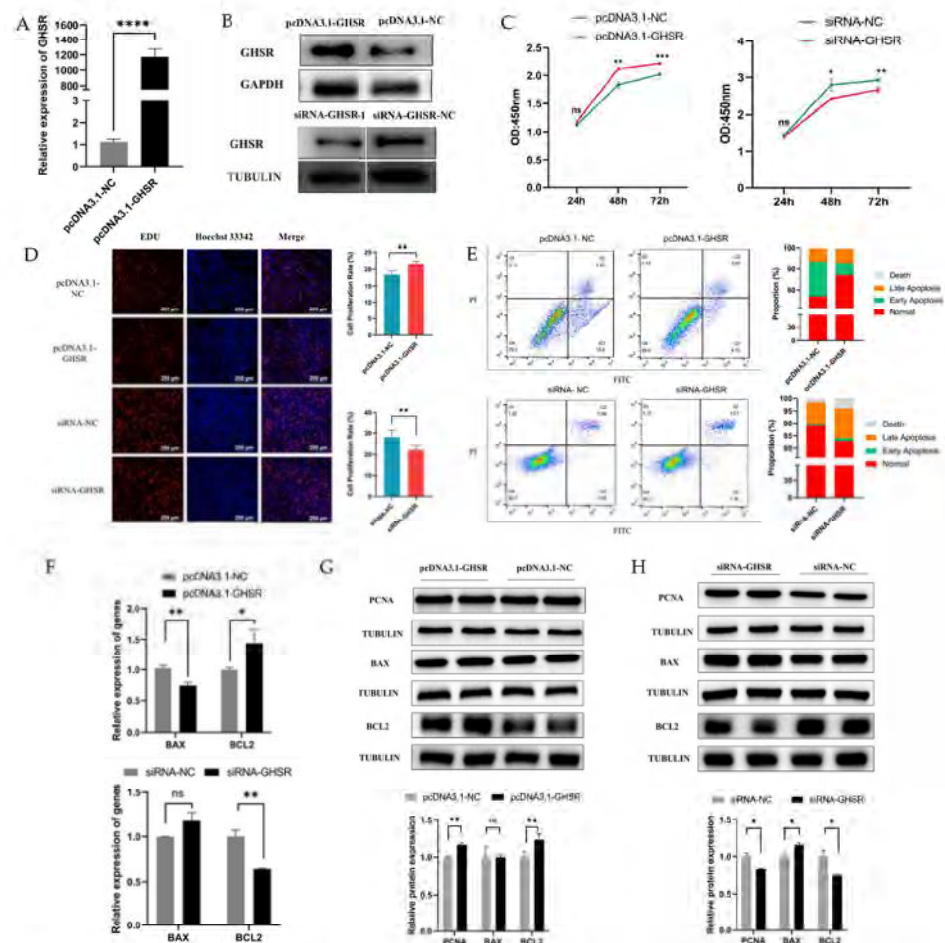


Figure 4. GHSR regulates GC proliferation and apoptosis. (A) Relative expression levels of *GHSR* after transfection into GCs using qRT-PCR. (B) Western blots of *GHSR* protein expression after overexpression and inhibition of *GHSR*. (C,D) GC proliferation after overexpression and inhibition of *GHSR* through CCK-8 and EDU assay. (E) Apoptosis rate of GCs measured via flow cytometry. (F) Relative mRNA expression levels of *BAX* and *BCL2* genes. (G,H) Effects of overexpression and inhibition of *GHSR* on *PCNA*, *BAX*, and *BCL2* protein expression in GCs determined by Western blot. Data are presented as mean \pm SD; ns, no significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

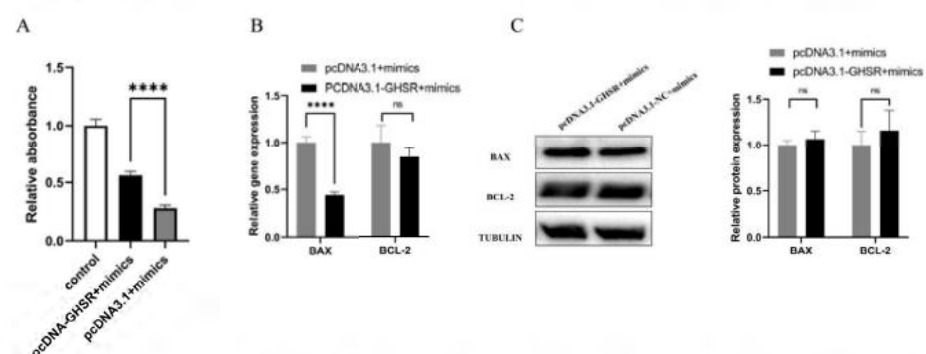


Figure 5. Analysis of the targeting relationship between miR-128-3p and its predicted target gene *GHSR*. (A) Compensatory expression of *GHSR* in KGN cells after the overexpression of miR-128-3p on cell proliferation vitality. (B) Influence of miR-128-3p overexpression and subsequent *GHSR* rescue on mRNA levels of *BAX* and *BCL2* genes in GCs. (C) Effects of miR-128-3p overexpression and subsequent *GHSR* rescue on the protein levels of *BAX* and *BCL2* in GCs. Data are presented as mean \pm SD; ns, no significance; **** $p < 0.0001$.

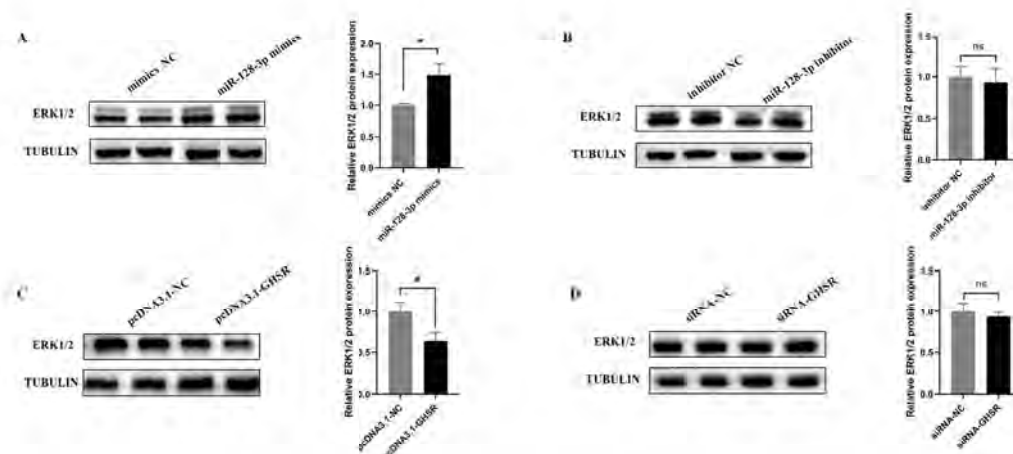


Figure 6. Effects of miR-128-3p and GHSR on ERK1/2 protein expression. (A) Influence of miR-128-3p mimics overexpression on ERK1/2 protein expression in GCs. (B) Influence of miR-128-3p inhibitor on ERK1/2 protein expression in GCs. (C) Effects of GHSR overexpression on ERK1/2 protein expression in GCs. (D) Influence of inhibiting GHSR expression on ERK1/2 protein expression in GCs. Data are presented as mean \pm SD; ns, no significance; * $p < 0.05$.

3. Discussion

Follicular development refers to a complex process in which genes in follicles constantly cause expression changes in response to follicular development. As regulators of gene expression, miRNAs play an important role in the development process. In this study, miR-128-3p inhibited the proliferation of GCs and promoted apoptosis, and the regulatory effect of GHSR on the proliferation and apoptosis of GCs was opposite that of miR-128-3p. We also observed that miR-128-3p can target the 3'UTR region of GHSR to inhibit its expression. In addition, miR-128-3p and GHSR regulated the ERK1/2 activity involved in follicular developmental regulation.

Ovaries can produce hormones that maintain the reproductive capacity of female animals. KEGG functional enrichment analysis of the predicted target gene of miR-128-3p revealed that the predicted target genes *cytochrome p450 family 11 subfamily a member 1* (*CYP11A1*), *adenylate cyclase 1* (*ADCY1*), and *adenylate cyclase 2* (*ADCY2*) of miR-128-3p can be enriched into the signaling pathway of ovarian steroid production. Production of steroid hormones, which mainly includes androgen, estrogen, progesterone, and so on, within the ovaries is essential for successful ovulation [34]. These hormones are synthesized in follicles with cholesterol as the original material [35]. The protein encoded by *CYP11A1* mainly converts cholesterol to pregnenolone, which is the first rate-limiting enzyme in steroid hormone synthesis and whose expression directly affects steroid hormone synthesis [36]. *ADCY1* and *ADCY2* belong to the adenylate cyclase family, and they mainly catalyze the conversion of adenosine-5'-triphosphate into 3',5'-adenosine monophosphate (cyclic AMP/cAMP) [37]. cAMP is a key factor in the synergistic activity of follicle-stimulating hormone and luteinizing hormone (LH) in promoting estrogen secretion, follicle development, and ovulation [38,39]. These studies suggest the possible involvement of miR-128-3p in the secretion of steroid hormones during follicular development and its great research potential.

Follicular atresia is an important physiological process during ovarian follicle development, maturation, and ovulation [40,41]. This process is affected by a combination of factors, among which the apoptosis of granule cells serves as one of the triggers of antral follicle atresia [42,43]. miRNAs play important roles in the proliferation and apoptosis of follicular GCs [44,45]. MiR-128-3p regulates cell proliferation and apoptosis in various tissues. In this study, miR-128-3p can inhibit the proliferation of KGN cells and promote apoptosis, which is consistent with the results reported by Ning Z. et al., who observed that miR-128-3p inhibited the proliferation and promoted the apoptosis of chicken primary GCs [46]. Furthermore, the inhibition of miR-128-3p in rats can alleviate the adrenaline-induced

apoptosis of GCs [47]. These results imply that the regulatory role of miR-128-3p in the proliferation and apoptosis of follicular GCs may be evolutionarily conserved. Therefore, in the late stage of follicular development, miR-128-3p may promote ovulation by inhibiting granulosa cell proliferation and promoting granulosa cell apoptosis. Additionally, upregulation of miR-128-3p has been observed in young women with premature ovarian failure, possibly due to the excessive expression of miR-128-3p inhibiting granulosa cell proliferation and leading to excessive apoptosis, causing follicular atresia and premature ovarian failure. This hypothesis requires specific experimental validation.

miRNAs can regulate the expression of target genes by influencing the stability of target gene mRNAs or blocking the translation process to exert their functions [48–50]. This study demonstrated that miR-128-3p in KGN cells can target GHSR to inhibit the expression of GHSR proteins. Furthermore, miR-128-3p and GHSR exhibited opposite effects on KGN cells, with GHSR promoting cell proliferation and inhibiting apoptosis. In addition, the overexpression of GHSR can alleviate the inhibitory effect of miR-128-3p on the proliferation of KGN cells. Previous transcriptomic sequencing of goat follicles revealed that compared with that in small follicles, the expression level of miR-128-3p was upregulated in large follicles, and that of the GHSR gene was downregulated [24]. This finding suggests that during follicle development, as the expression level of miR-128-3p increases, the expression of the GHSR gene is gradually suppressed, leading to the inhibition of granulosa cell proliferation. The protein encoded by GHSR, that is, GHSR1a, serves as the endogenous receptor for ghrelin. Currently, the functional role of GHSR in GCs has not been reported, with most studies focusing on the function of its endogenous ligand, which is ghrelin. A study revealed that ghrelin promoted the proliferation of porcine follicular granule cells and inhibited their apoptosis by affecting the expressions of *PCNA* and *BAX* genes [51,52]. Studies on chickens also showed that ghrelin promoted the proliferation of chicken follicle granule cells and inhibited their apoptosis through GHSR1a [53]. The observed results in KGN cells overexpressing the GHSR gene are consistent with these research findings. Therefore, it is possible to regulate the action of ghrelin hormone by modulating the expression of *GHSR* through miR-128-3p. However, further experimental validation is required to determine whether GHSR can exert its effects independently of ghrelin.

ERK1/2 plays a crucial role in mammalian follicle development. It regulates the proliferation and apoptosis of GCs and is an important component of the LH peak-induced cascade response [54,55]. Studies have shown that activation of ERK1/2 promotes cell proliferation. However, in this study, the overexpression of miR-128-3p promoted the expression of ERK1/2 proteins in KGN cells, and overexpression of GHSR inhibited their expression. This finding is in contrast to the result showing that elevated levels of ERK1/2 protein promoted cell proliferation. Moreover, in benign prostatic hyperplasia, the deficiency in neural epidermal growth factor-like protein 2 can inhibit cell proliferation by suppressing the activation of ERK1/2 through phosphorylation [56]. In KGN cells, miR-128-3p and its target gene, GHSR, regulate the protein expression of ERK1/2. However, whether changes in ERK1/2 protein levels affect cell proliferation in this context requires further investigation.

Follicle development is regulated by a complex and intricate network, and understanding follicle development at the cellular level is hindered by various limitations. In order to gain a better understanding of the role of miR-128-3p in follicular development and its molecular mechanisms, scientists must transfect miR-128-3p into animal ovaries or construct an ovarian tissue-specific miR-128-3p knockout animal model. Subsequently, the direct impact of miR-128-3p on follicular development will be observed through tissue sectioning, immunohistochemistry, and hormone measurements. Various omics techniques such as transcriptomic sequencing, ribosome profiling, proteomics, and metabolomics will then be employed to elucidate the molecular mechanisms by which miRNA-128-3p regulates follicular development. Animal experiments will provide a visual assessment of the regulatory role of miR-128-3p in follicular development, thereby laying a theoretical

foundation for a comprehensive understanding of follicular development and addressing ovarian diseases.

4. Materials and Methods

4.1. Cell Line Selection and Culture

KGN cells can respond to gonadotropins and exhibit high aromatase activity. Fas-mediated apoptosis has been observed in KGN cells. Therefore, KGN cells are considered a valuable model for the study of steroidogenesis, cell growth, and apoptosis regulation in human GCs [57,58]. Furthermore, it has been discovered that KGN cells express the GHSR gene and are responsive to stimulation by ghrelin analog growth hormone-releasing peptide-2 [59]. Therefore, this study selected KGN cells to explore the relationship between miR-128-3p and GHSR gene expression and their effects on the proliferation and apoptosis of KGN cells. Additionally, 293T cells are commonly used as a tool cell line for the investigation of dual-luciferase reporter activity. Hence, in this study, KGN and 293T cells were employed to investigate the relationship and function between miR-128-3p and the predicted target gene GHSR. They were cultured in Dulbecco's Modified Eagle's Medium/F12 (GIBCO, Shunyi district, Beijing, China) supplemented with 10% fetal bovine serum (Fisher, Grand Island, NY, USA) and 100 IU/mL penicillin/streptomycin (Gibco, Grand Island, NY, USA) at 37 °C in a humidified atmosphere of 5% CO₂.

4.2. Vector Construction

The plasmid was constructed using seamless cloning technology, and the primers were designed based on the target sequence of the inserted vector and the sequence near the enzymatic site of the planned insert of the vector, which was synthesized by BGI (Beijing, China), using CE Design (<https://crm.vazyme.com/cetool/simple.html> (accessed on 9 January 2023)), an online primer design software from Vazyme Biotech Co., Ltd. (Nanjing, Jiangsu, China). The designed primers were used to amplify the target fragment with homologous arm sequences from cDNA and recover it using the TIANGUICK Midi Purification Kit (Tiangen, Changping district, Beijing, China). pcDNA3.1 and pmirGLO were then digested using two endonucleases, and the cleaved plasmid was recovered using the TIANGUICK Midi Purification Kit (Tiangen, Changping district, Beijing, China). The above-purified target fragment and linearized vector were ligated using the ClonExpress Ultra One-Step Cloning Kit (Vazyme, Nanjing, Jiangsu, China). The constructed vector was transfected into DH5 α competent cells (Vazyme, Nanjing, Jiangsu, China) for screening and culture expansion, and finally, sequencing was performed to detect the successful construction of the vector. Table S1 provides detailed information on the primers used in this study.

4.3. Cell Transfection

Cell transfection was used to send foreign genes into cells for expression to explore gene function. miR-128-3p mimics, inhibitors, negative controls, and siRNA sets were designed and synthesized by Ribobio (Guangzhou, Guangdong, China). Supplementary Table S2 provides synthetic sequence information. The transfection operation was carried out when the cells grew to 60–80% and were in good condition. LipofectamineTM 3000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) was used to transfect miRNAs, siRNAs, and overexpression vectors into the cells in accordance with the manufacturer's instructions. A total of 100 nM/mL siRNA, miR-128-3p mimics, and inhibitor and 2 μ g/mL pcDNA3.1-GHSR and pcDNA3.1-NC plasmid were used in this study.

4.4. RNA Extraction and qRT-PCR Analysis

The total RNA in KGN cells was extracted using SteadyPure Universal RNA Extraction Kit (Agbio, Changsha, China) following the manufacturer's instructions. miRNA was reverse transcribed into cDNA using miRNA 1st-Strand cDNA Synthesis Kit (by stem-loop) (Vazyme, Nanjing, Jiangsu, China) via the stem-loop method, and cDNA of mRNA was

synthesized through reverse transcription using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara, Kusatsu, Shiga, Japan). ChamQ SYBR qPCR Master Mix (Low ROX Premixed) (Vazyme, Nanjing, Jiangsu, China) and QuantStudio 5 real-time PCR system (Thermo Fisher Scientific, Marsiling, Singapore) were used in qRT-PCR. Melting curve analyses confirmed that all primers were specific for their respective transcript. U6 (U6 snRNA) and β -actin were used as internal controls, and the results were calculated using the $2^{-\Delta\Delta C_t}$ method. Experiments were carried out in triplicate. All the primers were synthesized by BGI (Beijing, China), and the complete list of primers used is shown in Table S3.

4.5. Cell Counting Kit-8

Cell proliferation assay was conducted using a CCK-8 kit (Biosharp, Shunyi district, Beijing, China). Approximately 2000 cells were added to each well of the 96-well plate, and transfection was performed when the cells reached 50% growth. The cells were transfected for 24, 48, and 72 h afterward. Then, 10 μ L CCK-8 reagent was added to each well, and the plate was incubated for 0.5–4 h at 37 °C in a humidified atmosphere of 5% CO₂ in accordance with the manufacturer's instructions. The optical density for each well was measured using a microplate reader (Thermo, Pudong, Shanghai, China) at 450 nm.

4.6. EDU Proliferation Assay

The EDU cell proliferation assay was performed following the instructions provided in the Cell-Light™ EDU Apollo In Vitro Kit (Ribobio, Guangzhou, Guangdong, China). Briefly, the prepared EDU solution was added to the cell culture medium and incubated for 2 h. After removing the culture medium, the cells were fixed with cell fixation solution for 30 min, followed by permeabilization with permeabilization buffer for 10 min and washing with PBS. Subsequently, the cells were stained with Apollo dye and washed with PBS. Finally, the cell nuclei were stained with Hoechst dye.

4.7. Cell Apoptosis Analysis

Cell apoptosis was analyzed via flow cytometry (BD, Franklin, NJ, USA) using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (Vazyme, Nanjing, Jiangsu, China). Briefly, cells were transfected for 48 h, digested with 0.25% trypsin-ethylenediaminetetraacetic acid (Gibco, Grand island, NY, USA), collected through centrifugation, washed twice with cold Dulbecco's phosphate-buffered saline (DPBS) (Gibco, Shunyi district, Beijing, China), and centrifuged at 4 °C. Then, DPBS was discarded. Next, 100 μ L 1× Binding Buffer was added to each sample and mixed well. Then, 5 μ L of FITC and PI were added separately, the mixture was incubated for 15 min at room temperature, and then 400 μ L of 1× Binding Buffer was added. After the addition of 1× Binding Buffer, the mixture was mixed well. The samples were assayed via flow cytometry within 1 h. All data were analyzed using FlowJo software (version V10.8.1).

4.8. Western Blot

Protein expression levels in cells were detected using Western blot. Total protein was extracted from KGN cells using radioimmunoprecipitation assay lysis buffer I (Sangon Biotech, Songjiang, Shanghai, China) at 72 h after transfection, and its concentration was quantified using the Modified BCA Protein Assay kit (Sangon Biotech, Songjiang, Shanghai, China). The concentration of protein samples was kept constant through the addition of PBS to the protein sample. Then, the 4× protein sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (Takara, Beijing, China) was added to each protein sample, and the samples were denatured for 5 min at 100 °C. Denatured proteins were resolved through 10% SDS-PAGE and electrophoretically transferred to a 0.45 μ m polyvinylidene difluoride (PVDF) membrane (Sigma, Tullagreen, Carrigtwohill, Ireland). After 5 min of blocking with QuickBlock™ Blocking Buffer for Western blot (Beyotime, Shanghai, China), the PVDF membrane was incubated with the first antibody overnight

at 4 °C, followed by rinsing three times for 5 min each rinsing. Next, the membrane was incubated with the corresponding secondary antibody for 1 h at room temperature. After rinsing with Tris-buffered saline and Tween 20 three times for 5 min each time, the chemiluminescence horseradish peroxidase substrate (Invitrogen, Carlsbad, CA, USA) was used to visualize the intensity of labeled proteins.

4.9. Dual-Luciferase Reporter Assay

The wild-type and mutant 3'UTR sequences of GHSR were cloned into the pmirGLO vector containing the luciferase reporter gene. The constructed luciferase plasmid (the pmirGLO plasmid containing miR-128-3p at the binding site of the GHSR 3'UTR or a mutation at the binding site) was cotransfected with miR-128-3p mimics into 293T cells. After 48 h, luciferase activity was detected using a dual-luciferase reporter assay kit (Vazyme, Nanjing, China) following the manufacturer's instructions.

4.10. Statistical Analysis

All experiments were performed at least thrice. Comparisons between two and among multiple groups were performed using *t*-test and one-way variance analysis, respectively. All data were expressed as the mean \pm SD. GraphPad Prism (version 9.0) was used for all analyses (GraphPad Software, La Jolla, CA, USA). *p* values < 0.05 were considered statistically significant.

5. Conclusions

In vitro, miR-128-3p can target GHSR to regulate the expression of PCNA, BAX, and BCL2 genes and thereby impede KGN cell proliferation and promote KGN cell apoptosis (Figure 7).

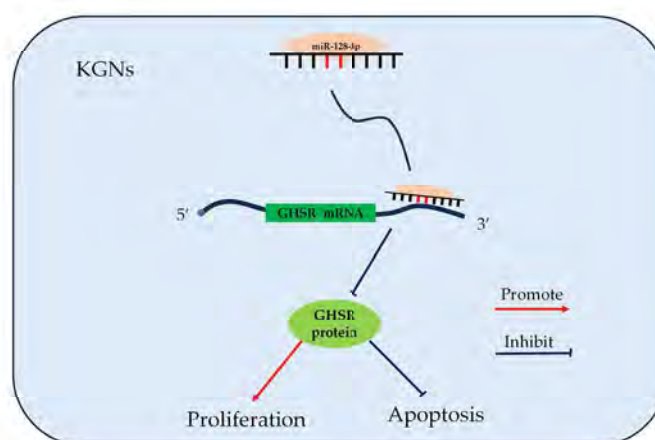


Figure 7. Schematic of miR-128-3p regulating the proliferation and apoptosis of KGN.

Supplementary Materials: The supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms25052720/s1>.

Author Contributions: Conceptualization, G.L. and S.D.; methodology, Y.L.; validation, D.L.; formal analysis, S.J.; resources, M.D.; data curation, B.H.; writing—original draft preparation, S.D.; writing—review and editing, Y.G., M.D., B.S. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Forestry Science and Technology Innovation Program of Guangdong Province (2023KJCX004), the Modern Agricultural Industrial Technology System of Guangdong Province (2023KJ127), the Guangdong Provincial Department of Agriculture and Rural Affairs Project (2022-XPY-00-009), and the Renhua County Cattle and Sheep Industrial Park Project (GDSCYY2021-018).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sánchez, F.; Smits, J. Molecular control of oogenesis. *Biochim. Biophys. Acta* **2012**, *1822*, 1896–1912. [\[CrossRef\]](#)
2. Matsuda, F.; Inoue, N.; Manabe, N.; Ohkura, S. Follicular growth and atresia in mammalian ovaries: Regulation by survival and death of granulosa cells. *J. Reprod. Dev.* **2012**, *58*, 44–50. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Regan, S.L.P.; Knight, P.G.; Yovich, J.L.; Leung, Y.; Arfuso, F.; Dharmarajan, A. Granulosa Cell Apoptosis in the Ovarian Follicle—A Changing View. *Front. Endocrinol.* **2018**, *9*, 61. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Duffy, D.M.; Ko, C.; Jo, M.; Brannstrom, M.; Curry, T.E. Ovulation: Parallels with Inflammatory Processes. *Endocr. Rev.* **2019**, *40*, 369–416. [\[CrossRef\]](#)
5. Jaffe, L.A.; Egbert, J.R. Regulation of Mammalian Oocyte Meiosis by Intercellular Communication within the Ovarian Follicle. *Annu. Rev. Physiol.* **2017**, *79*, 237–260. [\[CrossRef\]](#)
6. D'Aurora, M.; Sperduti, S.; Di Emidio, G.; Stuppia, L.; Artini, P.G.; Gatta, V. Inside the granulosa transcriptome. *Gynecol. Endocrinol. Off. J. Int. Soc. Gynecol. Endocrinol.* **2016**, *32*, 951–956. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Zhou, J.; Peng, X.; Mei, S. Autophagy in Ovarian Follicular Development and Atresia. *Int. J. Biol. Sci.* **2019**, *15*, 726–737. [\[CrossRef\]](#)
8. Tilly, J.L. Apoptosis and ovarian function. *Rev. Reprod.* **1996**, *1*, 162–172. [\[CrossRef\]](#)
9. Han, S.; Wang, J.; Cui, C.; Yu, C.; Zhang, Y.; Li, D.; Ma, M.; Du, H.; Jiang, X.; Zhu, Q.; et al. Fibromodulin is involved in autophagy and apoptosis of granulosa cells affecting the follicular atresia in chicken. *Poult. Sci.* **2022**, *101*, 101524. [\[CrossRef\]](#)
10. Wang, X.; Zhang, X.; Dang, Y.; Li, D.; Lu, G.; Chan, W.Y.; Leung, P.C.K.; Zhao, S.; Qin, Y.; Chen, Z.J. Long noncoding RNA HCP5 participates in premature ovarian insufficiency by transcriptionally regulating MSH5 and DNA damage repair via YB1. *Nucleic Acids Res.* **2020**, *48*, 4480–4491. [\[CrossRef\]](#)
11. Yu, Y.S.; Sui, H.S.; Han, Z.B.; Li, W.; Luo, M.J.; Tan, J.H. Apoptosis in granulosa cells during follicular atresia: Relationship with steroids and insulin-like growth factors. *Cell Res.* **2004**, *14*, 341–346. [\[CrossRef\]](#)
12. Hu, J.; Jin, J.; Qu, Y.; Liu, W.; Ma, Z.; Zhang, J.; Chen, F. ERO1 α inhibits cell apoptosis and regulates steroidogenesis in mouse granulosa cells. *Mol. Cell. Endocrinol.* **2020**, *511*, 110842. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Gershon, E.; Dekel, N. Newly Identified Regulators of Ovarian Folliculogenesis and Ovulation. *Int. J. Mol. Sci.* **2020**, *21*, 4565. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Wang, J.; Samuels, D.C.; Zhao, S.; Xiang, Y.; Zhao, Y.Y.; Guo, Y. Current Research on Non-Coding Ribonucleic Acid (RNA). *Genes* **2017**, *8*, 366. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Bushati, N.; Cohen, S.M. microRNA functions. *Annu. Rev. Cell Dev. Biol.* **2007**, *23*, 175–205. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 509–524. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Ahmed, K.; LaPierre, M.P.; Gasser, E.; Denzler, R.; Yang, Y.; Rülcke, T.; Kero, J.; Latreille, M.; Stoffel, M. Loss of microRNA-7a2 induces hypogonadotropic hypogonadism and infertility. *J. Clin. Investig.* **2017**, *127*, 1061–1074. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Zhang, X.; Xiao, H.; Zhang, X.; Qiukai, E.; Gong, X.; Li, T.; Han, Y.; Ying, X.; Cherrington, B.D.; Xu, B.; et al. Decreased microRNA-125b-5p disrupts follicle steroidogenesis through targeting PAK3/ERK1/2 signalling in mouse preantral follicles. *Metab. Clin. Exp.* **2020**, *107*, 154241. [\[CrossRef\]](#)
19. Bartolucci, A.F.; Uliasz, T.; Peluso, J.J. MicroRNA-21 as a regulator of human cumulus cell viability and its potential influence on the developmental potential of the oocyte. *Biol. Reprod.* **2020**, *103*, 94–103. [\[CrossRef\]](#)
20. Li, H.; Wang, X.; Mu, H.; Mei, Q.; Liu, Y.; Min, Z.; Zhang, L.; Su, P.; Xiang, W. Mir-484 contributes to diminished ovarian reserve by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis. *Int. J. Biol. Sci.* **2022**, *18*, 1008–1021. [\[CrossRef\]](#)
21. Qu, C.; Liu, X.; Guo, Y.; Fo, Y.; Chen, X.; Zhou, J.; Yang, B. MiR-128-3p inhibits vascular smooth muscle cell proliferation and migration by repressing FOXO4/MMP9 signaling pathway. *Mol. Med.* **2020**, *26*, 116. [\[CrossRef\]](#)
22. Zhao, J.; Li, D.; Fang, L. MiR-128-3p suppresses breast cancer cellular progression via targeting LIMK1. *Biomed. Pharmacother. = Biomed. Pharmacother.* **2019**, *115*, 108947. [\[CrossRef\]](#)
23. Du, X.; Li, Y.; Lian, B.; Yin, X. microRNA-128-3p inhibits proliferation and accelerates apoptosis of gastric cancer cells via inhibition of TUF1. *World J. Surg. Oncol.* **2023**, *21*, 47. [\[CrossRef\]](#)
24. Feng, G.; Liu, J.; Lu, Z.; Li, Y.; Deng, M.; Liu, G.; Sun, B.; Guo, Y.; Zou, X.; Liu, D. miR-450-5p and miR-202-5p Synergistically Regulate Follicle Development in Black Goat. *Int. J. Mol. Sci.* **2022**, *24*, 401. [\[CrossRef\]](#)
25. Woo, I.; Christenson, L.K.; Gunewardena, S.; Ingles, S.A.; Thomas, S.; Ahmady, A.; Chung, K.; Bendikson, K.; Paulson, R.; McGinnis, L.K. Micro-RNAs involved in cellular proliferation have altered expression profiles in granulosa of young women with diminished ovarian reserve. *J. Assist. Reprod. Genet.* **2018**, *35*, 1777–1786. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Gnanapavan, S.; Kola, B.; Bustin, S.A.; Morris, D.G.; McGee, P.; Fairclough, P.; Bhattacharya, S.; Carpenter, R.; Grossman, A.B.; Korbonits, M. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2988. [\[CrossRef\]](#) [\[PubMed\]](#)

27. Davis, J. Hunger, ghrelin and the gut. *Brain Res.* **2018**, *1693*, 154–158. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Lim, C.T.; Kola, B.; Korbonsits, M. The ghrelin/GOAT/GHS-R system and energy metabolism. *Rev. Endocr. Metab. Disord.* **2011**, *12*, 173–186. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Wang, M.; Qian, L.; Li, J.; Ming, H.; Fang, L.; Li, Y.; Zhang, M.; Xu, Y.; Ban, Y.; Zhang, W.; et al. GHSR deficiency exacerbates cardiac fibrosis: Role in macrophage inflammasome activation and myofibroblast differentiation. *Cardiovasc. Res.* **2020**, *116*, 2091–2102. [\[CrossRef\]](#)
30. Yuan, M.J.; Wang, T. The new mechanism of Ghrelin/GHSR-1a on autophagy regulation. *Peptides* **2020**, *126*, 170264. [\[CrossRef\]](#)
31. Schalla, M.A.; Stengel, A. The Role of the Gastric Hormones Ghrelin and Nesfatin-1 in Reproduction. *Int. J. Mol. Sci.* **2021**, *22*, 11059. [\[CrossRef\]](#)
32. Kheradmand, A.; Roshangar, L.; Taati, M.; Sirotkin, A.V. Morphometrical and intracellular changes in rat ovaries following chronic administration of ghrelin. *Tissue Cell* **2009**, *41*, 311–317. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Rak, A.; Szczepankiewicz, D.; Gregoraszczuk, E. Expression of ghrelin receptor, GHSR-1a, and its functional role in the porcine ovarian follicles. *Growth Horm. IGF Res. Off. J. Growth Horm. Res. Soc. Int. IGF Res. Soc.* **2009**, *19*, 68–76. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Chou, C.H.; Chen, M.J. The Effect of Steroid Hormones on Ovarian Follicle Development. *Vitam. Horm.* **2018**, *107*, 155–175. [\[CrossRef\]](#)
35. Andersen, C.Y.; Ezcurra, D. Human steroidogenesis: Implications for controlled ovarian stimulation with exogenous gonadotropins. *Reprod. Biol. Endocrinol. RBE* **2014**, *12*, 128. [\[CrossRef\]](#)
36. Guo, I.C.; Hu, M.C.; Chung, B.C. Transcriptional regulation of CYP11A1. *J. Biomed. Sci.* **2003**, *10*, 593–598. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Guo, R.; Liu, T.; Shasaltaneh, M.D.; Wang, X.; Imani, S.; Wen, Q. Targeting Adenylate Cyclase Family: New Concept of Targeted Cancer Therapy. *Front. Oncol.* **2022**, *12*, 829212. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Pan, B.; Li, J. The art of oocyte meiotic arrest regulation. *Reprod. Biol. Endocrinol. RBE* **2019**, *17*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Andrieu, T.; Pezzi, V.; Sirianni, R.; Le Bas, R.; Feral, C.; Benhaim, A.; Mitre, H. cAMP-dependent regulation of CYP19 gene in rabbit preovulatory granulosa cells and corpus luteum. *J. Steroid Biochem. Mol. Biol.* **2009**, *116*, 110–117. [\[CrossRef\]](#)
40. Bhardwaj, J.K.; Paliwal, A.; Saraf, P.; Sachdeva, S.N. Role of autophagy in follicular development and maintenance of primordial follicular pool in the ovary. *J. Cell. Physiol.* **2022**, *237*, 1157–1170. [\[CrossRef\]](#)
41. Chu, Y.L.; Xu, Y.R.; Yang, W.X.; Sun, Y. The role of FSH and TGF- β superfamily in follicle atresia. *Aging* **2018**, *10*, 305–321. [\[CrossRef\]](#)
42. Meng, L.; Jan, S.Z.; Hamer, G.; van Pelt, A.M.; van der Stelt, I.; Keijer, J.; Teerds, K.J. Preantral follicular atresia occurs mainly through autophagy, while antral follicles degenerate mostly through apoptosis. *Biol. Reprod.* **2018**, *99*, 853–863. [\[CrossRef\]](#)
43. Kumariya, S.; Ubba, V.; Jha, R.K.; Gayen, J.R. Autophagy in ovary and polycystic ovary syndrome: Role, dispute and future perspective. *Autophagy* **2021**, *17*, 2706–2733. [\[CrossRef\]](#)
44. Zhang, J.; Xu, Y.; Liu, H.; Pan, Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. *Reprod. Biol. Endocrinol. RBE* **2019**, *17*, 9. [\[CrossRef\]](#)
45. Li, Y.; Zhang, Z.; Wang, S.; Du, X.; Li, Q. miR-423 sponged by lncRNA NORHA inhibits granulosa cell apoptosis. *J. Anim. Sci. Biotechnol.* **2023**, *14*, 154. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Ning, Z.; Deng, X.; Li, L.; Feng, J.; Du, X.; Amevor, F.K.; Tian, Y.; Li, L.; Rao, Y.; Yi, Z.; et al. miR-128-3p regulates chicken granulosa cell function via 14-3-3 β /FoxO and PPAR- γ /LPL signaling pathways. *Int. J. Biol. Macromol.* **2023**, *241*, 124654. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Li, M.; Xue, L.; Xu, W.; Liu, P.; Li, F. rno-miR-128-3p promotes apoptosis in rat granulosa cells (GCs) induced by norepinephrine through Wilms tumor 1 (WT1). *Vitr. Cell. Dev. Biol. Anim.* **2021**, *57*, 775–785. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Stavast, C.J.; Erkeland, S.J. The Non-Canonical Aspects of MicroRNAs: Many Roads to Gene Regulation. *Cells* **2019**, *8*, 1465. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Rykova, E.; Ershov, N.; Damarov, I.; Merkulova, T. SNPs in 3'UTR miRNA Target Sequences Associated with Individual Drug Susceptibility. *Int. J. Mol. Sci.* **2022**, *23*, 13725. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Pu, M.; Chen, J.; Tao, Z.; Miao, L.; Qi, X.; Wang, Y.; Ren, J. Regulatory network of miRNA on its target: Coordination between transcriptional and post-transcriptional regulation of gene expression. *Cell. Mol. Life Sci. CMLS* **2019**, *76*, 441–451. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Sirotkin, A.V.; Alexa, R.; Harrath, A.H. Puncturevine (*Tribulus terrestris* L.) affects the proliferation, apoptosis, and ghrelin response of ovarian cells. *Reprod. Biol.* **2020**, *20*, 33–36. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Benco, A.; Sirotkin, A.V.; Vasicek, D.; Pavlová, S.; Zemanová, J.; Kotwica, J.; Darlak, K.; Valenzuela, F. Involvement of the transcription factor STAT1 in the regulation of porcine ovarian granulosa cell functions treated and not treated with ghrelin. *Reproduction* **2009**, *138*, 553–560. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Sirotkin, A.V.; Grossmann, R. Effects of ghrelin and its analogues on chicken ovarian granulosa cells. *Domest. Anim. Endocrinol.* **2008**, *34*, 125–134. [\[CrossRef\]](#)
54. Hsieh, M.; Lee, D.; Panigone, S.; Horner, K.; Chen, R.; Theologis, A.; Lee, D.C.; Threadgill, D.W.; Conti, M. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. *Mol. Cell. Biol.* **2007**, *27*, 1914–1924. [\[CrossRef\]](#)
55. Fan, H.Y.; Liu, Z.; Shimada, M.; Sterneck, E.; Johnson, P.F.; Hedrick, S.M.; Richards, J.S. MAPK3/1 (ERK1/2) in ovarian granulosa cells are essential for female fertility. *Science* **2009**, *324*, 938–941. [\[CrossRef\]](#)

56. Liu, J.; Liu, D.; Zhang, X.; Li, Y.; Fu, X.; He, W.; Li, M.; Chen, P.; Zeng, G.; DiSanto, M.E.; et al. NELL2 modulates cell proliferation and apoptosis via ERK pathway in the development of benign prostatic hyperplasia. *Clin. Sci.* **2021**, *135*, 1591–1608. [[CrossRef](#)]
57. Nishi, Y.; Yanase, T.; Mu, Y.; Oba, K.; Ichino, I.; Saito, M.; Nomura, M.; Mukasa, C.; Okabe, T.; Goto, K.; et al. Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. *Endocrinology* **2001**, *142*, 437–445. [[CrossRef](#)] [[PubMed](#)]
58. Ma, Y.; Zheng, L.; Wang, Y.; Gao, Y.; Xu, Y. Arachidonic Acid in Follicular Fluid of PCOS Induces Oxidative Stress in a Human Ovarian Granulosa Tumor Cell Line (KGN) and Upregulates GDF15 Expression as a Response. *Front. Endocrinol.* **2022**, *13*, 865748. [[CrossRef](#)] [[PubMed](#)]
59. Chao, Y.N.; Sun, D.; Peng, Y.C.; Wu, Y.L. Growth Hormone Releasing Peptide-2 Attenuation of Protein Kinase C-Induced Inflammation in Human Ovarian Granulosa Cells. *Int. J. Mol. Sci.* **2016**, *17*, 1359. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

RESEARCH

Open Access



Expression profile and bioinformatics analysis of circRNA and its associated ceRNA networks in longissimus dorsi from Lufeng cattle and Leiqiong cattle

Chuang Yang¹, Longfei Wu¹, Yongqing Guo¹, Yaokun Li¹, Ming Deng¹, Dewu Liu¹, Guangbin Liu^{1*} and Baoli Sun^{1*}

Abstract

This paper aims to explore the role of circRNA expression profiles and circRNA-associated ceRNA networks in the regulation of myogenesis in the longissimus dorsi of cattle breeds surviving under subtropical conditions in southern China by RNA sequencing and bioinformatics analysis. It also aims to provide comprehensive understanding of the differences in muscle fibers in subtropical cattle breeds and to expand the knowledge of the molecular networks that regulate myogenesis. With regard to meat quality indicators, results showed that the longissimus dorsi of LQC had lower pH ($P < 0.0001$), lower redness ($P < 0.01$), lower shear force ($P < 0.05$), and higher brightness ($P < 0.05$) than the longissimus dorsi of LFC. With regard to muscle fiber characteristics, the longissimus dorsi of LQC had a smaller diameter ($P < 0.0001$) and higher density of muscle fibers ($P < 0.05$). The analysis results show that the function of many circRNA-targeted mRNAs was related to myogenesis and metabolic regulation. Furthermore, in the analysis of the function of circRNA source genes, we hypothesized that *btacirc_00497* and *btacirc_034497* may regulate the function and type of myofibrils by affecting the expression of MYH6, MYH7, and NEB through competitive linear splicing.

Keywords RNA sequencing, circRNA, ceRNA network, Beef, Longissimus dorsi, Myofibers, Meat quality

Introduction

According to the Domestic Animal Diversity Information System (FAO-DAD-IS), there are over 70 cattle breeds widely distributed in China, with 53 of them being indigenous [1, 2]. Native breeds are divided into three categories in accordance with their geographical distribution, including northern-distributed group, central-distributed

group, and southern-distributed group, which are distributed in northern China, middle and lower reaches of the Yellow River, and southern China, respectively [3, 4]. Native Chinese cattle were once bred as draft animals, which has allowed them to adapt to the climate in which they grow and to be highly resistant to adversity [2]. Natural selection and environmental characteristics have shaped the genomes and expression profiles of cattle grown in each environment. Cattle that have been grown in southern China for a long time are adapted to hot and humid climates, and they have excellent meat quality and high disease resistance [5, 6].

Meat quality is an important element in the assessment of the meat value of livestock [7]. Beef myofibers

*Correspondence:

Guangbin Liu

gbliu@scau.edu.cn

Baoli Sun

baolisun@scau.edu.cn

¹College of Animal Science, South China Agricultural University, No.483 Wushan Road, Guangzhou 510642, China



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Table 1 Longissimus dorsi muscle quality about Leiqiong cattle and Lufeng cattle

Items	Varieties of cattle		P-value
	LQC	LFC	
pH (24 h)	5.66 ± 0.025 ^b	5.83 ± 0.032 ^a	0.000
L*	49.01 ± 3.937 ^a	40.01 ± 3.011 ^b	0.011
a*	15.35 ± 1.356 ^b	19.51 ± 1.234 ^a	0.004
b*	12.14 ± 0.494	11.64 ± 1.466	0.543
Tangential stress (Pa)	39.43 ± 14.228 ^b	65.26 ± 22.305 ^a	0.021
Cooking loss (%)	0.357 ± 0.008	0.343 ± 0.038	0.515

can affect the traits to a certain extent, such as tenderness, color, and pH drop of beef [8]. Thus, among the factors that can affect meat quality, myofiber is the strongest [9, 10]. However, the growth and development of muscle fibers include ontogeny at different embryonic stages, as well as hypertrophy and transformation at postnatal stages [9, 11]. In addition, the process of myogenesis is a complex biological process regulated by multiple factors, such as myogenic regulatory factors [12], signaling pathways [13], noncoding RNAs (ncRNAs) [14], and genes. Among these factors, circular RNA (circRNA) is an ncRNA with a covalently closed continuous loop structure that can play a role in gene regulation by competing with linear splicing [15], and this factor plays an important regulatory role in myogenesis [16]. In addition, the function of circRNA as a sponge of miRNA may indirectly affect the translation of mRNA [17]. CircRNA can be found in bovine muscle, and it is involved in the regulation of myogenesis [18–20].

Leiqiong cattle (LQN) and Lufeng cattle (LFN) are native cattle breeds in South China, which are primarily distributed in Guangdong and Hainan Provinces. These two kinds of cattle live in a subtropical climate environment for a long time, and they are less affected by artificial introduction. In addition, the breeding scale is

considerable. The genetic relationship between LQN and LFN cattle is close, but their physical appearance, such as is quite different [5]. In this study, we compared the circRNA transcripts in longissimus dorsi from two types of southern Chinese cattle, then constructed and analyzed the circRNA-associated ceRNA network. These results could provide comprehensive understanding of the differences in muscle fiber development in cattle grown under subtropical conditions and extend our understanding of the molecular networks that regulate beef meat quality.

Results

Characteristics in meat quality and myofiber

The result related to meat quality were shown in Table 1. Compared potential of hydrogen (pH) of longissimus dorsi from two varieties of cattle, pH value of LQC was significantly lower than LFC ($P < 0.0001$). As for the muscle color, the muscle lightness (L^*) of LFC was significantly lower than that of LQC ($P < 0.05$), and the muscle redness (a^*) of LFC was significantly higher than that of LQC ($P < 0.01$). It was no significantly differences between LFC and LQC on yellowness (b^*) ($P > 0.05$). In the assessment of tenderness, tangential stress of LQC is lower than LFC ($P < 0.05$), that may preliminarily indicate that beef from LQC is more tender than that from LFN.

Compared the longissimus dorsi fibers from two kinds of cattle (Fig. 1), in the same level viewing area, myofibers quantity of LQC was significantly more than that of LFC ($P < 0.05$). For the area and diameter of longissimus dorsi fibers, LQC has smaller myofibers area and smaller diameter compared with LFC ($P < 0.0001$).

miRNA and transcriptome expression analysis

miRNA-seq generated 24,041,508 row reads from 8 samples, and after filtering, 23,312,472 clean reads were

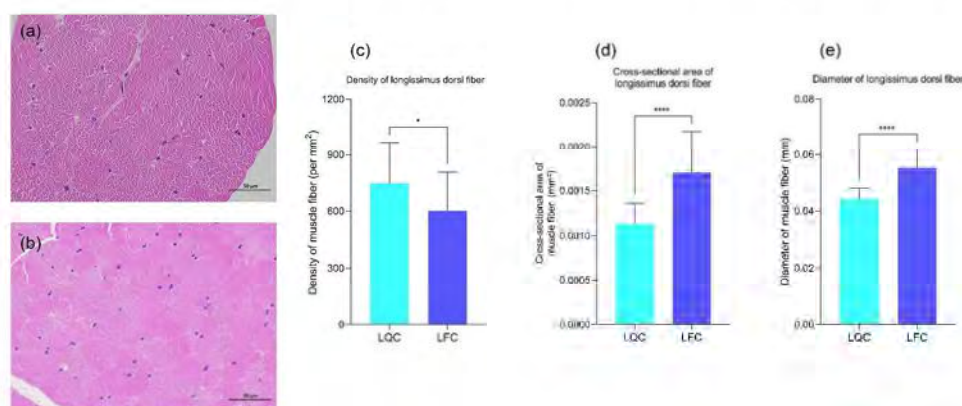


Fig. 1 Longissimus dorsi myofibers difference between Leiqiong cattle and Lufeng cattle: **(a)** Section of longissimus dorsi muscle of Lufeng cattle; **(b)** Section of longissimus dorsi muscle of Leiqiong cattle; **(c)** Density of longissimus dorsi myofibers between Lufeng cattle and Leiqiong cattle; **(d)** Cross-sectional area of longissimus dorsi myofibers between Lufeng cattle and Leiqiong cattle; **(e)** Diameter of longissimus dorsi myofibers between Lufeng cattle and Leiqiong cattle. * $P < 0.05$, **** $P < 0.0001$

obtained, accounting for $97.00 \pm 0.05\%$ of the raw reads. The reference genome, *Bos taurus*, gene data was downloaded in miRBase and analyzed using miRDeep2 software, $92.21 \pm 1.88\%$ of clean reads could be mapped to the reference genome, and a total of 493 known miRNAs as well as 538 miRNA precursors were obtained. Predictive analysis of sequences not annotated to any information using mireap yielded 34.88 ± 8.22 new miRNAs (Table S1). Among all the annotated miRNAs, 59 and 51 miRNAs were unique to LQC and LFC, respectively, and 572 miRNAs were shared (Fig. 2(a)). Further analysis of miRNAs differentially expressed (DESeq, Expression difference fold: $|\log_2\text{FoldChange}| > 1$, Significance: $P\text{-value} < 0.05$) between LQC and LFC by the longissimus dorsi, with LQC as the control group, revealed 7 up-regulated and 6 down-regulated (Fig. 2(b)& Table S2).

For the RNA sequencing of mRNA and circRNA, a total of 8 samples from LQC and LFC yielded an average of 108,794,942 reads per sample. After quality control and trimming using Cutadapt, there are 94,857,498 per sample clean reads were yielded. TopHat2's upgraded HISAT2 software (<http://ccb.jhu.edu/software/hisat2/index.shtml>) was used to map the reads, and $97.14\% \pm 0.28\%$ of the clean reads can be mapped to the reference genome, *Bos taurus* (Table S1). A total of 17,313 transcripts were found in all samples detected, of which 16,810 transcripts were found in samples from LQC and 993 were unique; 16,320 were found in samples from

LFC and 503 were unique; and 15,817 transcripts were shared by LQC and LFC (Fig. 2(d)). Differential analysis of gene expression was performed using DESeq (Expression difference fold: $|\log_2\text{FoldChange}| > 1$, Significance: $P\text{-value} < 0.05$), with LQC as the control group, 155 up-regulated and 444 down-regulated (Fig. 2(e)& Table S2). The PCA plot of miRNA and mRNA expression profile is shown in Fig. 2(c& f). GO (Gene Ontology) analysis revealed that these differentially expressed transcripts were significantly enriched ($P < 0.05$), and the vast majority (77.7%) of GO terms were Biological Process (Table S3).

circRNA identification

After alignment with the reference genome, the unmatched Reads double-end 20 bp from the HISAT2 alignment results were intercepted as Anchors sequences and re-matched to the genome using Bowtie2 to detection of circRNA. 4,660,322 of these sequences were re-matched to the genome, representing $85.80 \pm 3.50\%$ of all Anchors sequences. Using find_circ to identify and annotate circRNAs, a total of 5,715 annotated entries were obtained (Table S4). Expression profile about circRNAs in LQC and LFC was showed in Fig. 3. The sourcegenes of identified circRNA vast majority were annotated exons (annot exons), and the spliced length of most identified circRNAs were concentrated about 500 bp (Fig. 3(a& b)). In the other hand, identified circRNAs were abundant

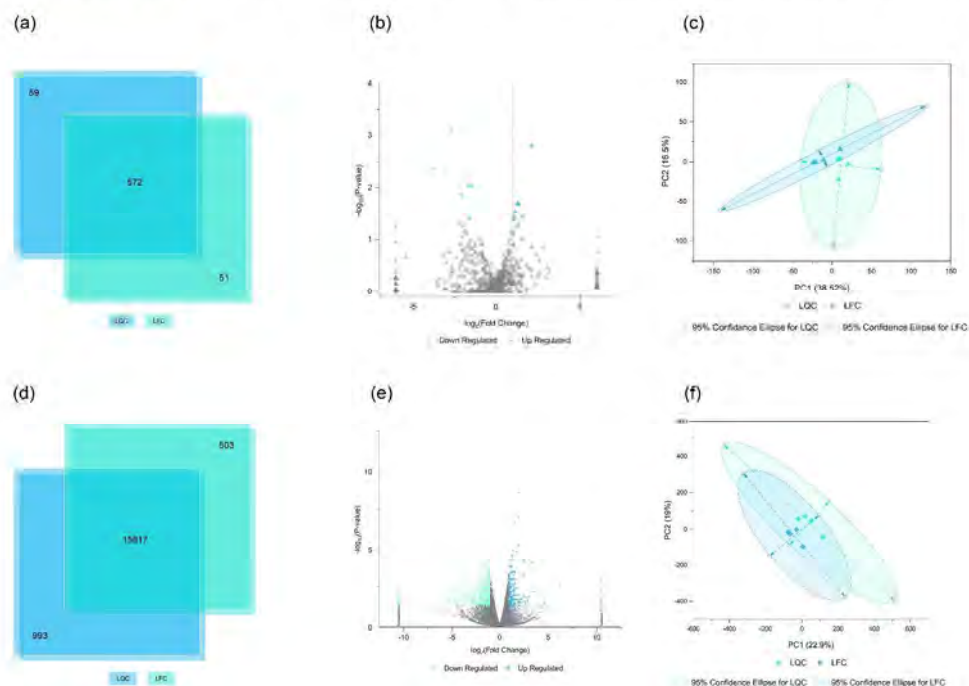


Fig. 2 DE miRNAs and transcripts expression analyses of Longissimus dorsi from Leiqiong cattle and Lufeng cattle: **(a)** Venn diagram of the number of miRNAs in longissimus dorsi from two types of cattle; **(b)** Volcano plot for DE miRNAs in Longissimus dorsi between two types of cattle; **(c)** PCA plot for miRNAs expression in each sample; **(d)** Venn diagram of the number of transcripts in longissimus dorsi from two types of cattle; **(e)** Volcano plot for DE transcripts in Longissimus dorsi between two types of cattle; **(f)** PCA plot for transcripts expression in each sample

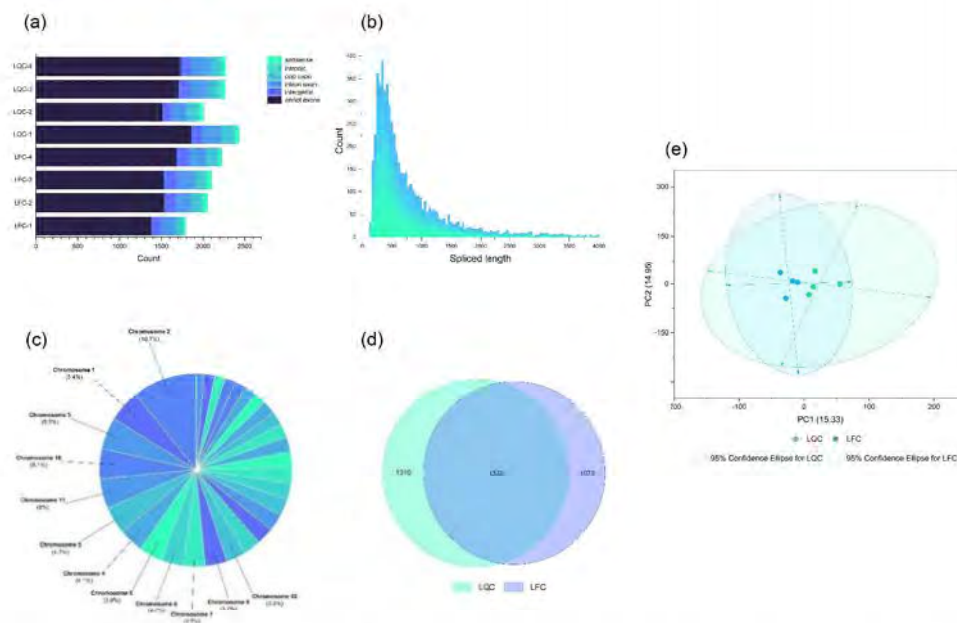


Fig. 3 Expression profile about circRNA in Longissimus dorsi from Leiqiong cattle and Lufeng cattle: (a) The types of sourcegenes of circRNA that identified in each sample; (b) The spliced length of circRNA identified; (c) The distribution on chromosomes of sourcegenes of circRNA identified; (d) Venn diagram of the number of circRNA in longissimus dorsi from two types of cattle; (e) PCA plot for circRNA expression in each sample

on chromosome 1, 2, 3, 10 and 11. Compared with each other, there were 1,310 unique expressed circRNAs in LQC and 1,075 in LFC, 3,330 circRNAs were shared (Fig. 3(c& d)). PCA plot (Fig. 3(e)) showed that obviously separation exist between the samples of LQC and the samples of LFC, indicating that there was a certain difference in expression of circRNAs between the two types of cattle.

Furthermore, differential expressed circRNAs (DE circRNAs) between LQC and LFC were showed in Fig. 4& Table S2 (DEseq, Expression difference fold: $|\log_2\text{Fold-Change}| > 1$, Significance: $P\text{-value} < 0.05$). Compared to LQC, 19 circRNAs were up regulated and 10 were down regulated in LFC (Fig. 4(a& c)), and accounts for 65.5% and 34.5% of all DE circRNAs (Fig. 4(b)), respectively.

Construction of ceRNA-networks and Enrichment analysis

After screening the DE circRNAs, psRobot and miRanda databases were used to predict targeted miRNA. As the co-expression networks in Fig. 5 and Table S5 shown, 13 miRNA and 135 mRNAs were associated with 8 circRNAs. Above them, there were 4 up-regulated and 4 down-regulated circRNAs, and influenced by them, 7 miRNAs and 35 mRNAs were up-regulated, and 6 miRNAs and 100 mRNAs were down-regulated.

GO and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis were performed on mRNAs that were indirectly influenced by circRNAs. As shown in Fig. 6(a& b) and Table S6, the top20 GO terms that targeted genes enriched in were mostly associated with Cellular Component and Biological Process. Further

refinement, the top20 GO terms were closely related to peptidyl-tyrosine phosphorylation, peptidyl-tyrosine modification, regulation of peptidyl-tyrosine phosphorylation, plasma membrane part, myelin sheath, intrinsic component of plasma membrane, external side of plasma membrane, integral component of plasma membrane, regulation of cytosolic calcium ion concentration, cellular calcium ion homeostasis, myelination, ensheathment of neurons, axon ensheathment, calcium ion homeostasis, regulation of biological quality, positive regulation of cytosolic calcium ion concentration, cellular divalent inorganic cation homeostasis, regulation of midbrain dopaminergic neuron differentiation, regulation of planar cell polarity pathway involved in axis elongation, negative regulation of planar cell polarity pathway involved in axis elongation and so on. And the KEGG enrichment analysis results shown by Fig. 6(c& d) and Table S7, top20 KEGG Orthologs distributed in 5 categories, included of Pyrimidine metabolism, Nicotinate and nicotinamide metabolism, Rap1 signaling pathway, Platelet activation, MAPK signaling pathway, Purine metabolism, Complement and coagulation cascades, Calcium signaling pathway, Thyroid hormone signaling pathway, Focal adhesion, Glycine, serine and threonine metabolism, Toxoplasmosis, Vitamin B6 metabolism, Ras signaling pathway, PI3K-Akt signaling pathway, Regulation of actin cytoskeleton, Vascular smooth muscle contraction, Central carbon metabolism in cancer, Human cytomegalovirus infection and so on.

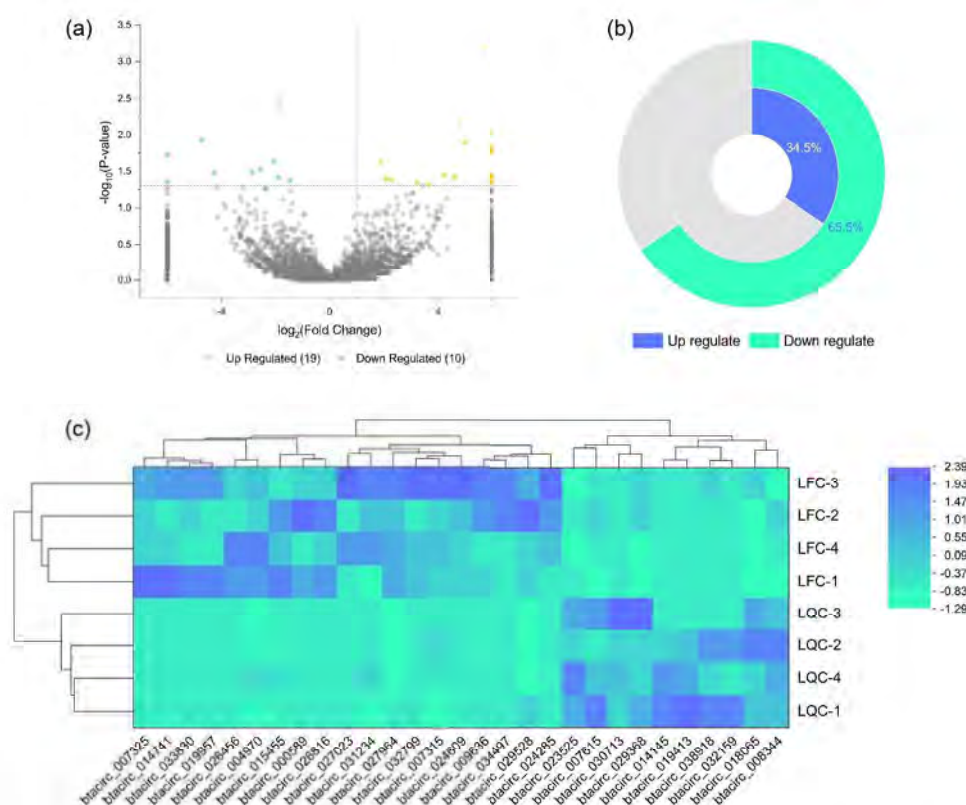


Fig. 4 DE circRNAs between Leiqiong cattle and Lufeng cattle: **(a)** Volcano plot for DE circRNA between two types of cattle; **(b)** Ring diagram about the percentage of DE circRNAs between two types of cattle; **(c)** Heatmap for DE circRNA between two types of cattle

Enrichment analysis of sourcegene of circRNAs

CircRNA is usually produced by exons or introns of their sourcegenes, and the cyclization of circRNA probably affects the expression of the sourcegene [21]. We analyzed sourcegenes of 29 DE circRNAs from LQC and LFC, then KEGG and GO enrichment analysis were performed on them (Fig. 7). The result of top 25 GO terms in Fig. 7(a) illustrate that most terms were enriched in Cellular Component and 6 terms were enriched in Biological Progress. The information of all terms that were enriched have been stored in Table S8, and it is worth noting that most of the enriched genes were associated with the function of myofibers, such as sarcomere, contractile fiber part, myofibril, contractile fiber, stress fiber, myosin complex, contractile actin filament bundle, actin filament bundle, actomyosin, muscle filament sliding, actin-myosin filament sliding and so on. Figure 7(b) and Table S9 shown that top 25 KEGG Orthology of related genes, among them, there were 1, 1, 2, 3, 5 and 13 KEGG Orthology were classified to Cellular Processes, Environmental Information Processing, Genetic Information Processing, Human Diseases, Organismal Systems and Metabolism, respectively.

In order to verify the reliability of the RNA sequencing results, 3 circRNAs, 3 miRNAs and 8 mRNAs that differentially expressed in LQC and LFC were randomly selected and analyzed by RT-qPCR. The LQC served as a control group, RNA-seq and RT-qPCR results were consistent in the expression levels of each selected genes (Figure S1). Information about the primers used to amplify the selected genes has been placed in the Table S10.

Discussion
Differences in meat quality between longissimus dorsi from LQC and LFC may be related to the differences in myofiber composition

LFC and LQC are native Chinese cattle breeds, which have adapted to the hot and humid climate of southern China after long evolution; therefore, it is important to enhance their meat value potential. Most of the indicators used for meat quality assessment, such as pH decline, meat color, and tenderness, are related to the structure and metabolism of the muscle fibers [22, 23]. The rate of pH decline is often related to the amount of glycogen reserves in muscle tissue before slaughter and

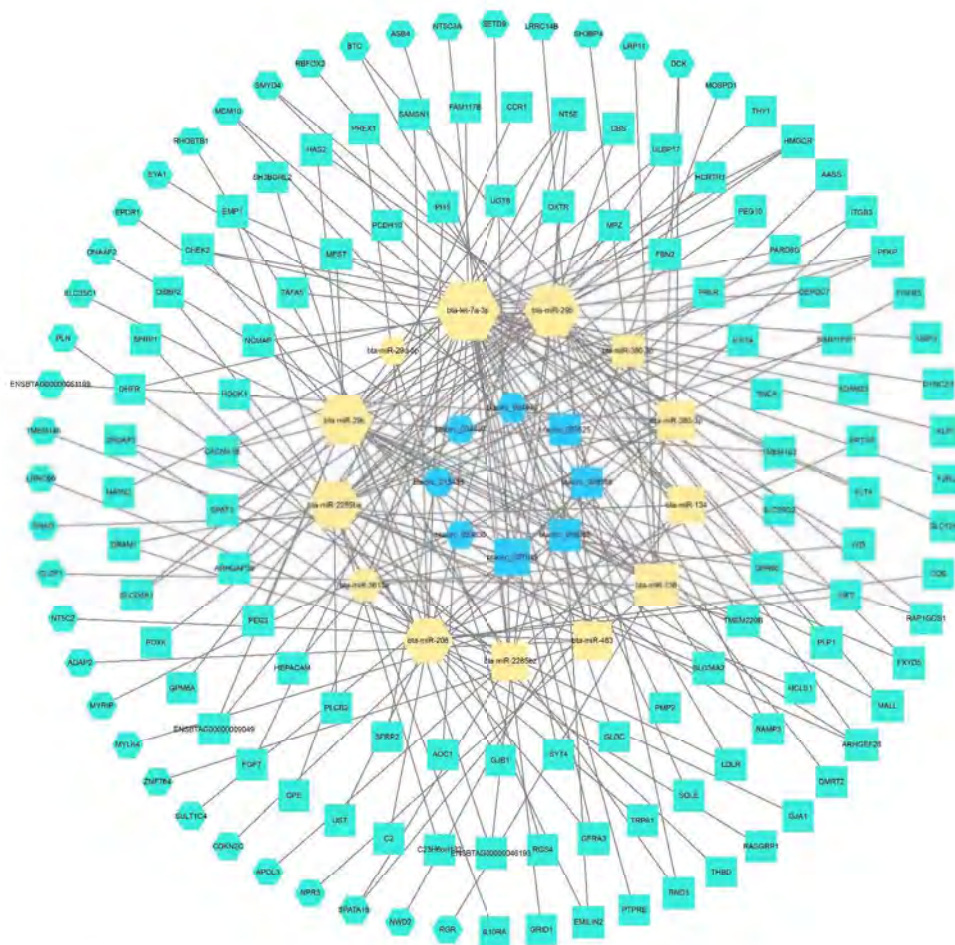


Fig. 5 Identification of circRNA-associated ceRNA networks

the content of mitochondria in different muscle fibers. When the glycogen level in muscle tissue is low, the rate of glycolysis decreases; therefore, the pH declines gradually because of the slow accumulation of lactic acid [24]. Muscles with more oxidative properties had a higher pH than those with more glycolytic properties after the animals were slaughtered [25], and the meat with a higher percentage of fast-twitch glycolytic fibers has a higher rate and degree of pH decline, it is commonly known in the field of red muscle. Furthermore, the rate of glycolysis and rate of pH decrease can affect changes of some proteins in muscle fibers at the post-mortem, such as tropomyosin, actin, troponin, and some enzymes on glycolysis, and these protein changes will be directly reflected in meat tenderness, flavor substances, color, and other measures of meat quality [8]. Meat color is an important point in determining meat quality, and it is a marketability criterion of meat because consumers often use this criterion to select and buy meat. Previous studies [26, 27] have shown that meat with low glycogen reserves tends to have higher ultimate pH, which leads to low light scattering and high oxygen consumption

in meat surface, resulting in low lightness of the meat. The hue and chromaticity of meat color are primarily dominated by myoglobin (Mb) because the changes in the biochemical state of Mb cause the changes in meat color, particularly the degree of oxidation and reduction of Mb. In addition, Mb in fresh meat is present in four states, namely, deoxymyoglobin, oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin, in which OxyMb and COMb provide the meat with a bright cherry red color that is typical of fresh meat [28]. However, the four redox states of Mb are not constant, and many meat-endogenous factors can affect the color of meat by influencing the state of Mb, of which pH, muscle source, presence of antioxidants, lipid oxidation, and mitochondrial activity and the most prominent [29]. Beef tenderness has received considerable attention because it affects the return decision and satisfaction of consumers. The characteristics of the muscle fibers largely affect the tenderness of the meat, although the tenderness and texture of beef are also affected by the connective tissue and intermuscular fat in the muscle [30]. The activity of some protein hydrolytic enzymes changes with the

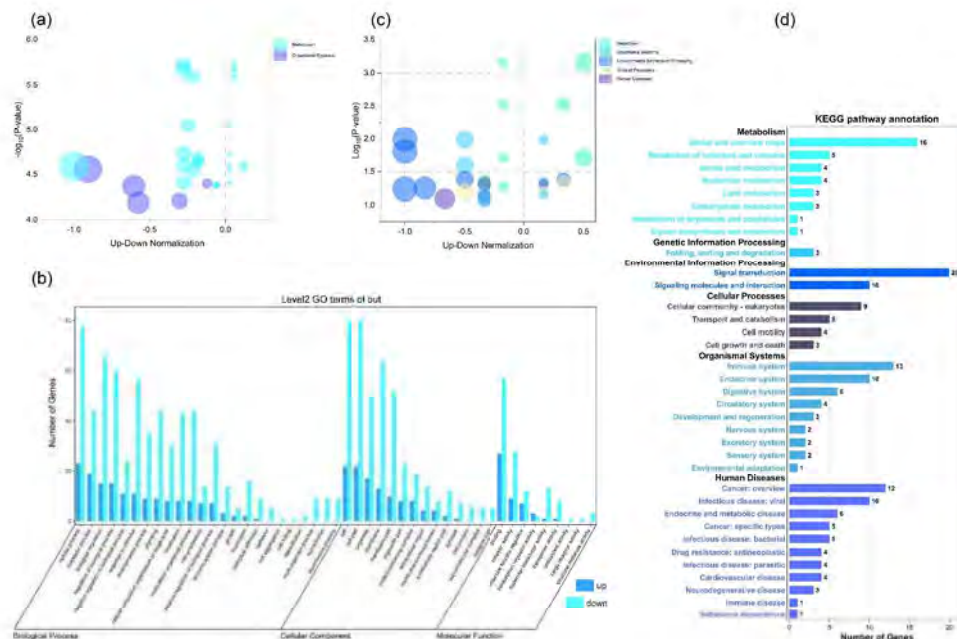


Fig. 6 GO and KEGG enrichment analysis for mRNAs that were influenced by circRNAs-targeted-miRNAs: (a) Bubble chart of Top20 GO terms for targeted genes related to DE circRNAs; (b) Bar plot of GO enrichment terms in BP, CC, and MF categories for targeted genes related to DE circRNAs; (c) Bubble chart of Top20 KEGG Orthologs for targeted genes related to DE circRNAs; (d) Bar plot of KEGG enrichment pathways for targeted genes related to DE circRNAs

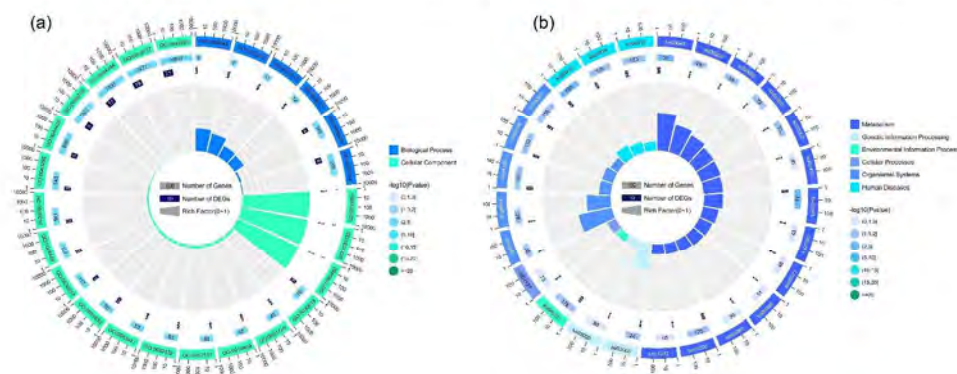


Fig. 7 GO and KEGG enrichment analysis for sourcegenes of DE circRNAs: (a) Top25 GO terms function enrichment for sourcegenes of DE circRNAs; (b) Top25 KEGG Orthologs function enrichment for sourcegenes of DE circRNAs

decrease of the rate of pH, which leads to differences in glycogen metabolism by muscle fibers that indirectly affect meat tenderness [31, 32]. In addition, the number of muscle fibers affect the tenderness of meat. In general, tender meat has more muscle fibers per unit area and a smaller diameter [33]. In the present study, the pH of the longissimus dorsi of LQC decreased faster than that of LFC, which may be one of the reasons for the high lightness of the longissimus dorsi of LQC. With regard to redness, the longissimus dorsi of LFC has a higher level of redness than that of LQC, which may be due to some factors that keep the Mb more in the state of OxyMb or COMb in LFC. By analyzing the quantitative characteristics of myofibers per unit area, we found that the number of myofibers in LQC was less than that in LFC, and the

diameter of myofibers was smaller. Such quantity characteristics of myofibers in LQC also confer a smaller shear force in LQC.

ceRNA network points to the regulation of PI3K-Akt, MAPK, and calcium pathways

Considering that muscle fibers profoundly influence meat quality, the regulatory mechanism of myogenesis has been discussed comprehensively and extensively. Myogenesis is a physiological process that is influenced by various internal or external factors starting from fetal life. Among the factors, circRNA as a ncRNA has received considerable attention for its wide range of regulatory functions. circRNAs are dynamically expressed and abundant in muscle tissue of many species, including

humans, cattle, goat, pig, chicken, and mice [9]. Although the functions of circRNAs remain largely unexplored, they can serve as miRNA sponges and further contribute to mRNA stability or protein production [34]. For example, circPTPN4 can sponge miR-499-3p, which regulates NAMPT expression, thereby promoting myoblast proliferation and differentiation and activating the fast-twitch muscle phenotype [35]. The overexpression of circCPE counteracts the inhibitory effect of miR-138 on cell proliferation and the accelerating effect on differentiation and apoptosis, and circRNAs can reduce the inhibitory effect of miR-138 on FOSCl, which is involved in myogenesis [36]. With the continuous improvement of sequencing technology and database, the construction of a ceRNA network has become an important tool for predicting circRNA function. Therefore, we performed Pearson correlation analysis to assess the association between DE circRNA and DE mRNA that exist between LQC and LFC, and enrichment analysis was performed for target mRNAs. Based on the results of KEGG analysis, we found that target mRNAs were primarily related to PI3K-Akt, MAPK, and calcium signaling pathways. PI3K-Akt has been widely proven to be associated with muscle hypertrophy and atrophy [37–40]. Several recent studies have also demonstrated the ability of circRNA to regulate the PI3K/AKT pathway, for example, the newly identified circRILPL1 as miR-145 sponge promotes myogenic cell growth by regulating the expression of the IGF1R gene to reduce the inhibitory effect of miR-145 on the PI3K/AKT signaling pathway [41]. The proliferation and differentiation of bovine myoblasts can be inhibited by circMEF2D, and circMEF2D can regulate the PI3K-AKT signaling pathway by competitively binding miR-486 [42]. Previous studies suggest that MAPK may be associated with myoblast cell cycle arrest, which is critical for initiating muscle differentiation in myogenic cells [43]. In addition, MAPK is a signaling pathway that drives the metabolic adaptation of skeletal muscle to exercise [44]. Numerous studies have shown that MAPK increases insulin-dependent glucose uptake and oxidative metabolism as well as mitochondrial oxidative phosphorylation in muscle during exercise [44–47]. In muscle tissue, intracellular stores of Ca^{2+} , upon release, trigger the formation of actin crossbeams and the generation of contractile force [48]. Myocytes can sense and respond to changes in workload and activation patterns by regulating the gene expression and cellular metabolism of calcium signaling pathways [49, 50]. Stored calcium influx has become a mechanism by which the calcium signaling pathway is activated in response to the changing demands of myocytes [48, 51]. Most of the target mRNAs identified by the ceRNA network using LQC and LFC longissimus dorsi were enriched in pathways related to muscle metabolism and myogenesis. Therefore, these mRNA-related circRNAs

and miRNAs might be effective tools for regulating muscle production and development.

Cyclization of btacirc_00497 and btacirc_034497 may affect myogenesis

Apart from being a sponge for miRNA, circRNA can compete with linear splicing to play a functional role in gene regulation [15]. In this study, we attempted to use GO and KEGG enrichment analyses to organize and predict the function of genes that were affected by and were the source of DE circRNA. Based on the results of enrichment analysis, MYH6, MYH7, and nebulin (NEB) have high frequency in multiple pathways and GO terms that may associate with myogenesis and meat quality. Myosin is a motor protein that plays an important role in the contraction of animal skeletal muscle. Myosin consists of six subunits, two of which are myosin heavy chain (MyHC) subunits. MyHC isoforms play an irreplaceable role in muscle contraction because they have ATPase activity, which provides energy for muscle contraction [22]. In mammals, 11 known genes can encode MyHC, and these genes are highly conserved during evolution [52]. The four MyHC isoforms, namely, I, IIa, IIx, and IIb, are classified by different coding genes, which leads to four myofiber types. MyHC I is expressed in type I fibers, and IIa, IIx, and IIb are expressed in IIA, IIX, and IIB fiber types, respectively [53, 54]. The two subunits of the MyHC I heavy chain are composed of β -cardiac and α -cardiac encoded by MYH7 and MYH6, respectively [55]. Theoretically, the high expression level of MyHC I indicates that muscle fiber metabolism will have less efficient glycolysis, resulting in less lactate accumulation [56]. Low levels of lactic acid in the muscle tissue of live cattle can lead to a gradual decrease in beef pH rate postmortem [24]. Insufficient pH reduction in beef impairs meat color, tenderness, and shelf life [57]. Many studies have shown that feeding high-energy diet before slaughter is an effective way to increase muscle glycogen content and improve muscle pH reduction after slaughter, and this strategy may not become dependent on muscle fibers that have high MyHC I expression level [58, 59]. In our study, btacirc_00497 in LFC longissimus dorsi has a high expression level, which is sourced from MYH6 and MYH7. However, our findings on the meat quality of LQC and LFC do not support our conjecture, that is, circRNA reduces β -MHC and α -MHC expression by competing for linear shearing of the transcripts of MYH6 and MYH7 and further influences the metabolic process of MyHC. We hypothesize that the expression level of MYH6 and MYH7 transcripts of LFC may be higher than that of LQC, so the probability of cyclization to btacirc_00497 is also increased. Thus, although the factors affecting the cyclization of btacirc_00497 are unclear, this gene may still serve as a potential lynchpin

for the regulation of muscle fiber types. In addition, we identified *btacirc_034497*, which shares the same origin gene as NEB. Actin is present and functionally important in most eukaryotic cells, and the control of actin filament organization and structure is critical for many cellular functions. NEB stabilizes actin filaments in thin filament architecture, thereby regulating the filament length [60, 61]. Moreover, NEB regulates skeletal muscle contraction in skeletal muscle, and muscles from NEB knockout mice produce significantly less force than their wild-type siblings [62, 63]. Therefore, the factors affecting *btacirc_034497* cyclization have great application potential as important tools for NEB regulation.

Materials and methods

Sample collection

All experimental animals were sourced from the breeding farm (Meixian Country, Meizhou City, Guangdong province). Four Leiqiong cattle (LQC) and four Lufeng cattle (LFC) each, four-month-old, were humanely slaughtered, and the cattle of the same breed are similar in weight and body condition and raised in the same farm environment. Then longissimus dorsi tissues from 8 cattle were collected and snap-frozen in liquid nitrogen immediately to extract total RNA. Another portion of the longissimus dorsi tissue was trimmed into 0.5×0.5×1.0 cm pieces and immediately fixed in 4% paraformaldehyde for observation in tissue sections. In addition, any anesthesia or euthanizing agent was not used in our study.

Analysis of muscle quality properties

After 24 hours of slaughter, pH was determined using Meat pH Meter HI99163 (HANNA, Italy) on the longissimus dorsi. The colorimetric parameters of the muscles were calculated using the $L^* a^* b^*$ system with a colorimeter OPTO-STAR Meat Color Tester (Matthaus, Germany) from the average of three random readings of each sample. And the evaluation of steaming losses was performed according to the project of Honikel [64]. During the shear force measurement, three samples of the longissimus dorsi after 72 hours of aging of each cattle (1cm×1cm×3cm) were measured three times perpendicular to the fiber direction. The final shear force was the average of the three readings, and the measurements were expressed in Pascal (Pa) [65].

Muscle fiber characteristics

The tissues were after fixing by 4% paraformaldehyde for 24h, and then immersed in xylene alcohol (1:1, v/v), infiltrated and embedded in paraffin. Cross sections of 3μm thickness were prepared, stained with hematoxylin and eosin, observed under a microscope, and photographed (400×magnification). The number of muscle fibers and total cross-sectional area were subsequently evaluated

with Image-Pro Plus V6.0 (Media Cybernetics Inc., Rockville, MD, USA).

Preparation and sequencing for RNA-seq

TRIzol (Thermo Fisher, Shanghai, China) used to extract total RNA from the tissue samples. RNA quantity and purity were determined using Agilent 2100 Bioanalyzer and the RNA 6000 Nano Labchip Kit (Agilent, Santa Clara, USA). Epicentre Ribo-Zero[™] rRNA Removal Kit (Epicentre, America) were used to remove rRNA before the RNA-seq library construction. Then, the rRNA-depleted RNAs were then fragmented and reverse-transcribed to obtain cDNA libraries using the Truseq[™] RNA sample prep Kit (Illumina, America). Small RNA-seq library construction was performed according to the instructions of NEBNext Multiplex Small RNA library Prep (Illumina, America), which was followed by TRIzol extraction. Then, the library was amplified and enriched using PCR technique, proprietary indexed adapters were then ligated to 5'- and 3'-termini, and later electrophoresed using 15% concentration of agarose gel to obtain the target fragments. Finally, all sequenced libraries were sent to the sequencing company (Personalbio, Shanghai, China) and sequenced by Illumina Hiseq4000 platform for paired-end sequencing after quality control.

Bioinformatics Primary Analysis

The reference genome used in this experiment was *Bos taurus* ftp:([ftp.ensembl.org/pub/release-101/fasta/bos_taurus/dna](ftp://ftp.ensembl.org/pub/release-101/fasta/bos_taurus/dna)). For miRNA, Hiseq Single-End mode sequencing data were mapped between reference genome sequences after quality control using miRDeep2 [66] software. The mature miRNA and precursor sequences of the species miRNAs were downloaded from miRbase (<https://www.mirbase.org/>). Then the de-duplicated sequences were mapped and annotate. Sequences that were not annotated with any information were analyzed using mireap (<http://sourceforge.net/projects/mireap>). DESeq V1.18.0 [67] was performed to analyze miRNAs for differential expression and filtered by $|\log_2\text{Fold-Change}| > 1$ and $P\text{-value} < 0.05$ to filter out differentially conserved miRNAs.

For the circRNA and mRNA, downstream data were used for initial evaluation of raw data using FastQC v0.11.9 software after RNA-seq was completed. Cutadapt v2.6 was used to filter lower quality data as well as adapters. The obtained clean data were used for the subsequent analysis of circRNA and mRNA. During the analysis of mRNA, the upgraded HISAT2 (<http://ccb.jhu.edu/software/hisat2/index.shtml>) software of TopHat2 was used to map reads with reference genes. The differentially expressed transcripts were analyzed using DESeq, and the differentially expressed genes were screened for $|\log_2\text{FoldChange}| > 1$, $P\text{-value} < 0.05$. During the

circRNA prediction analysis, the 20 bp of two ends of the reads that were not matched on the HISAT2 were realignment and used as Anchors. Ffind_circ [68] was used for circRNA identification. CircRNA expression was analyzed for differential expression, and the screened standard was $|\log_2\text{FoldChange}| > 1$ and $P\text{-value} < 0.05$. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis [69–71] were performed using DAVID (<https://david.ncifcrf.gov/>). In addition, miRNA targeting relationships to circRNA and mRNA were predicted using miRanda and psRobot software.

RT-qPCR validation

After obtained sequencing results, RNA was extracted from the longissimus dorsi tissue using TRIzol (Thermo Fisher, Shanghai, China). RNA was reverse-transcribed to cDNA using the method provided in the introductions of PrimeScript RT Reagent Kit-Prefect Real Time (Takara, Beijing, China) for validation of mRNA and circRNA. miRNA 1st Strand cDNA Synthesis Kit (by stem-loop) (Vazyme, Nanjing, China) was used for miRNA reverse transcription. 2×Ultra SYBR Green qPCR Mix (CISTRO, Shanghai, China) was used for RT-qPCR in an Applied Biosystems QuantStudio 5 (Thermo Fisher, Shanghai, China). The reaction mixture containing, reaction conditions and primer sequences for the RT-qPCR validation procedure are stored in Table S10. The results were statistically analyzed using the $2^{-\Delta\Delta CT}$ method [72]. All data are expressed as the average of 4 independent experiments.

Statistical analysis

The comparative analysis of two groups was performed using unpaired independent t-test. SPSS 25.0 (SPSS Inc., Chicago, IL) and GraphPad prism 9 was used for statistical analyses. Results were considered statistically significant differences at $P < 0.05$ and were expressed as the mean \pm Standard Deviation unless otherwise stated.

Conclusions

In this study, we found that the pH drop and brightness of beef from LQC were significantly higher than those from LFC. In addition, the small cross-sectional area and diameter of muscle fibers produced better tenderness for beef from LQC. However, LFC was found to have superior redness compared with LQC. Furthermore, we identified several circRNAs related to muscle production and metabolism by ceRNA network building and analysis. By analyzing the source genes of DE circRNAs, we found that *btacirc_00497* and *btacirc_034497* may regulate the expression of MYH6, MYH7, and NEB by competing for linear shear, thereby altering the muscle fiber structure. Therefore, the ceRNA network-related genes and

circRNA-derived genes identified in this study could be used as tools for regulating muscle production and meat quality of cattle grown in a subtropical environment.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-023-09566-0>.

Supplementary Material 1 Figure S1. Test of molecular biological validation about RNA sequencing

Supplementary Material 2 Table S1. RNA sequencing information statistics

Supplementary Material 3 Table S2. Differentially Expressed Gene Catalog

Supplementary Material 4 Table S3. Differential expressed mRNA enrichment analysis

Supplementary Material 5 Table S4. circRNA information from find_circ

Supplementary Material 6 Table S5. Co-expression Network Relationships and Network Analysis

Supplementary Material 7 Table S6. Target mRNA GO Enrichment Analysis

Supplementary Material 8 Table S7. Target mRNA KEGG enrichment analysis

Supplementary Material 9 Table S9. circRNA source gene KEGG enrichment analysis

Supplementary Material 10 Table S10. Primer and reaction system information

Supplementary Material 11 Table S8. circRNA source gene GO enrichment analysis

Acknowledgements

We gratefully acknowledge the invaluable help from lab technicians of Gene Bank of Guangdong Livestock and Poultry in the sample collection process and providing computational resources in this work.

Authors' contributions

C. Yang, L. Wu, Y. Guo and M. Deng conceived and designed the study; C. Yang, L. Wu, Y. Guo, Y. Li and D. Liu performed the experiments; G. Liu and Y. Guo organized the database and performed the statistical analysis; C. Yang, B. Sun, wrote the manuscript; C. Yang, B. Sun visualized the results; Y. Guo and B. Sun revised the manuscript.

Funding

This research has received funding from the Key Realm R&D Program of Guangdong Province (2022B0202110002), Guangdong Basic and Applied Basic Research Foundation of China (2019B1515210020).

Data Availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (CRA007241 and CRA007242) that are publicly accessible at <https://ngdc.cncb.ac.cn/gsa>.

Declarations

Ethical approval

Our study was conducted in accordance with ARRIVE guidelines and no anesthetic or euthanasia agents were used in our study. All experimental animal procedures were in accordance with the relevant guidelines and regulations for the management and welfare of experimental animals approved by the Ethics Committee of South China Agricultural University. This study was approved by the Ethics Committee of South China Agricultural University in Guangdong Province.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 11 November 2022 / Accepted: 8 August 2023

Published online: 29 August 2023

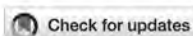
References

- Zhang W, Gao X, Zhang Y, Zhao Y, Zhang J, Jia Y, et al. Genome-wide assessment of genetic diversity and population structure insights into admixture and introgression in Chinese indigenous cattle. *BMC Genet.* 2018;19(1):114. <https://doi.org/10.1186/s12863-018-0705-9>.
- Huai Q, Zhiyong J, Jihie C. A survey of cattle production in China. *World Anim Rev.* 1993;76.
- Zeng L, Chen N, Ning Q, Yao Y, Chen H, Dang R, et al. PRLH and SOD1 gene variations associated with heat tolerance in Chinese cattle. *Anim Genet.* 2018;49(5):447–51. <https://doi.org/10.1111/age.12702>.
- Hong C, Huai Q. Studies on sex chromosome polymorphism of four local cattle (*Bos taurus*) breeds in China. *Heredity.* 1993.
- Liu Y, Xu L, Yang L, Zhao G, Li J, Liu D, et al. Discovery of genomic characteristics and selection signatures in Southern Chinese local cattle. *Front Genet.* 2020;11:533052. <https://doi.org/10.3389/fgene.2020.533052>.
- Lu X, Arbab AAI, Zhang Z, Fan Y, Han Z, Gao Q, et al. Comparative transcriptomic analysis of the Pituitary gland between cattle breeds differing in growth: Yunling cattle and Leiqiong cattle. *Anim (Basel).* 2020;10(8). <https://doi.org/10.3390/ani10081271>.
- Santiago GG, Siqueira F, Cardoso FF, Regitano LCA, Ventura R, Sollero BP, et al. Genomewide association study for production and meat quality traits in Canchim beef cattle. *J Anim Sci.* 2017;95(8):3381–90. <https://doi.org/10.2527/jas.2017.1570>.
- Picard B, Gagaoua M. Muscle Fiber Properties in cattle and their Relationships with meat qualities: an overview. *J Agric Food Chem.* 2020;68(22):6021–39. <https://doi.org/10.1021/acs.jafc.0c02086>.
- Zhuang X, Lin Z, Xie F, Luo J, Chen T, Xi Q, et al. Identification of circRNA-associated ceRNA networks using longissimus thoracis of pigs of different breeds and growth stages. *BMC Genomics.* 2022;23(1):294. <https://doi.org/10.1186/s12864-022-08515-7>.
- Lee SH, Joo ST, Ryu YC. Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. *Meat Sci.* 2010;86(1):166–70. <https://doi.org/10.1016/j.meatsci.2010.04.040>.
- Lefaucheur L. Myofiber typing and pig meat production. *Slovenski Veterinar-ski Zbornik.* 2001;5–29.
- He S, Fu T, Yu Y, Liang Q, Li L, Liu J, et al. IRE1 α regulates skeletal muscle regeneration through myostatin mRNA decay. *J Clin Invest.* 2021;e143737. <https://doi.org/10.1172/JCI143737>.
- Coudert L, Osseni A, Gangloff YG, Schaeffer L, Leblanc P. The ESCRT-0 subcomplex component Hrs/Hgs is a master regulator of myogenesis via modulation of signaling and degradation pathways. *BMC Biol.* 2021;19(1):153. <https://doi.org/10.1186/s12915-021-01091-4>.
- Archacka K, Ciemerych MA, Florkowska A, Romanczuk K. Non-coding RNAs as regulators of myogenesis and postexercise muscle regeneration. *Int J Mol Sci.* 2021;22(21). <https://doi.org/10.3390/ijms222111568>.
- Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanani M, et al. circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell.* 2014;56(1):55–66. <https://doi.org/10.1016/j.molcel.2014.08.019>.
- Zhang P, Chao Z, Zhang R, Ding R, Wang Y, Wu W, et al. Circular RNA regulation of myogenesis. *Cells.* 2019;8(8). <https://doi.org/10.3390/cells8080885>.
- Yue B, Wang J, Song C, Wu J, Cao X, Huang Y, et al. Biogenesis and ceRNA role of circular RNAs in skeletal muscle myogenesis. *Int J Biochem Cell Biol.* 2019;117:105621. <https://doi.org/10.1016/j.biocel.2019.105621>.
- Liu R, Liu X, Bai X, Xiao C, Dong Y. Identification and characterization of circRNA in Longissimus Dorsi of different breeds of cattle. *Front Genet.* 2020;11:565085. <https://doi.org/10.3389/fgene.2020.565085>.
- Huang K, Chen M, Zhong D, Luo X, Feng T, Song M, et al. Circular RNA profiling reveals an abundant circEch1 that promotes myogenesis and differentiation of bovine skeletal muscle. *J Agric Food Chem.* 2021;69(1):592–601. <https://doi.org/10.1021/acs.jafc.0c06400>.
- Zhang R-M, Pan Y, Zou C-X, An Q, Cheng J-R, Li P-J, et al. CircUBE2Q2 promotes differentiation of cattle muscle stem cells and is a potential regulatory molecule of skeletal muscle development. *BMC Genomics.* 2022;23(1):267. <https://doi.org/10.1186/s12864-022-08518-4>.
- Zhang Y, Yang Y, Ju H, He X, Sun P, Tian Y, et al. Comprehensive profile of circRNAs in formaldehyde induced heart development. *Food Chem Toxicol.* 2022;162:112899. <https://doi.org/10.1016/j.fct.2022.112899>.
- Lefaucheur L. A second look into fibre typing—relation to meat quality. *Meat Sci.* 2010;84(2):257–70. <https://doi.org/10.1016/j.meatsci.2009.05.004>.
- Klont RE, Brocks L, Eikelenboom G. Muscle fibre type and meat quality. *Meat Sci.* 1998;49S1:219–S229.
- Wicks J, Beline M, Gomez JFM, Luzardo S, Silva SL, Gerrard D. Muscle energy metabolism, growth, and meat quality in beef cattle. *Agriculture-Basel.* 2019;9(9). <https://doi.org/10.3390/agriculture9090195>.
- Whipple G, Koohmaria M, Dikeman ME, Crouse JD. Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. *J Anim Sci.* 1990;68(12):4193–9. <https://doi.org/10.2527/1990.68124193x>.
- Hughes JM, Clarke FM, Purslow PP, Warner RD. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. *Compr Rev Food Sci Food Saf.* 2020;19(1):44–63. <https://doi.org/10.1111/1541-4337.12509>.
- Purslow PP, Warner RD, Clarke FM, Hughes JM. Variations in meat colour due to factors other than myoglobin chemistry: a synthesis of recent findings (invited review). *Meat Sci.* 2020;159:107941. <https://doi.org/10.1016/j.meatsci.2019.107941>.
- Suman SP, Joseph P. Myoglobin chemistry and meat color. *Annu Rev Food Sci Technol.* 2013;4:79–99. <https://doi.org/10.1146/annurev-food-030212-182623>.
- Mancini RA, Hunt MC. Current research in meat color. *Meat Sci.* 2005;71(1):100–21. <https://doi.org/10.1016/j.meatsci.2005.03.003>.
- Listrat A, Gagaoua M, Normand J, Gruffat D, Andueza D, Mairesse G, et al. Contribution of connective tissue components, muscle fibres and marbling to beef tenderness variability in longissimus thoracis, rectus abdominis, semimembranosus and semitendinosus muscles. *J Sci Food Agric.* 2020;100(6):2502–11. <https://doi.org/10.1002/jsfa.10275>.
- White A, O'Sullivan A, Troy DJ, O'Neill EE. Manipulation of the pre-rigor glycolytic behaviour of bovine M. longissimus dorsi in order to identify causes of inconsistencies in tenderness. *Meat Sci.* 2006;73(1):151–6. <https://doi.org/10.1016/j.meatsci.2005.11.021>.
- Gagaoua M, Picard B, Monteils V. Assessment of cattle inter-individual cluster variability: the potential of continuum data from the farm-to-fork for ultimate beef tenderness management. *J Sci Food Agric.* 2019;99(8):4129–41. <https://doi.org/10.1002/jsfa.9643>.
- Albrecht E, Teuscher F, Ender K, Wegner J. Growth- and breed-related changes of muscle bundle structure in cattle. *J Anim Sci.* 2006;84(11):2959–64. <https://doi.org/10.2527/jas.2006-345>.
- Li X, Yang L, Chen L-L. The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol Cell.* 2018;71(3):428–42. <https://doi.org/10.1016/j.molcel.2018.06.034>.
- Cai B, Ma M, Zhou Z, Kong S, Zhang J, Zhang X, et al. circPTPN4 regulates myogenesis via the miR-499-3p/NAMPT axis. *J Anim Sci Biotechnol.* 2022;13(1):2. <https://doi.org/10.1186/s40104-021-00664-1>.
- Ru W, Qi A, Shen X, Yue B, Zhang X, Wang J, et al. The circular RNA circCPE regulates myoblast development by sponging miR-138. *J Anim Sci Biotechnol.* 2021;12(1):102. <https://doi.org/10.1186/s40104-021-00618-7>.
- Glass DJ. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat Cell Biol.* 2003;5(2):87–90. <https://doi.org/10.1038/ncb0203-87>.
- Yu M, Wang H, Xu Y, Yu D, Li D, Liu X, et al. Insulin-like growth factor-1 (IGF-1) promotes myoblast proliferation and skeletal muscle growth of embryonic chickens via the PI3K/Akt signalling pathway. *Cell Biol Int.* 2015;39(8):910–22. <https://doi.org/10.1002/cbin.10466>.
- Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyeva Y, Kline WO, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell.* 2004;14(3):395–403. [https://doi.org/10.1016/s1097-2765\(04\)00211-4](https://doi.org/10.1016/s1097-2765(04)00211-4).
- Kumar A, Xie L, Ta CM, Hinton AO, Gunasekar SK, Minerath RA, et al. SWELL1 regulates skeletal muscle cell size, intracellular signaling, adiposity and glucose metabolism. *Elife.* 2020;9:e58941. <https://doi.org/10.7554/eLife.58941>.
- Shen X, Tang J, Jiang R, Wang X, Yang Z, Huang Y, et al. CircRILP1 promotes muscle proliferation and differentiation via binding miR-145 to activate

- IGF1R/PI3K/AKT pathway. *Cell Death Dis.* 2021;12(2):142. <https://doi.org/10.1038/s41419-021-03419-y>.
42. Zhang XY, Yang SL, Kang ZH, Ru WX, Shen XM, Li M et al. circMEF2D negatively regulated by HNRNP1 inhibits proliferation and differentiation of Myoblasts via miR-486-PI3K/AKT Axis. *J Agric Food Chem* 8145–63. <https://doi.org/10.1021/acs.jafc.2c01888>.
43. Lee J, Hong F, Kwon S, Kim SS, Kim DO, Kang HS, et al. Activation of p38 MAPK induces cell cycle arrest via inhibition of Raf/ERK pathway during muscle differentiation. *Biochem Biophys Res Commun.* 2002;298(5):765–71. [https://doi.org/10.1016/S0006-291X\(02\)02562-7](https://doi.org/10.1016/S0006-291X(02)02562-7).
44. Bengal E, Aviram S, Hayek T. p38 MAPK in glucose metabolism of skeletal muscle: beneficial or harmful? *Int J Mol Sci.* 2020;21(18). <https://doi.org/10.3390/ijms21186480>.
45. Somwar R, Koterski S, Sweeney G, Sciotti R, Djuric S, Berg C, et al. A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation. *J Biol Chem.* 2002;277(52):50386–95. <https://doi.org/10.1074/jbc.M205277200>.
46. Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, et al. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem.* 2005;280(20):19587–93. <https://doi.org/10.1074/jbc.M408862200>.
47. Pogozelski AR, Geng T, Li P, Yin X, Lira VA, Zhang M, et al. p38gamma mitogen-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. *PLoS ONE.* 2009;4(11):e7934. <https://doi.org/10.1371/journal.pone.0007934>.
48. Kuo IY, Ehrlich BE. Signaling in muscle contraction. *Cold Spring Harb Perspect Biol.* 2015;7(2):a006023. <https://doi.org/10.1101/cshperspect.a006023>.
49. Tarasova NV, Vishnyakova PA, Logashina YA, Elchaninov AV. Mitochondrial calcium Uniporter structure and function in different types of muscle tissues in Health and Disease. *Int J Mol Sci.* 2019;20(19):4823. <https://doi.org/10.3390/ijms20194823>.
50. Stiber JA, Rosenberg PB. The role of store-operated calcium influx in skeletal muscle signaling. *Cell Calcium.* 2011;49(5):341–9. <https://doi.org/10.1016/j.ceca.2010.11.012>.
51. Berridge MJ. The Inositol Trisphosphate/Calcium Signaling Pathway in Health and Disease. *Physiol Rev.* 2016;96(4):1261–96. <https://doi.org/10.1152/physrev.00006.2016>.
52. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev.* 2011;91(4):1447–531. <https://doi.org/10.1152/physrev.00031.2010>.
53. Bottinelli R, Reggiani C. Human skeletal muscle fibres: molecular and functional diversity. *Prog Biophys Mol Biol.* 2000;73(2–4):195–262. [https://doi.org/10.1016/S0079-6107\(00\)00006-7](https://doi.org/10.1016/S0079-6107(00)00006-7).
54. Ebarb SM, Phelps KJ, Drouillard JS, Maddock-Carlin KR, Vaughn MA, Burnett DD, et al. Effects of anabolic implants and ractopamine-HCl on muscle fiber morphometrics, collagen solubility, and tenderness of beef longissimus lumborum steaks. *J Anim Sci.* 2017;95(3):1219–31. <https://doi.org/10.2527/jas.2016.1263>.
55. Scheffler TL, Leitner MB, Wright SA. Technical note: protocol for electrophoretic separation of bovine myosin heavy chain isoforms and comparison to immunohistochemistry analysis. *J Anim Sci.* 2018;96(10):4306–12. <https://doi.org/10.1093/jas/sky283>.
56. Bao G, Liu X, Wang J, Hu J, Shi B, Li S, et al. Effects of Slaughter Age on myosin heavy chain isoforms, muscle fibers, fatty acids, and Meat Quality in muscle of Tibetan Sheep. *Front Vet Sci.* 2021;8:689589. <https://doi.org/10.3389/fvets.2021.689589>.
57. Immonen K, Ruusunen M, Hissa K, Puolanne E. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. *Meat Sci.* 2000;55(1):25–31. [https://doi.org/10.1016/S0309-1740\(99\)00121-7](https://doi.org/10.1016/S0309-1740(99)00121-7).
58. Della Rosa MM, Pouzo LB, Pavan E. Meat and fat quality traits of grazing steers supplemented with corn grain and increasing amounts of flaxseed. *Livest Sci.* 2018;208:51–4. <https://doi.org/10.1016/j.livsci.2017.12.004>.
59. McGilchrist P, Alston CL, Gardner GE, Thomson KL, Pethick DW. Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. *Meat Sci.* 2012;92(4):474–80. <https://doi.org/10.1016/j.meatsci.2012.05.014>.
60. Witt CC, Burkart C, Labeit D, McNabb M, Wu Y, Granzier H, et al. Nebulin regulates thin filament length, contractility, and Z-disk structure in vivo. *EMBO J.* 2006;25(16):3843–55. <https://doi.org/10.1038/sj.emboj.7601242>.
61. Conover GM, Gregorio CC. The desmin coil 1B mutation K190A impairs nebulin Z-disc assembly and destabilizes actin thin filaments. *J Cell Sci.* 2011;124(Pt 20):3464–76. <https://doi.org/10.1242/jcs.087080>.
62. Gokhin DS, Bang M-L, Zhang J, Chen J, Lieber RL. Reduced thin filament length in nebulin-knockout skeletal muscle alters isometric contractile properties. *Am J Physiol Cell Physiol.* 2009;296(5):C1123. <https://doi.org/10.1152/ajpcell.00503.2008>.
63. Bang M-L, Li X, Littlefield R, Bremner S, Thor A, Knowlton KU, et al. Nebulin-deficient mice exhibit shorter thin filament lengths and reduced contractile function in skeletal muscle. *J Cell Biol.* 2006;173(6):905–16. <https://doi.org/10.1083/jcb.200603119>.
64. Honikel KO. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* 1998;49(4):447–57. [https://doi.org/10.1016/S0309-1740\(98\)00034-5](https://doi.org/10.1016/S0309-1740(98)00034-5).
65. Wang Y, Wang Z, Hu R, Peng Q, Xue B, Wang L. Comparison of carcass characteristics and meat quality between Simmental crossbred cattle, cattle-yaks and Xuanhan yellow cattle. *J Sci Food Agric.* 2021;101(9):3927–32. <https://doi.org/10.1002/jsfa.11032>.
66. Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N. miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res.* 2012;40(1):37–52. <https://doi.org/10.1093/nar/gkr688>.
67. Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biol.* 2010;11(10):R. <https://doi.org/10.1186/gb-2010-11-10-r106>.
68. Gao Y, Wang J, Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. *Genome Biol.* 2015;16:4. <https://doi.org/10.1186/s13059-014-0571-3>.
69. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* 2023;51(D1):D587. <https://doi.org/10.1093/nar/gkac963>.
70. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* 2019; 28(11):1947–1951. <https://doi.org/10.1002/pro.3715>.
71. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 2000;28(1):27–30. <https://doi.org/10.1093/nar/28.1.27>.
72. Bustin SA, Benes V, Garson JA, Helleman J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem.* 2009;55(4):611–22. <https://doi.org/10.1373/clinchem.2008.112797>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



OPEN ACCESS

EDITED BY

Mohammad Reza Bakhtiarzadeh,
University of Tehran, Iran

REVIEWED BY

Xiaoyun He,
Chinese Academy of Agricultural
Sciences, China
Zezhang Liu,
Beijing Genomics Institute (BGI), China
Gan Shangquan,
Xinjiang Academy of Agricultural and
Reclamation Sciences (XAARS), China

*CORRESPONDENCE

Dewu Liu,
✉ dwliu@scau.edu.cn
Guangbin Liu,
✉ gblu@scau.edu.cn

RECEIVED 27 September 2023

ACCEPTED 01 December 2023

PUBLISHED 12 December 2023

CITATION

Mao S, Dong S, Hou B, Li Y, Sun B, Guo Y,
Deng M, Liu D and Liu G (2023),
Transcriptome analysis reveals pituitary
lncRNA, circRNA and mRNA affecting
fertility in high- and low-yielding goats.
Front. Genet. 14:1303031.
doi: 10.3389/fgene.2023.1303031

COPYRIGHT

© 2023 Mao, Dong, Hou, Li, Sun, Guo,
Deng, Liu and Liu. This is an open-access
article distributed under the terms of the
Creative Commons Attribution License
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original author(s)
and the copyright owner(s) are credited
and that the original publication in this
journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Transcriptome analysis reveals pituitary lncRNA, circRNA and mRNA affecting fertility in high- and low-yielding goats

Shuaixiang Mao, Shucan Dong, Biwei Hou, Yaokun Li, Baoli Sun,
Yongqing Guo, Ming Deng, Dewu Liu* and Guangbin Liu*

College of Animal Science, South China Agricultural University, Guangzhou, China

The pituitary gland serves as the central endocrine regulator of growth, reproduction, and metabolism and plays a crucial role in the reproductive process of female animals. Transcriptome analysis was conducted using pituitary gland samples from Leizhou goats with varying levels of fecundity to investigate the effects of long noncoding RNA (lncRNA), circular RNA (circRNA), and mRNA regulation on pituitary hormone secretion and its association with goat fecundity. The analysis aimed to identify lncRNAs, circRNAs, and mRNAs that influence the fertility of Leizhou goats. GO and KEGG enrichment analyses were performed on differentially expressed lncRNAs, circRNAs, and mRNAs and revealed considerable enrichment in pathways, such as regulation of hormone secretion, germ cell development, and gonadotropin-releasing hormone secretion. The pituitary lncRNAs (ENSCHIT00000010293, ENSCHIT00000010304, ENSCHIT00000010306, ENSCHIT00000010290, ENSCHIT00000010298, ENSCHIT00000006769, ENSCHIT00000006767, ENSCHIT00000006921, and ENSCHIT00000001330) and circRNAs (chicirc_029285, chicirc_026618, chicirc_129655, chicirc_018248, chicirc_122554, chicirc_087101, and chicirc_078945) identified as differentially expressed regulated hormone secretion in the pituitary through their respective host genes. Additionally, differential mRNAs (GABBR2, SYCP1, HNF4A, CBLN1, and CDKN1A) influenced goat fecundity by affecting hormone secretion in the pituitary gland. These findings contribute to the understanding of the molecular mechanisms underlying pituitary regulation of fecundity in Leizhou goats.

KEYWORDS

Leizhou goat, pituitary, fertility, lncRNA, circRNA, mRNA

1 Introduction

Goats are among the earliest domesticated animals and widely distributed worldwide as livestock. Given their small size and high productivity, they have no competition with humans in terms of food, and they can provide human beings with meat, milk, and other daily necessities (Ahlawat et al., 2015; Yao et al., 2023). They also play a crucial role in animal husbandry. With the rapid development of the social economy and the improvement of peoples living standards, meat consumption has increased substantially. However, the slow development of the mutton goat industry has led to a widening gap between production and consumption (Kantonio et al., 2021; Li et al., 2022). Genetic enhancement of the reproductive

rate in low-yield animals can help fill this production–demand gap (Islam et al., 2020). Litter size is a crucial economic indicator in the goat industry. However, goats generally have a low reproductive rate, with an average litter size of 1–2, and their low fecundity greatly inhibits the development of the goat breeding industry (Wang et al., 2012). Leizhou goats, a superior goat breed in southern China, are known for their early maturity, fast growth, and high-quality meat (Dong et al., 2023). However, a difference in fertility exists between low- and high-fertility populations of Leizhou goats. The use of high-throughput sequencing technology to identify genes associated with reproductive traits in Leizhou goats can provide valuable insights into the genetic factors that influence goat fertility at the molecular level.

The litter size of goats is closely related to the number of mature oocytes released during the ovulation cycle (Zhao et al., 2015). Follicle development and maturation are regulated by the secretion of various hormones from the hypothalamic–pituitary–gonadal axis and by several growth factors and cytokines expressed by the ovaries and follicles (Chronowska, 2014). The pituitary gland is a key regulator of the hypothalamic–pituitary–gonadal axis (Wan et al., 2022b) and the central endocrine regulator of growth, reproduction, and metabolism. The secretion of synthetic hormones in adenohypophysis is not only regulated by gonadotropin-releasing hormones (GnRHs; Li et al., 2019), but also influenced by certain noncoding RNAs present in pituitary cells (Wan et al., 2022a). However, research on the underlying molecular regulatory mechanisms is limited.

The regulation of goat reproduction is a complex biological process that involves coordinated gene interactions. The pituitary gland plays an essential role in regulating reproductive performance, and this regulation involves coding and noncoding RNAs. Studies on the effects of long noncoding and circular RNAs on reproductive performance have focused on the ovaries (Miao et al., 2017; Liu et al., 2021; Zhang et al., 2022) and granulosa cells (Li et al., 2018; Li X. et al., 2021; Zhao et al., 2023), and research on their effects on reproductive performance in the pituitary gland is limited (Yang et al., 2020). Long noncoding RNA (lncRNA) is transcribed by RNA polymerase II/III and is a type of RNA molecule whose length exceeds 200 bp. It is widely present in eukaryotes but cannot encode functional proteins (Zhang et al., 2020; Lee and Kang, 2022). Circular RNA (circRNA) is an endogenous, single-stranded, covalently closed noncoding RNA. Its unique circular structure renders circRNA more stable than linear RNA (Du Toit, 2013; Gao et al., 2023). Noncoding RNAs influence the expression of coding genes through competitive regulatory networks, thereby affecting various biological processes (Qiannan et al., 2020). Transcriptome sequencing technology provides information on nearly all coding and noncoding genes, thus enabling us to explore the mutual regulatory relationships between genes and elucidate the underlying molecular mechanisms (Costa et al., 2010). Therefore, transcriptome technology can help in understanding the molecular mechanisms of pituitary regulation of animal reproduction by identifying genes associated with pituitary regulation of reproductive performance.

This study utilized transcriptome technology to investigate the pituitary glands of low- and high-fertility Leizhou goats. The objective was to identify differentially expressed functional genes

that may affect the reproductive performance of Leizhou goats. The findings can serve as a reference for understanding the molecular mechanisms that regulate the reproduction of Leizhou goats and provide a theoretical foundation for studying the prolific traits of this breed.

2 Materials and methods

2.1 Experimental animals and sample collection

Seven healthy Leizhou female goats aged between 3.5 and 4.5 years were selected for this study. All the goats had more than three previous litters and were managed under consistent-feeding and management conditions. The goats were divided into two groups on the basis of fertility: high-fertility group ($n = 3$) where ewes had two or more litters per pregnancy and low-fertility group ($n = 4$) where ewes had only one litter per pregnancy (Supplementary Table S11). After synchronized estrus, all seven Leizhou goats were slaughtered. The pituitary glands were collected, immediately placed in frozen tubes, flash-frozen using liquid nitrogen, and stored for the long term at -80°C in a refrigerator.

2.2 RNA extraction, cDNA library preparation, and sequencing

Total RNA was extracted from the samples by using the Trizol reagent (Thermo Fisher, Shanghai, China) in accordance with the manufacturer's instructions, and ribosomal RNA in total RAN was removed with the Ribo-Zero rRNA Removal Kit (Illumina, Inc.). The absence of any genomic DNA contamination was confirmed by 1% agarose gel electrophoresis for RNA degradation. The total amount of RNA and its integrity were determined using the Agilent 2100 Bioanalyzer and RNA 6000 Nano LabChip Kit (Agilent, Santa Clara, United States). The samples that met the test requirements were sent to Shanghai Personalbio Technology Co., Ltd. (Shanghai, China). Sequencing was performed using the Illumina HiSeq 2500 platform. Exactly 1 μL of total RNA from each sample was used for library construction, and the RNA was cleaved into 200- to 300-bp fragments, followed by first-strand cDNA synthesis using random hexamer primers and reverse transcriptase and second-strand cDNA synthesis. Double-end sequencing (150 bp) was performed with the NEB Next Ultra Directional RNA Library Prep Kit for Illumina (NEB, Ipswich, United States).

2.3 Quality assessment of raw sequencing data and assembly of transcripts

After the raw image data generated by the HiSeq platform were converted into raw data in FASTQ format, the raw sequence data were quality checked using Cutadapt to remove joints and low-quality reads. The filtered clean reads were aligned to the *Capra hircus* reference genome by using HiSAT2. After the comparison bam file was obtained, String Tie software was used to align the reads

on the genome, count the expression levels of transcripts in each sample, and standardize lncRNA and mRNA as transcripts per kilobase million (FPKM) and circRNA as transcripts per million (TPM).

2.4 Screening of lncRNAs and circRNAs

In the transcript assembly results, single-exon transcripts with low expression were filtered out. Transcripts with an exon number greater than or equal to 2 and a length of at least 200 bp were selected. Cuffcompare software was used to screen the transcripts with overlapping exon regions of the database annotation, and the lncRNA in the database with overlapping exon regions of this spliced transcript was included in the subsequent analysis as the database annotation lncRNA. The transcripts without coding ability judged by PLEK, CNCI, and Pfamscan software were high-confidence lncRNAs. The expression level of lncRNA in each sample was determined and homogenized by FPKM. After alignment with the reference genome, the nonalignment reads were used to identify circRNAs. After alignment of the anchor sequence of each sample with the reference genome, we combined the alignment results of all the samples and used find_circ to identify circRNAs. Then, the high-confidence circRNAs were filtered based on the following criteria: 1) breakpoints = 1; 2) anchor_overlap \leq 2; 3) edit \leq 2; 4) n_uniq > 2, n_uniq > samples, and n_uniq > int (1/2 samples); 5) best_qual_A > 35 or best_qual_B > 35; and 6) circRNA < 100 k in length. The expression level of circRNAs was estimated by TPM.

2.5 Differential expression and enrichment analyses

Differential analysis of gene expression in the two groups was performed using the R language DEseq package, and genes that met the differential fold $|\log_2 \text{Fold Change}| > 1$ and $p < 0.05$ were screened as differentially expressed genes. Gene ontology (GO) enrichment analysis was performed using top GO. The significantly enriched GO term was determined by the hypergeometric distribution method (the criterion for significant enrichment was $p < 0.05$), and KEGG pathway enrichment analysis was performed using the Cluster Profiler software for significant enrichment (the criterion for significant enrichment was $p < 0.05$). On the basis of the GO and KEGG enrichment analysis results and biological significance, the target genes were selected for follow-up study.

2.6 lncRNA–mRNA network co-expression construction

Cis-target gene regulation of lncRNAs depends on their nearby protein-coding genes, which are usually considered to be their target genes with 100 kb located upstream and downstream. The Pearson correlation test was performed to calculate the correlation coefficients of pituitary DE lncRNAs and DE mRNAs, with Spearman correlation coefficient > 0.8 and $p < 0.05$ as a

TABLE 1 Primer sequences for RT-qPCR.

Name	Primer type	Primer sequence
MSTRG.52870.6	Forward	ACAACAGTGTGACGATCCGT
	Reverse	TTGGAGTCCCATTATGGCGG
MSTRG.33887.2	Forward	TGTTTCTCGACGTGACCTG
	Reverse	AAGACGACTTTGGGCTGGAG
ENSCHIT0000008199	Forward	TGTTGCTTCTGACCTGCAT
	Reverse	GCCGCACCATCTATTCTGC
chicirc_061339	Forward	GAGAAATCTCAGCAGGCGGT
	Reverse	GTTCATGGGCTTTTGGCAG
chicirc_002310	Forward	CGTTGTACGATCACGCATC
	Reverse	AGCCTCGAAATCCAGCACAA
chicirc_131778	Forward	TGGTGACGTGGAAAAGACCC
	Reverse	CTCCTCGTCCGTGGTGTTC
CDKN1A	Forward	CCAGACCAGCATGACAGATTTC
	Reverse	GTGACAGCAAGCAGCGTATG
SYCP1	Forward	ATCTGCGTACACCTGCCAAA
	Reverse	TCCTCTGAAACCATGCTCAAA
RIMS4	Forward	GGAGTTTGTCTGGCATCGGA
	Reverse	CCTTGATGTAGCCGCTGG

conditional screen. The results were visualized using Cytoscape software (v3.9.1).

2.7 Construction of protein–protein interaction network

The interaction between predicted mRNA translation proteins was predicted using the STRING database with a confidence score ≥ 0.4 . Protein–protein interaction (PPI) network regulation was visualized by Cytoscape (v3.9.1).

2.8 RNA-seq validation by quantitative real-time PCR

In accordance with the sequencing results, three lncRNA, circRNA, and mRNA were selected for expression verification. GAPDH was used as the reference gene of lncRNA, circRNA, and mRNA, and reverse transcription was performed using a Takara reverse transcription kit. The 2 \times Ultra SYBR Green qPCR Mix fluorescence quantitative kit was employed to detect the gene expression. The real-time quantitative PCR (RT-qPCR) cycling parameters were as follows: predenaturation at 95°C for 10 min, followed by 40 cycles of 5 s at 95°C and 60°C for 20 s. Three biological replicates were used for each assay. The relative expression of the target genes was analyzed using the $2^{-\Delta\Delta CT}$ method. The primer sequences are shown in Table 1.

TABLE 2 Pituitary RNA sequencing profiles of low- and high-fertility Leizhou goats.

Sample	LZ_L1	LZ_L2	LZ_L3	LZ_L4	LZ_H1	LZ_H2	LZ_H3
Raw reads	105,065,052	103,763,540	106,273,534	102,644,634	106,205,704	106,878,816	103,869,098
Clean reads	104,649,542	103,389,924	105,891,248	102,277,412	105,838,156	106,542,068	103,497,872
Clean reads (%)	99.6	99.63	99.64	99.64	99.65	99.68	99.64
Q30 (%)	93.02	93.31	93.46	93	92.92	93.32	92.48
Q20 (%)	97.13	97.26	97.32	97.16	97.13	97.3	96.93
Total_Mapped (%)	84.42	88.02	87.43	85.34	86.96	87.62	84.1
Multiple_Mapped (%)	1.78	2.18	3.88	1.83	1.95	3.61	2.7
Uniquely_Mapped (%)	98.22	97.82	96.12	98.17	98.05	96.39	97.3

3 Results

3.1 Quality control of RNA-seq sequencing data

Prior to further analysis, quality control analyses were performed on raw reads from the low- and high-fertility groups. Seven independent cDNA libraries were constructed from pituitary tissue RNA of the low-fertility group (LZ_L) and high-fertility group (LZ_H). The sequencing profiles are shown in Table 2. A total of 734,700,378 raw reads were generated from the seven sequencing libraries. After quality control, 732,086,222 clean reads were left. The number of bases with 99.9% or higher accuracy per sample was 92.48%–93.46% of the total number of bases. Clean data were aligned to the reference genome, and over 84.1% of the reads were accurately aligned with a high matching rate. About 1.78%–3.88% of these clean reads had multiple aligned positions, and 96.12%–98.22% of them had single aligned positions. These data show that the sequencing results were of high quality and could be used for the subsequent analysis.

3.2 Differential expression analysis of lncRNA, circRNA, and mRNA

Differences in the gene or transcript expression of the pituitary, as the central regulator of the goat hypothalamic–pituitary–ovarian axis, may affect the reproductive ability of Leizhou goats. We screened for DE lncRNAs, DE circRNAs, and DE mRNAs between the low- and high-fertility groups on the basis of $|\log_2 \text{Fold Change}| > 1$ and $p < 0.05$. The low-fertility pituitary samples were used as the control group, and a total of 4,472 lncRNAs were detected in the low- and high-fertility group pituitary samples. Among these lncRNAs, 11 differentially expressed lncRNAs were identified, including 5 upregulated lncRNAs and 6 downregulated lncRNAs. The five upregulated lncRNAs mainly included MSTRG.52870.6, ENSCHIT00000005480, and MSTRG.45842.5, and the six downregulated lncRNAs mainly included ENSCHIT00000009877, ENSCHIT00000009854, and ENSCHIT00000008199 (Supplementary Table S1; Figure 1A).

The volcano plot revealed the identification of 23,671 circRNAs in the two groups. Six differentially expressed circRNAs were

present compared with the low-fertility group, all of which were upregulated genes and mainly included chicirc_061339, chicirc_002310, and chicirc_131778 (Supplementary Table S1; Figure 1B).

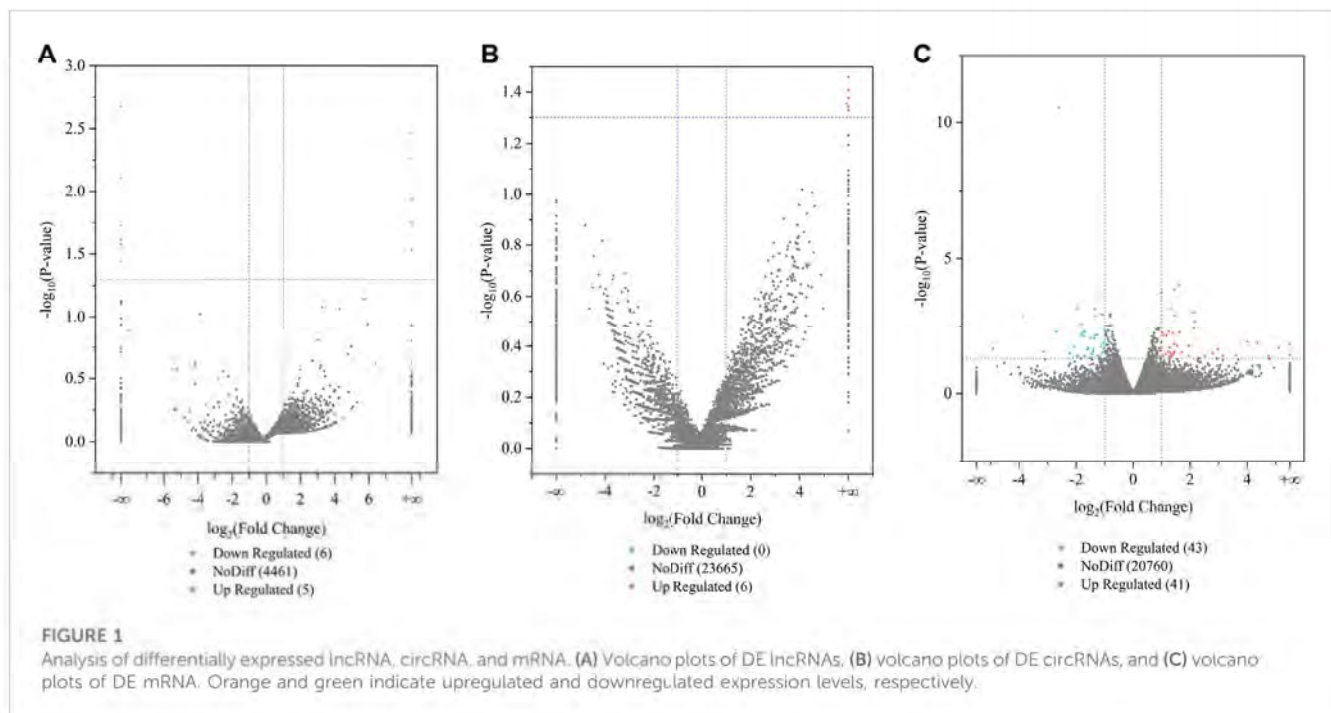
The volcano plot results also revealed the identification of 20,844 mRNAs in the two groups. A total of 84 mRNAs were differentially expressed, 41 mRNAs were upregulated, and 43 mRNAs were downregulated compared with the low-fertility group of Leizhou goats. The upregulated mRNAs mainly included RIMS4, SYCP1, LMNTD2, and NAP1L2, and the downregulated mRNAs mainly included SERPINE1, IL33, SLC44A5, and BAZ1A (Supplementary Table S1; Figure 1C). These differentially expressed transcripts are valuable for further studies on the reproductive performance of Leizhou goats.

3.3 Enrichment analysis of the pituitary function

3.3.1 Functional enrichment analysis of the target genes of differentially expressed lncRNAs

To thoroughly understand the biological functions of differentially expressed lncRNAs in the pituitary of low- and high-fertility groups, we predicted potential targets in terms of cis-regulatory relationships. We searched for 100-kb protein-coding genes upstream and downstream of the differentially expressed lncRNAs and performed GO function enrichment analysis on the target genes of the 11 differentially expressed lncRNAs. We found that the target genes of the differentially expressed lncRNAs were enriched to 853 GO terms. Differential significant enrichment analysis of the enrichment results yielded a total of 306 GO terms ($p < 0.05$) that were significantly enriched, of which 275 GO terms were for biological processes, 8 were for cellular composition, and 23 were for molecular functions. The four biological processes with the highest gene enrichment were cellular, metabolic, biological regulation, and regulation of the biological process, and the four cellular components were cell, cell part, extracellular region part, and extracellular region. The four most enriched molecular functions were nucleic acid binding transcription factor activity, binding, signal transducer factor activity and catalytic activity (Supplementary Table S2; Figures 2A, B).

KEGG enrichment analysis of the 11 differentially expressed lncRNA target genes resulted in 13 pathways, with seven



significantly enriched KEGG pathways ($p < 0.05$), including folate biosynthesis, spinocerebellar ataxia, Fc gamma R-mediated phagocytosis, and Wnt signaling pathway (Supplementary Table S3; Figures 2C, D).

3.3.2 Functional enrichment analysis of host genes of differentially expressed circRNAs

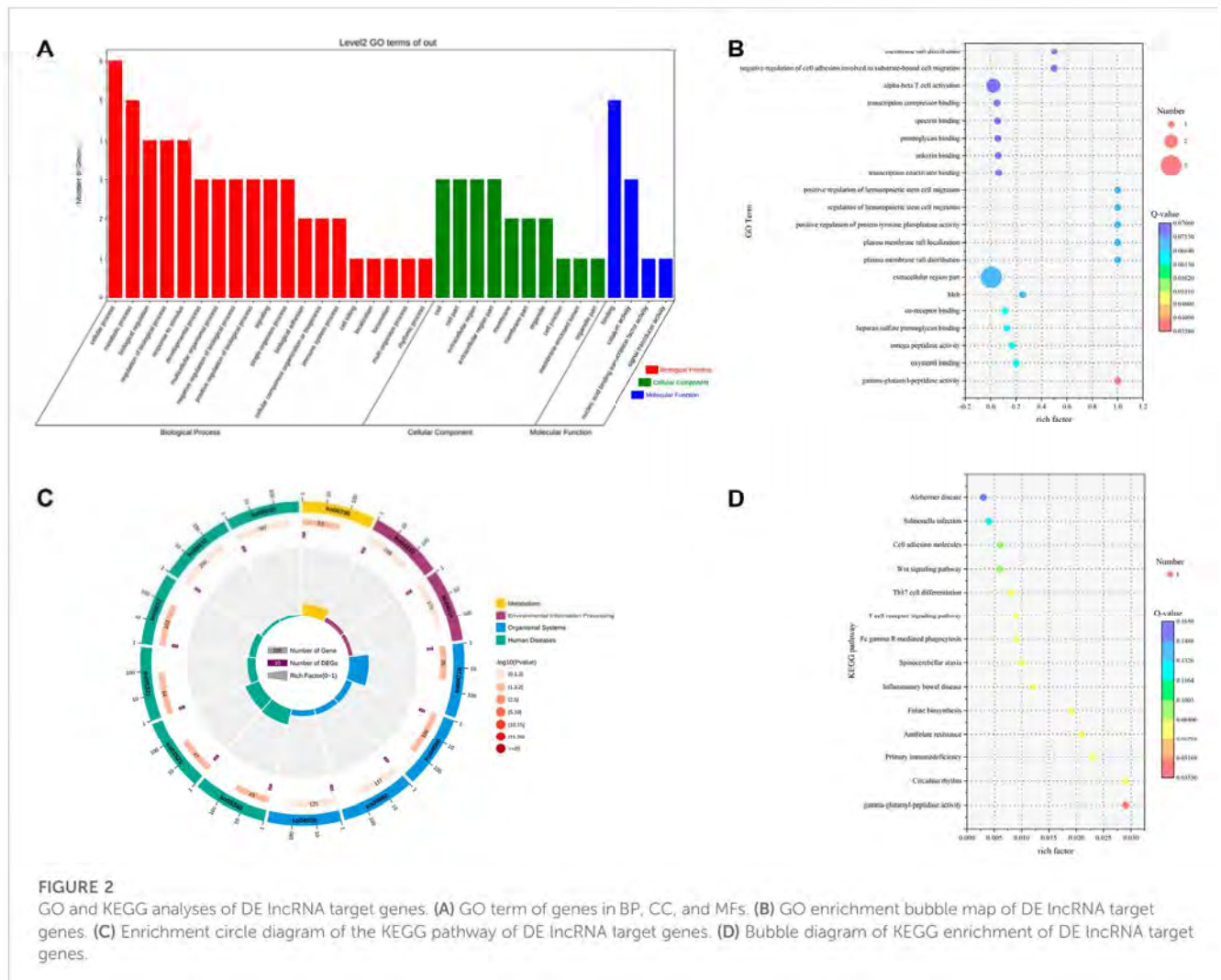
To explore the functions of differential circRNAs in the pituitary of Leizhou goats with high and low fertility, we performed GO functional enrichment analysis on the host genes of the six differentially expressed circRNAs and found that the host genes of the differentially expressed circRNAs were enriched to 693 GO terms. Differential significant enrichment analysis was performed on the enrichment results. A total of 322 GO terms ($p < 0.05$) were significantly enriched, of which 268 GO terms were for biological processes, 19 were for cellular components, and 35 were for molecular functions. The four biological processes with the highest gene enrichment were single-organism process, cellular component organization or biogenesis, cellular process, and localization, and the four cellular components with the highest gene enrichment were cell, cell part, macromolecular complex, and membrane. The four most enriched molecular functions were molecular transducer activity, binding, signal transducer activity and catalytic activity (Supplementary Table S4; Figures 3A, B).

KEGG enrichment analysis was performed on the host genes of six differentially expressed circRNAs. A total of 25 pathways were enriched, of which 14 were significantly enriched ($p < 0.05$). These pathways included the MAPK signaling pathway-fly, TGF-beta signaling pathway, FoxO signaling pathway, and autophagy-animal (Supplementary Table S5; Figures 3C, D).

3.3.3 Functional enrichment analysis of differentially expressed mRNAs

GO functional enrichment analysis was performed on the 84 differentially expressed mRNAs to predict the potential biological function of differential mRNAs, and the results showed that the differentially expressed mRNAs were enriched to 2,187 GO terms. The differential significant enrichment analysis of the enrichment results revealed that a total of 502 GO terms ($p < 0.05$) were significantly enriched, of which 412 GO terms were for biological processes, 35 were for cellular components, and 55 were for molecular functions. The four biological processes with the highest gene enrichment were single-organism process, biological regulation, cellular process, and regulation of the biological process, and the four cellular components with the highest gene enrichment were cell, cell part, membrane part, and membrane. The four most enriched molecular functions were molecular function regulator, binding, signal transducer activity, and catalytic activity (Supplementary Table S6; Figures 4A, B). The genes enriched to reproduction-related genes were GABBR2, SYCP1, HNF4A, CBLN1, and CDKN1. These genes may play an important role in the regulation of reproduction in Leizhou goats.

KEGG enrichment analysis was performed on the 84 differentially expressed mRNAs to further clarify the contribution of specific signaling pathways to goat fecundity, and the results showed that a total of 101 pathways were enriched, including 11 significantly enriched KEGG pathways ($p < 0.05$). These pathways included the p53 signaling pathway, cytokine-cytokine receptor interaction, cellular senescence, and arachidonic acid metabolism. These signaling pathways may play a role in reproduction and require further research (Supplementary Table S7; Figures 4C, D).



3.4 Construction of lncRNA–mRNA and circRNA–mRNA regulatory networks

To further explore the interactions of DE lncRNAs, DE circRNAs, and DEMRNAs in the high- and low-fertility groups, we intersected the predicted cis-targeted regulatory lncRNAs with the differential mRNAs of pituitary samples to predict genes that might be cis-targeted by lncRNAs. The predicted cis-targeted regulatory relationships showed that a total of 25 differentially expressed mRNAs were screened, and 51 lncRNAs were involved in gene regulation through cis-targeted regulatory effects (Supplementary Table S8; Figure 5A). The predicted circRNA–mRNA regulatory relationships revealed that a total of 31 differentially expressed mRNAs were screened, and 90 circRNAs were involved in gene regulation (Supplementary Table S9; Figure 5B).

3.5 Protein interaction network of differentially expressed mRNAs

Protein interaction networks were constructed using the STRING database to analyze the pairs of differentially expressed

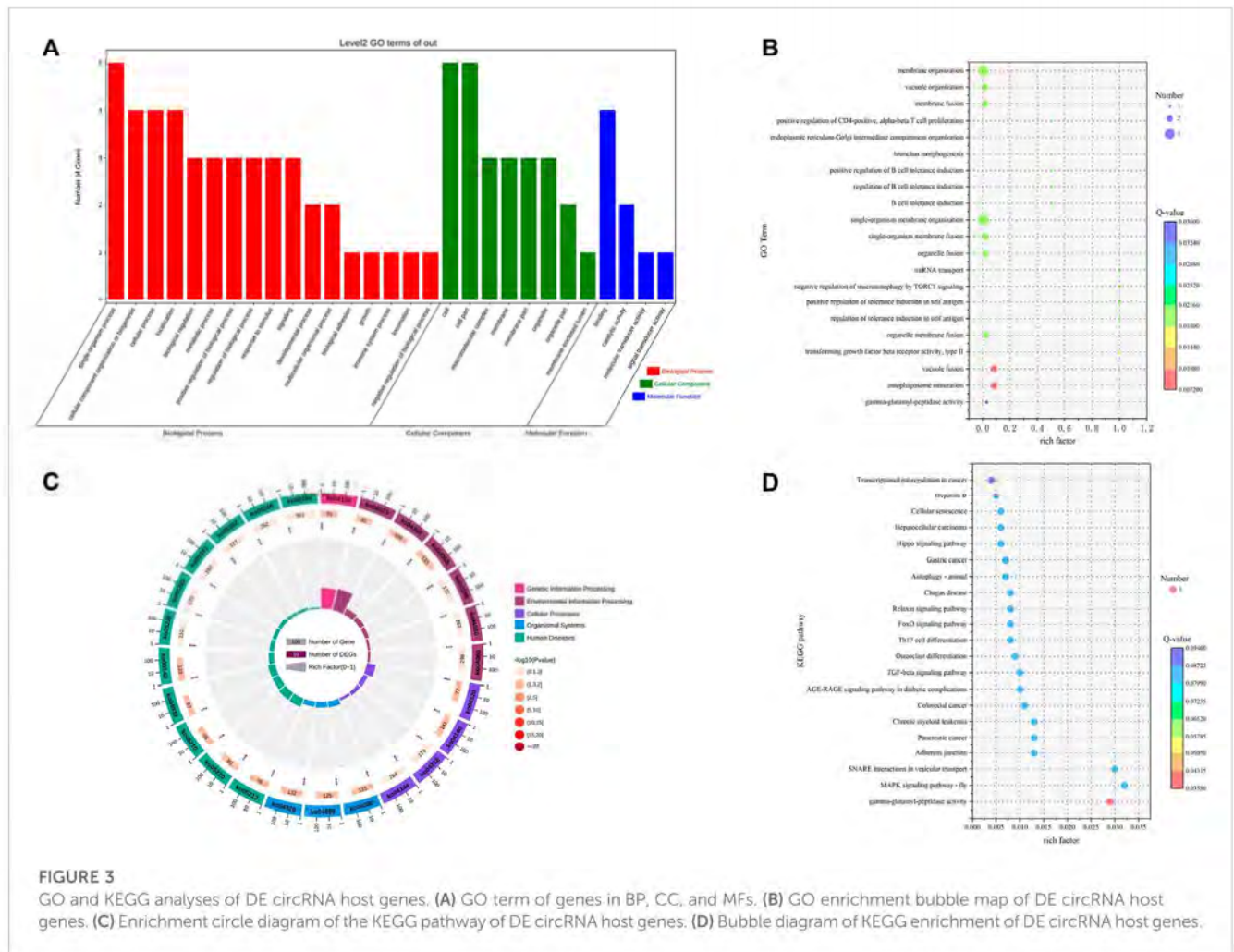
mRNAs and explore the significance of protein interrelationships in the pituitary in the low- and high-fertility groups. The differential mRNAs were enriched to a total of 84 proteins, and after hiding the proteins that did not contain interactions among them, 24 had interactions. Among them, SERPINE1 (degree = 4), ATF3 (degree = 4), TAC1 (degree = 3), and TNFRSF12A (degree = 3) were strongly correlated with the other proteins that may play an important role in pituitary regulation of fertility (Supplementary Table S10; Figure 6).

3.6 Sequencing data validation

The nine differentially expressed lncRNAs, circRNAs, and mRNAs were validated by RT-qPCR (Figure 7) to validate the RNA-seq results. The results showed that the expression levels of the two differed, but their trends were consistent, indicating that the sequencing results were credible.

4 Discussion

Goats have good adaptability to harsh environments, so they are widely distributed worldwide. Goats are one of the important



sources of meat and milk, and they have high economic value in many regions (Fatet et al., 2011; Pulina et al., 2018; Muriuki et al., 2019). The pituitary gland can influence the ovarian function and follicular development by synthesizing follicle-stimulating hormone (FSH) and luteinizing hormone (LH; Sheng et al., 2021). Therefore, the pituitary gland's influence on goat fertility can be studied by examining the pituitary function and the levels of the hormones that it regulates to obtain insights into the effects of the pituitary gland on goat fertility. The ovary, as a reproductive organ in female animals, also plays an essential role in the reproductive system, but due to the complexity of the internal structure of the ovary, determining the site that plays a role in regulating fertility in goats is difficult, and it may be affected by numerous interfering factors. Therefore, we chose to study the pituitary gland rather than the ovary to investigate high-fertility traits in Leizhou goats. Increasing evidence has shown that noncoding RNA and mRNA play an important role in goat reproduction (Ling et al., 2017; Li Y. et al., 2021). However, studies on noncoding RNA and mRNA in goat pituitary are relatively limited. Therefore, screening for genes associated with hormonal regulation is necessary to promote follicle development, increase ovulation numbers and litter size, and enhance goat fertility.

LncRNA is a new type of regulatory RNA, which is a ncRNA with a length of more than 200 bp. LncRNA is an important component of ncRNA (Shi et al., 2022), and it plays an essential

role in goat ovulation and kidding mainly by regulating transcription and post-transcription (Lian et al., 2020). LncRNA also plays an important role in the regulation of the fertility network. The main ways through which lncRNA affects goat ovulation are cis (Ma et al., 2018), trans, and competitive endogenous RNA. lncRNA can also influence goat ovulation by affecting hormone secretion. In the present study, 11 differentially expressed lncRNAs were identified in the cis-regulatory effects in the pituitary gland of the low- and high-fertility groups. Enrichment analysis of the target genes of the 11 differentially expressed lncRNAs revealed that TAC1, DUSP5, and MKRN3 were enriched to pathways associated with hormonal regulation of the gonadal axis. Substance P (SP) and neurokinin A (NKA) are tachykinins encoded by the TAC1 gene. SP and NKA can regulate the secretion of GnRH and LH. They play a neuromodulatory role in reproductive processes in vertebrate animals (Arisawa et al., 1989; Ogawa et al., 2021). SP signaling in TAC1 neurons is linked to kisspeptin signaling at the level of GnRH neurons to regulate fertility in male mice (Maguire et al., 2017), but the regulation of GnRH by SP may be the opposite in different species; for example, in crested newt, SP has been shown to downregulate GnRH and attenuate the pituitary secretion of LH (Gobbetti et al., 2000). The differential spliceosome of TAC1 can produce two isoforms, which encode tachykinins that regulate prolactin (PRL) release (Pan et al., 2021).

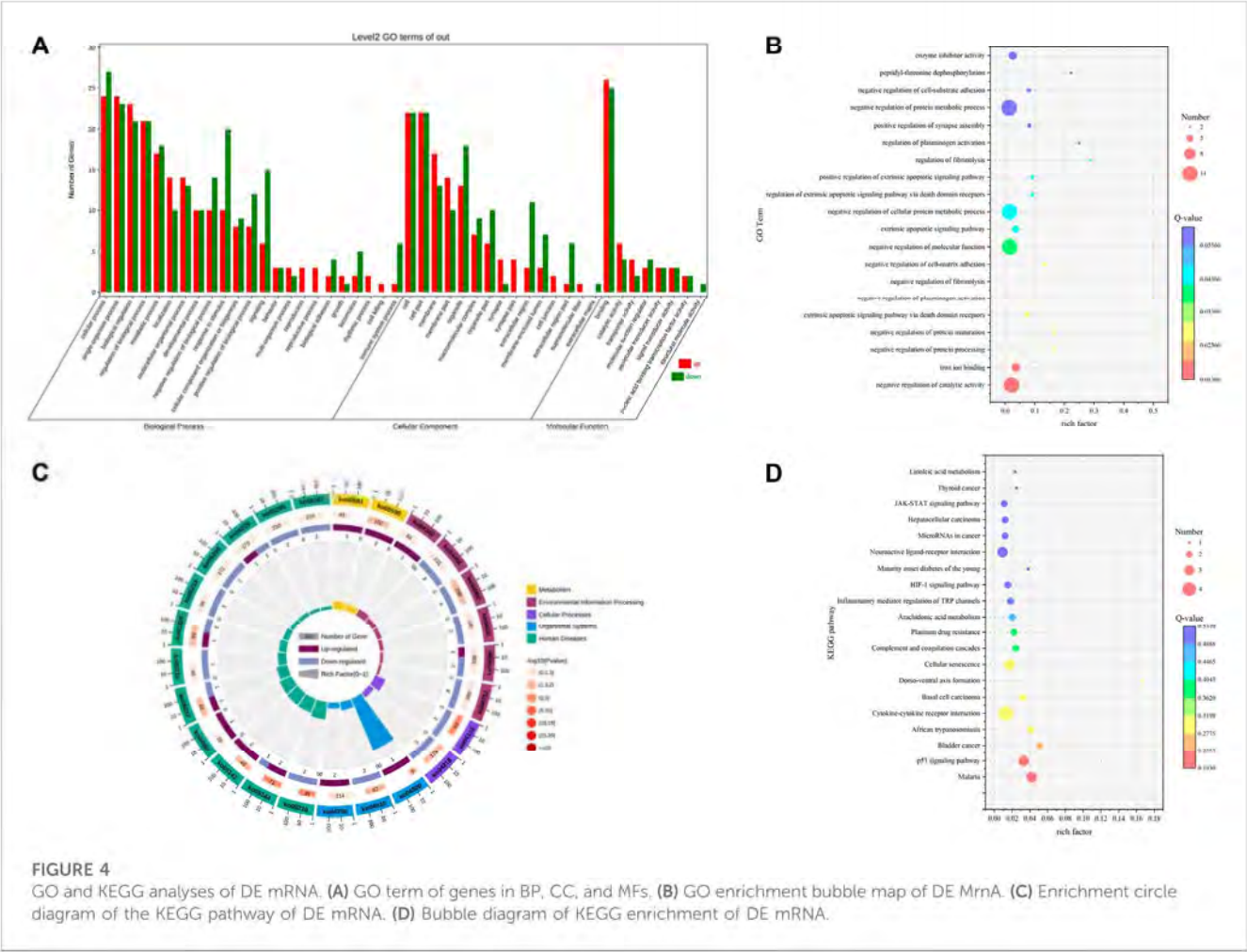


FIGURE 4
GO and KEGG analyses of DE mRNA. **(A)** GO term of genes in BP, CC, and MFs, **(B)** GO enrichment bubble map of DE MrnA. **(C)** Enrichment circle diagram of the KEGG pathway of DE mRNA. **(D)** Bubble diagram of KEGG enrichment of DE mRNA.

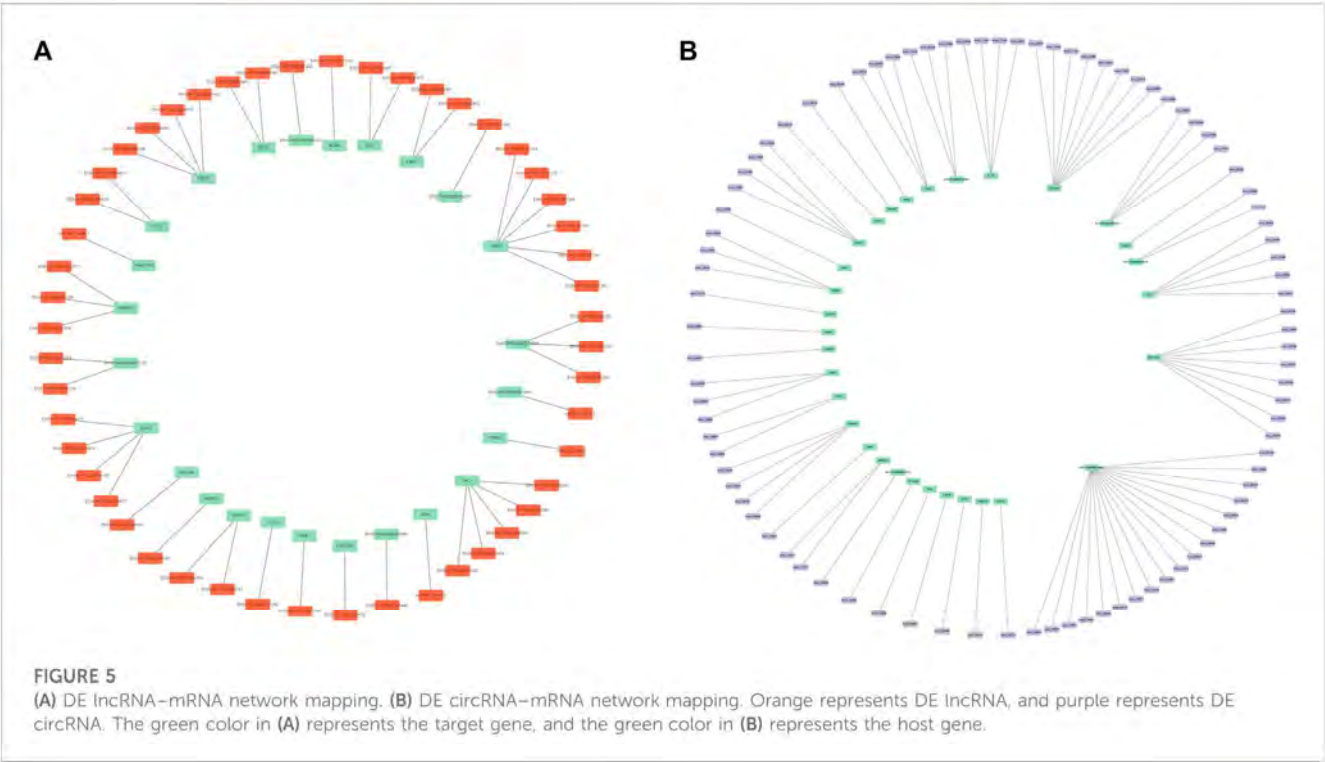


FIGURE 5
(A) DE lncRNA-mRNA network mapping. **(B)** DE circRNA-mRNA network mapping. Orange represents DE lncRNA, and purple represents DE circRNA. The green color in **(A)** represents the target gene, and the green color in **(B)** represents the host gene.

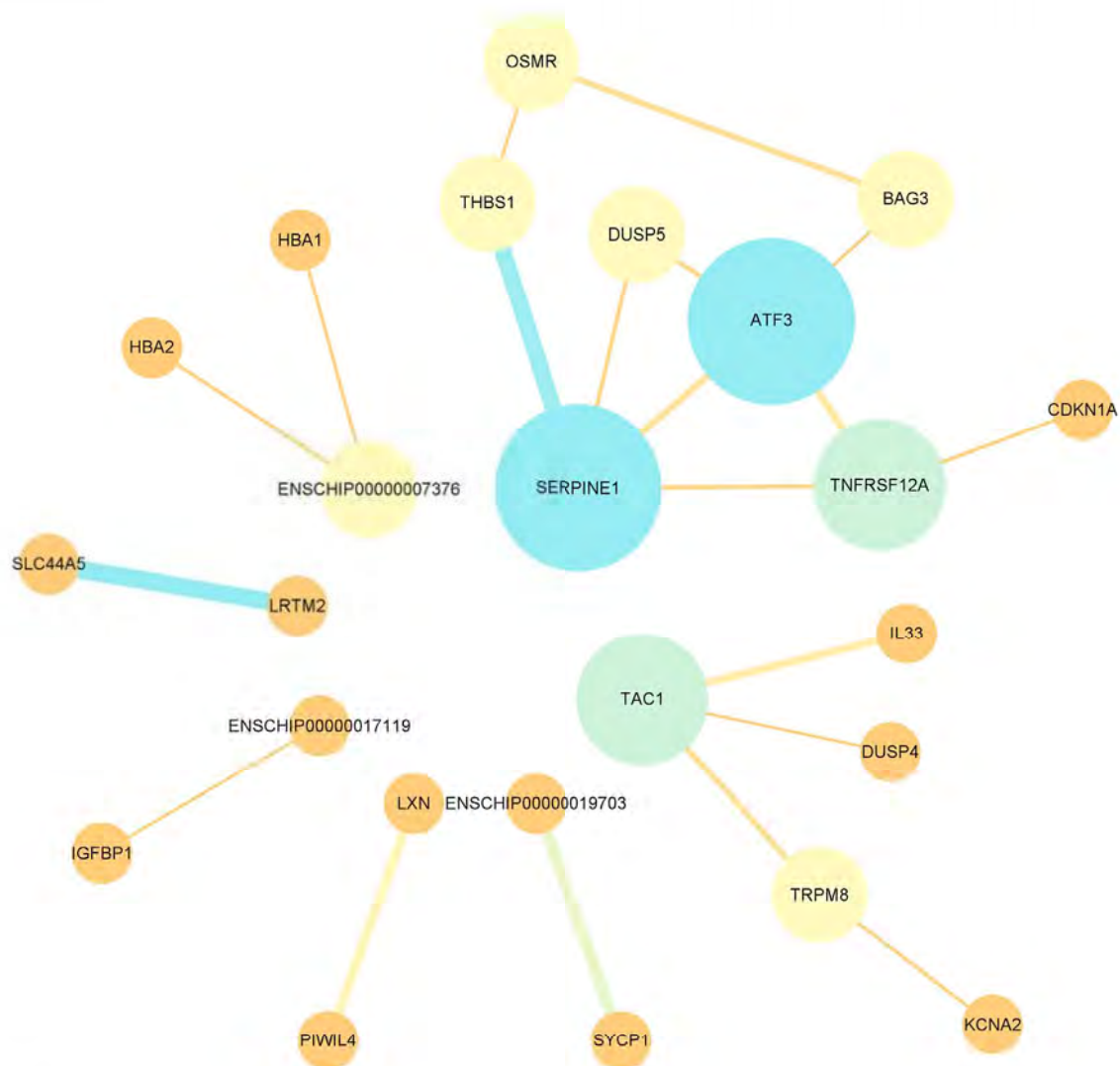


FIGURE 6

Protein-protein interaction network of DE mRNAs. Nodes in the network represent proteins, the width of the line in the node indicates the interaction between two proteins, and a wide line indicates a strong interaction.

Increased PRL interferes with the hypothalamus' secretion of GnRH, leading to decreased pituitary secretion of LH and FSH (Matuszewska et al., 2023). In our study, we found that lncRNAs (ENSCHIT00000010293, ENSCHIT00000010304, ENSCHIT00000010306, ENSCHIT00000010290, and ENSCHIT00000010298) may act through cis-targeted regulation to regulate TAC1. Therefore, the downregulation of ENSCHIT00000010293, ENSCHIT00000010304, and ENSCHIT00000010306 and the upregulation of ENSCHIT00000010290 and ENSCHIT00000010298 may cause the downregulation of TAC1, which may increase the secretion of GnRH and LH and decrease the secretion of PRL, thus enhancing the fertility of Leizhou goats. DUSP5 is a bispecific phosphatase 5 (Ni et al., 2023), and a study showed a 3.6-fold increase in the mRNA level of DUSP5 after 1 h of treatment with GnRH in hypothalamic neurons GT1-7 cells (Higa et al., 2018). Precise regulation of the synthesis and secretion of LH and FSH secreted in the pituitary gland is essential for the reproductive function of goats, and because

GnRH is the most important factor in regulating the synthesis and secretion of LH and FSH, DUSP5 may play an important role in the regulation of LH and FSH secretion. In our study, we found that lncRNAs (ENSCHIT00000006769 and ENSCHIT00000006767) may regulate DUSP5 through cis-regulatory effects. MKRN3 exerts a repressive effect on GnRH secretion, and MKRN3 inhibits GnRH secretion by suppressing the transcription of KISS1 and TAC3 in hypothalamic kiss1 neurons (Abreu et al., 2021; Liu et al., 2023). According to our sequencing results, lncRNA (ENSCHIT00000006921) cis-targeted and regulated MKRN3. Therefore, the upregulation of ENSCHIT00000006921 may lead to the downregulation of MKRN3, thereby attenuating its inhibitory effect on GnRH secretion as a means to regulate the secretion of LH and FSH by the pituitary gland of Leizhou goats to enhance the fecundity of these goats. RORA is enriched to the pathway associated with steroid secretion, and it is a transcriptional regulator of the steroid hormone receptor superfamily, which transcribes steroid-related genes to

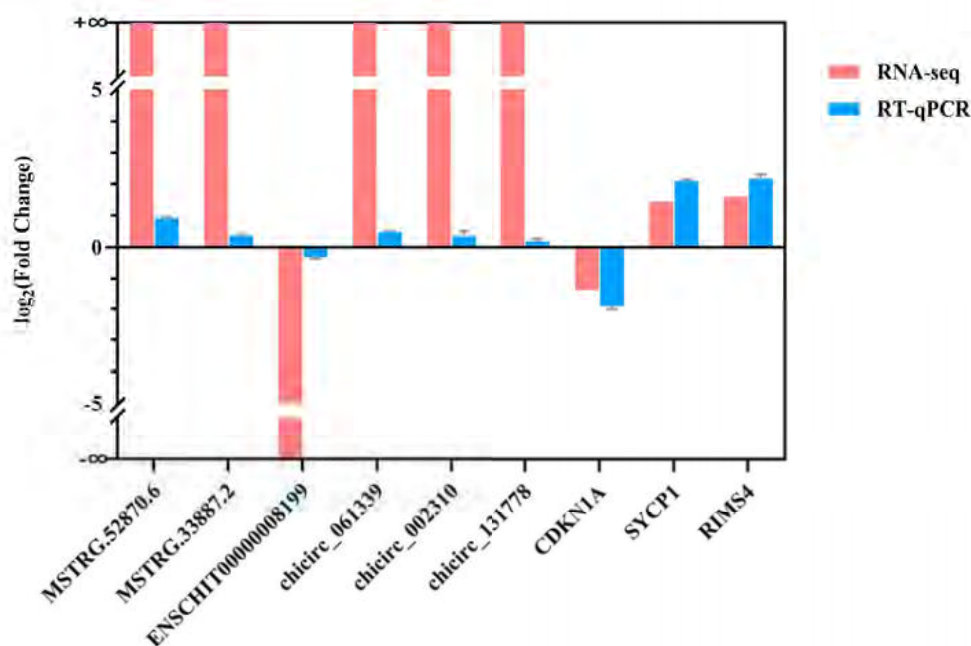


FIGURE 7

RT-qPCR verification of RNA-seq results. The X-axis represents DE lncRNAs, DE circRNAs, and DE mRNAs, and the Y-axis represents the log₂ (fold change) of RT-qPCR and RNA-Seq.

regulate estrogen synthesis (Yang et al., 2022). RORA is a potential target of estrogen and androgen receptors, and it can be involved in neurodevelopment, metabolism, and immunity. Some studies have shown that mutations in RORA cause problems with fertility in sheep (Yigit et al., 2023). RORA regulates estrogen and androgen synthesis by regulating the transcription of CYP19A1 and HSD17B10 (Sarachana and Hu, 2013). According to our sequencing results, lncRNA (ENSCHIT000000001330) cis-targeted RORA. Upregulation of ENSCHIT00000000133 leads to upregulation of RORA, which promotes the synthesis of estrogen and androgen, thereby enhancing the fertility of Leizhou goats. Therefore, these DE lncRNAs may play an important role in regulating female reproductive performance.

CircRNAs are a special class of noncoding RNAs (ncRNAs) that, unlike linear RNAs, are covalently closed-loop structures produced by reverse splicing of pre-mRNAs (Xiong et al., 2022). CircRNAs are highly stable and conserved across species because of their cyclization specificity (Zhang et al., 2019), and circRNAs can interact with RNA-binding proteins to regulate gene expression (Wang et al., 2020). Therefore, studying the function of circRNAs is worthwhile. In this study, six differentially expressed circRNAs were found to regulate the pituitary gland in the low- and high-fertility groups. Enrichment analysis of the host genes of these six differentially expressed circRNAs showed that tumor suppressor M (OSMR) was enriched in pathways related to hormone secretion. The mRNA abundance of OSMR increased after GnRH injection in ovulatory cattle, and the abundance of the X-linked inhibitor of apoptosis protein (XIAP) decreased in FSH-treated granulosa cells induced by OSM treatment. XIAP can inhibit granulosa cell apoptosis (Martins et al., 2019), so OSMR may regulate

granulosa cell apoptosis under the action of hormones, such as GnRH and FSH. According to our sequencing results, OSMR expression was higher in the low-fertility group than in the high-fertility group, so OSMR may also regulate apoptosis in cells that regulate the secretion of hormones by the pituitary gland. Chicirc_029285, chicirc_026618, and chicirc_129655 jointly regulated the expression of OSMR. Analysis of the regulatory network showed that GABBR2 was related to gonadotropin secretion (Walker et al., 2012), so GABBR2 may affect the regulation of LH and FSH secretion. According to our sequencing results, chicirc_018248, chicirc_122554, chicirc_087101, and chicirc_078945 co-regulated GABBR2. Therefore, these DE circRNAs may play an important role in regulating female reproductive performance.

A total of 20,844 mRNAs were identified in the pituitary tissues of Leizhou goats in the low- and high-fecundity groups. Among them, 84 DE mRNAs were significantly different. GO and KEGG enrichment analyses of the 84 genes showed that GABBR2, SYCP1, HNF4A, CBLN1, and CDKN1A were enriched in pathways related to the regulation of gonadal axis hormones and the regulation of reproductive performance. GABBR2 belongs to the G protein-coupled receptor (GPCR) family and the GABA-B receptor subfamily. Mouse GPCR binds to relaxin-3 to increase the plasma levels of LH, which is important for follicular development because it promotes the production of androgens by follicular membranous cells; then, the follicular granulosa cells convert the androgens produced by the membranous cells into estrogens in response to the stimulation of the hormone FSH to promote follicular development. The peaks of LH are essential for ovulation in

female mammals. In addition, GABBR2 is highly expressed in tilapia FSH cells and may be involved in the regulation of FSH secretion. In our sequencing results, GABBR2 was upregulated by 3.07-fold in the high-fertility group, and it may affect follicular development and ovulation in goats by influencing the secretion of LH and FSH, resulting in differences in goat reproductive performance (McGowan et al., 2008; Bathgate et al., 2013; Hollander-Cohen et al., 2021). SYCP1 plays an important role in meiosis. SYCP1 is required for the formation of crossovers in prophase meiosis, and it plays a role in regulating oocyte development (Tao et al., 2021). HNF4A has an active role in ovarian follicle differentiation and a positive role in ovarian follicular differentiation. FSH may optimize lipid metabolism in follicular tissue through the expression of HNF4A, thereby maintaining normal ovarian function (Khan et al., 2016). CBLN1 is involved in the proliferation and differentiation of nerve cells (Chen et al., 2021). CBLN1 transcription is affected by local changes in steroids (Whiley et al., 2020), so CBLN1 may play an important role in regulating goat fecundity. In our sequencing results, SYCP1, HNF4, and CBLN1 were upregulated by 2.71-fold, 20.65-fold, and 2.52-fold, respectively, in the high-fertility group. The role they play in the pituitary gland has not been reported yet, and we hypothesize that it may regulate the hypothalamic-pituitary-gonadal axis by modulating the activities of some pituitary neurons, thus affecting the secretion of related reproductive hormones and influencing the fertility of Leizhou goats. Studies have shown that the expression of CDKN1A varies at different follicular stages, and the expression of CDKN1A in the granulosa cells of dominant follicles is lower than that in the granulosa cells of preovulatory follicles (Jiang et al., 2015). CDKN1A can reduce the proliferation of granulosa cells, but it can promote the differentiation of granulosa cells (Wissing et al., 2014). Elevated CDKN1A expression may lead to pituitary hypoplasia (Gergics et al., 2015). CDKN1A was downregulated by 0.39-fold in the high-fecundity group in our sequencing data, so CDKN1A may affect pituitary development and thus reduce fecundity in Leizhou goats. The PPI network showed that SERPINE1, TAC1, and ATF3 were strongly correlated with other proteins, and they may play an important role in the pituitary regulation of fecundity. SERPINE1 is an inhibitor of plasminogen activation 1, and it is a tissue fibrinolytic activator and major inhibitor of urokinase (Chen et al., 2022). Studies have shown that SERPINE1 is expressed in preovulatory follicles in a tissue-specific manner and can inhibit FSH-induced PGE2 production by porcine cumulus granulosa cells (Blaha et al., 2019). Research has also provided evidence of an association between estrogen agonists and SERPINE1 levels (Ghaderian et al., 2021). SERPINE1 can be used as a biomarker for the prediction of pituitary dysfunction in patients with traumatic brain injury (Freundl et al., 2017). Therefore, the mechanism for the 0.07-fold downregulation of SERPINE1 in the high-fertility group may be that SERPINE1 attenuates the regulation of the levels of other hormones that regulate the fertility of Leizhou goats by affecting the inhibitory effect of FSH secreted by the pituitary gland. TAC1 is commonly expressed in interneurons and involved in the regulation of local circuits in the brain nucleus

(Burbach, 2016). The regulation of pituitary hormone secretion by TCA1 has been discussed above. ATF3 selectively stimulates FSHB expression during *in vitro* experiments, but FSH synthesis is not impaired in ATF3 knockout mice under *in vivo* conditions, so ATF3 may play a secondary role in the GnRH induction of FSHB transcription *in vivo* (Alonso et al., 2023). Moreover, ATF3 acts as a transcriptional repressor in gonadotropins (Mayer et al., 2008), and this study's sequencing showed that ATF3 was downregulated by 0.37-fold in the high-fertility group. Thus, the reduction of ATF3 in the high-fertility group may have attenuated the repressive effect on gonadotropins. Although the roles of some central proteins in the PPI network in the pituitary have not been reported, we hypothesize that these central proteins may affect the secretion of reproductive hormones by regulating the hypothalamic-pituitary-gonadal axis. The effects of these central proteins on the number of lambs produced by Leizhou goats deserve further investigation.

5 Conclusion

In this study, the pituitary glands of Leizhou goats in low- and high-fecundity groups were utilized as the research objects. LncRNA, circRNA, and mRNA in the pituitary glands of Leizhou goats in the low- and high-fecundity groups were identified by RNA sequencing technology, and 11 differentially expressed lncRNAs were obtained after differential expression analysis. Six differentially expressed circRNAs and 84 differentially expressed mRNAs were identified. The target gene prediction and target relationship were further analyzed to explore the molecular mechanism of pituitary regulation of fecundity, and lncRNA-mRNA homeopathy targeting the regulatory network and the circRNA-mRNA regulatory network was established. DE lncRNAs, DE circRNAs, and DE mRNAs that regulated GnRH, LH, and FSH secretion and reproductive traits were screened out. The results can help reveal the molecular mechanisms of pituitary regulation of fertility in Leizhou goats and serve as a theoretical basis for investigating the fecundity of Leizhou goats.

Data availability statement

The data presented in the study are deposited in the NCBI repository: <https://www.ncbi.nlm.nih.gov/>, accession number PRJNA1043736.

Ethics statement

The animal studies were approved by Ethics Committees of the Laboratory Animal Center of South China Agricultural University (permit number: SYXK-2014-0136). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

SM: Writing—original draft. SD: Software, Writing—review and editing. BH: Supervision, Writing—review and editing. YL: Formal Analysis, Writing—review and editing. BS: Methodology, Writing—review and editing. YG: Validation, Writing—review and editing. MD: Visualization, Writing—review and editing. DL: Funding acquisition, Writing—review and editing. GL: Resources, Writing—review and editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Forestry Science and Technology Innovation Program of Guangdong Province (2023KJCX004), Natural Science Foundation of Guangdong Province (2021A151010636), Modern Agricultural Industrial Technology System of Guangdong Province (2023KJ127) and Guangdong Provincial Department of Agriculture and Rural Affairs Project (2022-XPY-00-009).

References

- Abreu, A. P., Toro, C., Song, Y. B., Navarro, V., Bosh, M. A., Ercn, A., et al. (2021). MKRN3 inhibits the reproductive axis through actions in kisspeptin-expressing neurons. *Yearb. Paediatr. Endocrinol.* doi:10.1530/ey.18.7.11
- Ahluwat, S., Sharma, R., Maitra, A., and Tantia, M. S. (2015). Current status of molecular genetics research of goat fecundity. *Small Ruminant Res.* 125, 34–42. doi:10.1016/j.smallrumres.2015.01.027
- Alonso, C. A. L., David, C. D., Toufaily, C., Wang, Y., Zhou, X., Ongaro, L., et al. (2023). Activating transcription factor 3 stimulates follicle-stimulating hormone- β expression *in vitro* but is dispensable for follicle-stimulating hormone production in murine gonadotropes *in vivo*. *Endocrinology* 164, bqad050. doi:10.1210/endo/bqad050
- Arisawa, M., Snyder, G., Palatis, L., Ho, R., Xu, R., Pan, G., et al. (1989). Role of substance P in suppressing growth hormone release in the rat. *Proc. Natl. Acad. Sci. U. S. A.* 86, 7290–7294. doi:10.1073/pnas.86.18.7290
- Bathgate, R. A. D., Oh, M. H. Y., Ling, W. J., Kaas, Q., Hossain, M. A., Gooley, P. R., et al. (2013). Elucidation of relaxin-3 binding interactions in the extracellular loops of RXFP3. *Front. Endocrin.* 4, 13. doi:10.3389/fendo.2013.00013
- Blahe, M., Nevala, J., and Prochazka, R. (2019). The serine protease inhibitors and SERPINE1/2 disrupt prostaglandin E2 production and hyaluronic acid retention in FSH-stimulated pig cumulus-oocyte complexes. *Reprod. Dom. Anim.* 54, 65–68. doi:10.1111/rda.13497
- Burbach, J. P. H. (2016). "The neuroendocrine genome: neuropeptides and related signaling peptides," in *Molecular neuroendocrinology*. Editors D. Murphy and H. Gainer (Chichester, UK: John Wiley and Sons, Ltd), 25–56. doi:10.1002/9781118760369.ch2
- Chen, S., Guo, X., He, X., Di, R., Zhang, X., Zhang, J., et al. (2021). Transcriptome analysis reveals differentially expressed genes and long non-coding RNAs associated with fecundity in sheep hypothalamus with different FecB genotypes. *Front. Cell Dev. Biol.* 9, 633747. doi:10.3389/fcell.2021.633747
- Chen, S., Li, Y., Zhu, Y., Fei, J., Song, L., Sun, G., et al. (2022). SERPINE1 overexpression promotes malignant progression and poor prognosis of gastric cancer. *J. Oncol.* 2022, 2647825. doi:10.1155/2022/2647825
- Chronowska, E. (2014). High-throughput analysis of ovarian granulosa cell transcriptome. *BioMed Res. Int.* 2014, 213570. doi:10.1155/2014/213570
- Costa, V., Angelini, C., Feis, L., and Ciccodicola, A. (2010). Uncovering the complexity of transcriptomes with RNA-SEQ. *J. Biomed. Biotechnol.* 2010, 853916. doi:10.1155/2010/853916
- Dong, S., Hou, B., Yang, C., Li, Y., Sun, B., Guo, Y., et al. (2023). Comparative hypothalamic transcriptome analysis reveals crucial mRNAs, lncRNAs, and circRNAs affecting litter size in goats. *Genes* 14, 444. doi:10.3390/genes14020444
- Du Toit, A. (2013). Circular RNAs as miRNA sponges. *Nat. Rev. Mol. Cell Biol.* 14, 195. doi:10.1038/nrm3557
- Fatet, A., Pellicer-Rubio, M.-T., and Leboeuf, B. (2011). Reproductive cycle of goats. *Animal Reproduction Sci.* 124, 211–219. doi:10.1016/j.anireprosci.2010.08.029
- Frendl, I., Katko, M., Galgoczi, E., Boda, J., Zsidos, N., Nemeti, Z., et al. (2017). Plasminogen activator inhibitor type 1: a possible novel biomarker of late pituitary dysfunction after mild traumatic brain injury. *J. Neurotrauma* 34, 3238–3244. doi:10.1089/neu.2017.5198
- Gao, Y., Yang, L., Chen, Y., Peiwen, L., Zhou, Y., Chen, X., et al. (2023). Aal-circRNA-407 regulates ovarian development of *Aedes albopictus*, a major arbovirus vector, via the miR-9a-5p/Foxl axis. *PLoS Pathog.* 19, e1011374. doi:10.1371/journal.ppat.1011374
- Gergics, P., Brinkmeier, M. L., and Camper, S. A. (2015). Lhx4 deficiency: increased cyclin-dependent kinase inhibitor expression and pituitary hypoplasia. *Mol. Endocrinol.* 29, 597–612. doi:10.1210/me.2014-1380
- Ghaderian, S., Akbarzadeh, R., and Salehpour, S. (2021). Involvement of single nucleotide polymorphisms in ovarian poor response. *J. Assisted Reproduction Genet.* 38, 2405–2413. doi:10.1007/s10815-021-02242-w
- Gobbetti, A., Petrelli, C., and Zerani, M. (2000). Substance P downregulates basal and gonadotropin-releasing hormone-induced gonadotropin *in vitro* secretion by pituitary gland of crested newt, *Triturus cristatus*. *J. Neuroendocrinol.* 12, 1160–1166. doi:10.1046/j.1365-2826.2000.00576.x
- Higa, T., Takahashi, H., Higa-Nakamine, S., Suzuki, M., and Yamamoto, H. (2018). Up-regulation of DUSP5 and DUSP6 by gonadotropin-releasing hormone in cultured hypothalamic neurons, GT1-7 cells. *Biomed. Res.* 39, 149–158. doi:10.2220/biomedres.39.149
- Hollander-Cohen, L., Golan, M., and Levavi-Sivan, B. (2021). Differential regulation of gonadotropins as revealed by transcriptomes of distinct LH and FSH cells of fish pituitary. *Int. J. Mol. Sci.* 22, 6478. doi:10.3390/ijms22126478
- Islam, R., Liu, X., Gebreselassie, G., Abied, A., Ma, Q., and Ma, Y. (2020). Genome-wide association analysis reveals the genetic locus for high reproduction trait in Chinese Arbas Cashmere goat. *Genes and Genomics* 42, 893–899. doi:10.1007/s13258-020-00937-5
- Jiang, L., Huang, J., Li, L., Chen, Y., Chen, X., Zhao, X., et al. (2015). MicroRNA-93 promotes ovarian granulosa cells proliferation through targeting CDKN1A in polycystic ovarian syndrome. *J. Clin. Endocrinol. Metabolism* 100, E729–E738. doi:10.1210/jc.2014-3827
- Kantono, K., Hamid, N., Ma, Q., Chadha, D., and Oey, I. (2021). Consumers' perception and purchase behaviour of meat in China. *Meat Sci.* 179, 108548. doi:10.1016/j.meatsci.2021.108548
- Khan, D. R., Landry, D. A., Fournier, É., Vigneault, C., Blondin, P., and Sirard, M.-A. (2016). Transcriptome meta-analysis of three follicular compartments and its correlation with ovarian follicle maturity and oocyte developmental competence in cows. *Physiol. Genomics* 48, 633–643. doi:10.1152/physiolgenomics.00050.2016
- Lee, J., and Kang, H. (2022). Role of MicroRNAs and long non-coding RNAs in sarcopenia. *Cells* 11, 187. doi:10.3390/cells11020187
- Li, M., Lu, X., Xia, H., Zhang, C., Wang, X., Chen, Z., et al. (2019). In-depth characterization of the pituitary transcriptome in Simmental and Chinese native cattle. *Domest. Anim. Endocrinol.* 66, 35–42. doi:10.1016/j.domaniend.2018.09.003

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2023.1303031/full#supplementary-material>

- Li, S., Li, X., Ma, Q., Wang, Z., Fang, F., and Zhang, D. (2022). Consumer preference, behaviour and perception about lamb meat in China. *Meat Sci.* 192, 108878. doi:10.1016/j.meatsci.2022.108878
- Li, X., Gao, F., Fan, Y., Xie, S., Li, C., Meng, L., et al. (2021a). A novel identified circ-ANKHD1 targets the miR-27a-3p/SFRP1 signaling pathway and modulates the apoptosis of granulosa cells. *Environ. Sci. Pollut. Res.* 28, 57459–57469. doi:10.1007/s11356-021-14699-4
- Li, Y., Wang, H., Zhou, D., Shuang, T., Zhao, H., and Chen, B. (2018). Up-regulation of long noncoding RNA SRA promotes cell growth, inhibits cell apoptosis, and induces secretion of estradiol and progesterone in ovarian granular cells of mice. *Med. Sci. Monit.* 24, 2384–2390. doi:10.12659/MSM.907138
- Li, Y., Xu, X., Deng, M., Zou, X., Zhao, Z., Huang, S., et al. (2021b). Identification and comparative analysis of long non-coding RNAs in high- and low-fecundity goat ovaries during estrus. *Front. Genet.* 12, 648158. doi:10.3389/fgene.2021.648158
- Lian, Z., Zou, X., Han, Y., Deng, M., Sun, B., Guo, Y., et al. (2020). Role of mRNAs and long non-coding RNAs in regulating the litter size trait in Chuanzhong black goats. *Reproduction Domest. Animals* 55, 486–495. doi:10.1111/rda.13642
- Ling, Y., Xu, L., Zhu, L., Sui, M., Zheng, Q., Li, W., et al. (2017). Identification and analysis of differentially expressed long non-coding RNAs between multiparous and uniparous goat (*Capra hircus*) ovaries. *PLOS ONE* 12, e0183163. doi:10.1371/journal.pone.0183163
- Liu, A., Chen, X., Liu, M., Zhang, L., Ma, X., and Tian, S. (2021). Differential expression and functional analysis of CircRNA in the ovaries of low and high fecundity hanper sheep. *Animals* 11, 1863. doi:10.3390/ani11071863
- Liu, J., Li, T., Peng, M., Luo, M., Gui, Z., Long, S., et al. (2023). The key roles of makorin RING finger protein 3 (MKRN3) during the development of pubertal initiation and central Precocious Puberty (CPP). *CMM* 23, 668–677. doi:10.2174/1566524022666220624105430
- Ma, M., Cai, B., Jiang, L., Abdalla, B. A., Li, Z., Nie, Q., et al. (2018). lncRNA-Six1 is a target of miR-1611 that functions as a ceRNA to regulate Six1 protein expression and fiber type switching in chicken myogenesis. *Cells* 7, 243. doi:10.3390/cells7120243
- Maguire, C., Song, Y. B., Wu, M., Leon, S., Carroll, R., Alreja, M., et al. (2017). Tac1 signaling is required for sexual maturation and responsiveness of GnRH neurons to kisspeptin in the male mouse. *Endocrinology* 158, 2319–2329. doi:10.1210/en.2016-1807
- Martins, K. R., Haas, C. S., Ferst, J. G., Rovani, M. T., Goetten, A. L. F., Duggavathi, R., et al. (2019). Oncostatin M and its receptors mRNA regulation in bovine granulosa and luteal cells. *Theriogenology* 125, 324–330. doi:10.1016/j.theriogenology.2018.11.021
- Matuszewska, A., Kowalski, K., Jawień, P., Tomkalski, T., Gawel-Dąbrowska, D., Merwid-Ląd, A., et al. (2023). The hypothalamic-pituitary-gonadal Axis in men with schizophrenia. *Int. J. Mol. Sci.* 24, 6492. doi:10.3390/ijms24076492
- Mayer, S. I., Dexheimer, V., Nishida, E., Kitajima, S., and Thiel, G. (2008). Expression of the transcriptional repressor ATF3 in gonadotrophs is regulated by Egr-1, CREB, and ATF2 after gonadotropin-releasing hormone receptor stimulation. *Endocrinology* 149, 6311–6325. doi:10.1210/en.2008-0251
- McGowan, B. M., Stanley, S. A., Donovan, J., Thompson, E. L., Patterson, M., Semjonous, N. M., et al. (2008). Relaxin-3 stimulates the hypothalamic-pituitary-gonadal axis. *Am. J. Physiology-Endocrinology Metabolism* 295, E278–E286. doi:10.1152/ajpendo.00028.2008
- Miao, X., Luo, Q., Zhao, H., and Qin, X. (2017). An integrated analysis of miRNAs and methylated genes encoding mRNAs and lncRNAs in sheep breeds with different fecundity. *Front. Physiology* 8, 1049. doi:10.3389/fphys.2017.01049
- Muriuki, C., Bush, S. J., Salavati, M., McCulloch, M. E. B., Lisowski, Z. M., Agaba, M., et al. (2019). A mini-atlas of gene expression for the domestic goat (*Capra hircus*). *Front. Genet.* 10, 1080. doi:10.3389/fgene.2019.01080
- Ni, R., Liu, H., Song, G., Fu, X., Deng, B., Xu, Z., et al. (2023). MiR-216a-3p inhibits the proliferation and invasion of fibroblast-like synoviocytes by targeting dual-specificity phosphatase 5. *Int J Rheum Dis* 26, 699–709. doi:10.1111/1756-185X.14622
- Ogawa, S., Ramadasan, P. N., Anthonysamy, R., and Parhar, I. S. (2021). Sexual dimorphic distribution of hypothalamic Tachykinin1 cells and their innervations to GnRH neurons in the zebrafish. *Front. Endocrinol.* 11, 534343. doi:10.3389/fendo.2020.534343
- Pan, X., Li, Q., Chen, D., Gong, W., Li, N., Jiang, Y., et al. (2021). Alternative splicing dynamics of the hypothalamus-pituitary-ovary Axis during pubertal transition in gilts. *Front. Genet.* 12, 592669. doi:10.3389/fgene.2021.592669
- Pulina, G., Milán, M. J., Lavín, M. P., Theodoridis, A., Morin, E., Capote, J., et al. (2018). Invited review: current production trends, farm structures, and economics of the dairy sheep and goat sectors. *J. Dairy Sci.* 101, 6715–6729. doi:10.3168/jds.2017-14015
- Qiannan, E., Wang, C., Gu, X., Gan, X., Zhang, X., Wang, S., et al. (2020). Competitive endogenous RNA (ceRNA) regulation network of lncRNA-miRNA-mRNA during the process of the nickel-induced steroidogenesis disturbance in rat Leydig cells. *Toxicol. Vitro* 63, 104721. doi:10.1016/j.tiv.2019.104721
- Sarachana, T., and Hu, V. W. (2013). Differential recruitment of coregulators to the RORA promoter adds another layer of complexity to gene (dys) regulation by sex hormones in autism. *Mol. Autism* 4, 39. doi:10.1186/2040-2392.4.39
- Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueifar, M., Hale, T. M., et al. (2021). The hypothalamic-pituitary-adrenal Axis: development, programming actions of hormones, and maternal-fetal interactions. *Front. Behav. Neurosci.* 14, 601939. doi:10.3389/fnbeh.2020.601939
- Shi, H., He, Y., Li, X., Du, Y., Zhao, J., and Ge, C. (2022). Regulation of non-coding RNA in the growth and development of skeletal muscle in domestic chickens. *Genes* 13, 1033. doi:10.3390/genes13061033
- Tao, L., He, X., Wang, X., Di, R., and Chu, M. (2021). Litter size of sheep (*Ovis aries*): inbreeding depression and homozygous regions. *Genes* 12, 109. doi:10.3390/genes12010109
- Walker, D. M., Kirson, D., Perez, L. F., and Gore, A. C. (2012). Molecular profiling of postnatal development of the hypothalamus in female and male Rats1. *Biol. Reproduction* 87 (129), 1–12. doi:10.1095/biolreprod.112.102798
- Wan, Z., Yang, H., Cai, Y., Ma, J., Cheng, P., Wang, Z., et al. (2022a). Comparative transcriptomic analysis of Hu sheep pituitary gland prolifacy at the follicular and luteal phases. *Genes* 13, 440. doi:10.3390/genes13030440
- Wan, Z., Yang, H., Chen, P., Wang, Z., Cai, Y., Yao, X., et al. (2022b). The novel competing endogenous long noncoding RNA SM2 regulates gonadotropin secretion in the Hu sheep anterior pituitary by targeting the oar-miR-16b/TGF-β/SMAD2 signaling pathway. *Cells* 11, 985. doi:10.3390/cells11060985
- Wang, C.-J., Gao, F., Huang, Y.-J., Han, D.-X., Zheng, Y., Wang, W.-H., et al. (2020). circAkap17b acts as a miR-7 family molecular sponge to regulate FSH secretion in rat pituitary cells. *J. Mol. Endocrinol.* 65, 135–148. doi:10.1530/JME-20-0036
- Wang, J.-Y., Lan, Z.-R., and Zhang, X.-M. (2012). Advances in molecular breeding research of goat fecundity. *J. Animal Veterinary Adv.* 11, 449–453. doi:10.3923/javaa.2012.449.453
- Whiley, P. A. F., O'Donnell, L., Moody, S. C., Handelsman, D. J., Young, J. C., Richards, E. A., et al. (2020). Activin A determines steroid levels and composition in the fetal testis. *Endocrinology* 161, bqaa058. doi:10.1210/endo/bqaa058
- Wissing, M., Kristensen, S., Andersen, C., Mikkelsen, A., Hoest, T., Borup, R., et al. (2014). Identification of new ovulation-related genes in humans by comparing the transcriptome of granulosa cells before and after ovulation triggering in the same controlled ovarian stimulation cycle. *Hum. Reprod.* 29, 997–1010. doi:10.1093/humrep/deu008
- Xiong, J., Zhang, H., Wang, Y., Cheng, Y., Luo, J., Chen, T., et al. (2022). Rno_circ_0001004 acts as a miR-709 molecular sponge to regulate the growth hormone synthesis and cell proliferation. *IJMS* 23, 1413. doi:10.3390/ijms23031413
- Yang, H., Ma, J., Wang, Z., Yao, X., Zhao, J., Zhao, X., et al. (2020). Genome-wide analysis and function prediction of long noncoding RNAs in sheep pituitary gland associated with sexual maturation. *Genes* 11, 320. doi:10.3390/genes11030320
- Yang, Y., Hu, H., Mao, C., Jiang, F., Lu, X., Han, X., et al. (2022). Detection of the 23-bp nucleotide sequence mutation in retinoid acid receptor related orphan receptor alpha (RORA) gene and its effect on sheep litter size. *Anim. Biotechnol.* 33, 70–78. doi:10.1080/10495398.2020.1770273
- Yao, Z., Zhang, S., Wang, X., Guo, Y., Xin, X., Zhang, Z., et al. (2023). Genetic diversity and signatures of selection in BoHuai goat revealed by whole-genome sequencing. *BMC Genomics* 24, 116. doi:10.1186/s12864-023-09204-9
- Yigit, S., Kul, S., Aci, R., Keskin, A., Tuygun, T., and Duman, E. (2023). The effect of RORA (RAR-Related Orphan Receptor Alpha) receptors on litter size in Akkaraman sheep breed. *Mol. Biol. Res. Commun.* 12, 109–115. doi:10.22099/mbrc.2023.47336.1827
- Zhang, H., Hu, B., Xiong, J., Chen, T., Xi, Q., Luo, J., et al. (2019). Genomewide analysis of circular RNA in pituitaries of normal and heat-stressed sows. *BMC Genomics* 20, 1013. doi:10.1186/s12864-019-6377-7
- Zhang, H., Wang, Y., Liu, X., and Li, Y. (2020). Progress of long noncoding RNAs in anti-tumor resistance. *Pathology - Res. Pract.* 216, 153215. doi:10.1016/j.prp.2020.153215
- Zhang, J., Wang, C., Jia, C., Zhang, Y., Qing, X., Zhang, Y., et al. (2022). The role of circular RNAs in the physiology and pathology of the mammalian ovary. *Int. J. Mol. Sci.* 23, 15204. doi:10.3390/ijms232315204
- Zhao, X., Li, H., Chen, X., Wu, Y., Wang, L., and Li, J. (2023). Long non-coding RNA MSTRG.5970.28 regulates proliferation and apoptosis of goose follicle granulosa cells via the miR-133a-3p/ANOS1 pathway. *Poult. Sci.* 102, 102451. doi:10.1016/j.psj.2022.102451
- Zhao, Z. Q., Wang, L. J., Sun, X. W., Zhang, J. J., Zhao, Y. J., Na, R. S., et al. (2015). Transcriptome analysis of the *Capra hircus* ovary. *PLoS One* 10, e0121586. doi:10.1371/journal.pone.0121586



Article



Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats

Shucan Dong, Biwei Hou, Chuang Yang, Yaokun Li, Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu and Guangbin Liu



Article

Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats

Shucan Dong, Biwei Hou, Chuang Yang, Yaokun Li , Baoli Sun, Yongqing Guo, Ming Deng, Dewu Liu and Guangbin Liu 

Guangdong Laboratory for Lingnan Modern Agriculture, College of Animal Science, South China Agricultural University, Guangzhou 510642, China

* Correspondence: gblu@scau.edu.cn

Abstract: Litter size is an important indicator to measure the reproductive performance of goats, which is affected by the reproductive function of animals. The hypothalamus, as the regulatory center of the endocrine system, plays an important role in the reproduction of female animals. Here, we performed high-throughput RNA sequencing using hypothalamic tissue from high-fecundity and low-fecundity Leizhou goats to explore critical functional genes associated with litter size. Differentially expressed mRNA, lncRNA, and circRNAs were screened using DESeq and were enriched, and then analyzed by Gene Ontology and Kyoto Encyclopedia of Gene and Genome. Results showed that some of these differentially expressed mRNAs could be enriched in reproductive processes, jak-STAT, prolactin signaling pathway, and other signaling pathways related to reproduction, such as SOCS3. Furthermore, the central proteins POSTN, MFAP5, and DCN from protein–protein interaction may regulate animal reproductive activity by affecting cell proliferation and apoptosis. lncRNA MSTRG.33887.2 as well as circRNAs chirc_098002, chirc_072583, and chirc_053531 may be able to influence animal reproduction by participating in folate metabolism and energy metabolism homeostasis through their respective target genes. Our results expand the molecular mechanism of hypothalamic regulation on animal reproduction.

Keywords: litter size; goat; lncRNAs; circRNAs; fertility



Citation: Dong, S.; Hou, B.; Yang, C.; Li, Y.; Sun, B.; Guo, Y.; Deng, M.; Liu, D.; Liu, G. Comparative Hypothalamic Transcriptome Analysis Reveals Crucial mRNAs, lncRNAs, and circRNAs Affecting Litter Size in Goats. *Genes* **2023**, *14*, 444. <https://doi.org/10.3390/genes14020444>

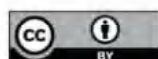
Academic Editor: Shaochen Sun

Received: 14 January 2023

Revised: 6 February 2023

Accepted: 7 February 2023

Published: 9 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The litter size of goats is an important economic index to measure the production performance of goats, which affects the economic benefit of farmers [1]. The litter size of livestock can be increased by improving the level of feeding and management, but the influence of these strategies is limited [2]. Improving the fecundity of livestock is the key to select excellent varieties with a large number of offspring by genetic breeding. With the development of high-throughput sequencing, variety selection has entered the era of molecular breeding, and many candidate genes related to animal litter size have been excavated continuously [3–5]. Leizhou goats primarily live in Leizhou Peninsula in Guangdong Province, China, and they have excellent characteristics such as early sexual maturity and high reproductive capacity [6]. Therefore, the study of the molecular mechanism of high-fertility Leizhou goats is useful to obtain comprehensive understanding of the reproductive process of animals and to improve the efficiency of goat industry.

The litter size of livestock is closely related to the number of mature oocytes excreted by the dam in each reproductive cycle [7]. Ovulation in livestock is strictly regulated by the hypothalamus–pituitary–ovary (HPO) axis [8]. As the initial organ of the HPO axis, the hypothalamus can regulate the secretion of luteinizing hormone (LH) and follicle-stimulating hormone from the pituitary by secreting gonadotropin-releasing hormone (GnRH), thereby

controlling gonadal development and sex hormone secretion [9]. In addition, the hypothalamus can influence animal reproduction by coordinating energy metabolism and biological rhythms [10–12]. Therefore, the hypothalamus is essential for animal reproduction. However, although the hypothalamus is essential for animal reproduction, little is known about the molecular mechanism by which the hypothalamus regulates animal reproduction.

The regulation of animal reproduction by the hypothalamus requires the participation of coding RNA and non-coding RNA. Previously, non-coding RNAs (ncRNAs) were considered as transcriptional noise. However, at present, many studies have shown that ncRNAs have a remarkable variety of biological functions, regulating gene expression at the level of transcription, RNA processing, and translation [13,14]. A previous study has revealed the mRNA–microRNAs network in the hypothalamus that affects litter size in goats by hypothalamic transcriptomics, but information on long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) is scarce [15]. circRNAs are ncRNAs that are widely expressed in organisms, which play a role in the regulation of gene expression through circularization and splicing against one another, and their expression is specific to tissue and developmental stages [16,17]. circRNAs are associated with the occurrence of many diseases, but their regulatory mechanisms for reproduction in the hypothalamus are lacking [18]. lncRNAs, a class of non-encoded proteins larger than 200 bp, are considered as key regulators of various biological functions [19]. These coding RNAs interact with ncRNAs to form a complex regulatory network. Investigating this network has important implications for understanding the regulation of reproduction by the hypothalamus. RNA sequencing (RNA-Seq) can obtain almost all mRNA and ncRNA sequence information and expression information of a specific tissue or cell; thus, it is widely used in the mining of molecular biological functions [20,21]. The use of RNA-Seq to mine differential mRNA, lncRNA, and circRNA in the hypothalamus of high- and low-fertility goats is of great significance for a comprehensive understanding of the gene networks in the hypothalamus, which regulate animal reproduction.

At present, information on the regulation of reproduction by circRNA and lncRNA in the hypothalamus of goats is lacking. Thus, we used RNA-Seq to analyze the differential expression of mRNAs (DE mRNAs), lncRNAs (DE lncRNAs), and circRNAs (DE circRNAs) in the hypothalamus of Leizhou goats with high and low fecundity and predicted the network interaction of DE lncRNA and DE circRNA with mRNA. Our research contributes to a comprehensive understanding of the regulatory role of the hypothalamus in animal reproduction.

2. Materials and Methods

2.1. Animals and Sample Collection

Seven healthy Leizhou goat of similar age and body condition were selected and divided into the high-fecundity group ($n = 3$) and low-fecundity group ($n = 4$) based on the litter size. The high-fecundity group had more than one lamb per litter, whereas the low-fecundity group had only one lamb, and three lambs were born to these goats. The female goats were injected with 0.1 mg of cloprostenol to induce estrus [22,23]. After 2 days of injection, the male goat test method (the vas deferens has been ligated) and vaginal observation method were used to check whether the female goat was in heat, and the second estrus identification was carried out on the test female goat at about 18 days. All female goats received estrus synchronization. After the second confirmation of estrus, these seven goats were slaughtered and dissected simultaneously within 24 h to collect hypothalamic tissue. The collected hypothalamus was immediately washed with PBS, after which it was placed in liquid nitrogen for short-term storage and transport, and finally stored at -80°C [15].

2.2. Extraction of Total RNA, Construction of cDNA Library, and Transcriptome Sequencing

Trizol (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract total RNA from the hypothalamus, following the manufacturer's instructions. NanoDrop ND-2000 (Thermo Science, Wilmington, DE, United States) was used to measure RNA concentration and purity. OD A260/A280 (>1.8) and A260/A230 (>1.6) were used to assess the purity of RNA, and agarose gel electrophoresis was used to detect the integrity of RNA. After passing the abovementioned indexes, 3 μ L of total RNA was selected from each sample for the construction of cDNA library. Paired-end sequencing of these libraries was performed using Next-Generation Sequencing based on the Illumina HiSeq sequencing platform. cDNA library construction and transcriptome sequencing were performed by Shanghai Personalbio Technology Co., Ltd. (Shanghai, China).

2.3. Quality Control, Alignment, and Quantification of RNA-Seq Data

The sequencing data contained some adapters and low-quality reads, and these sequences could have caused great interference with subsequent information analysis; thus, further filtering of the sequencing data was required. Our filtering condition was as follows: the joints at the 3' end were removed by Cutadapt, and the read with an average mass fraction lower than Q20 was also removed. Reference genome indexes were built by Bowtie2, and then filtered Reads were compared with the goat reference genome (Capra_hircus.ARS1.DNA.toplevel.fa) using Tophat2. The data source for the reference genome is the Ensembl database (<http://www.ensembl.org/>, accessed on 28 August 2018). The transcript of each sample was assembled from the mapped reads by StringTie based on the annotated transcript file from ENSEMBL.

We identified all the clipped forms of transcripts in accordance with StringTie and used gffcompare to compare with the reference genome to find new transcript regions. We identified all sheared forms of transcripts based on StringTie and used gffcompare to compare with the reference genome and identify new transcript regions. The coding potential of new transcripts was predicted using Coding-Non-Coding-Index, Pfam Scan, and CPC to screen for new mRNAs or lncRNAs. Subsequently, fragments per kilobase transcript per million mapped fragments of mRNAs and lncRNAs were calculated by StringTie. When identifying circRNA, the 20 bp at both ends of the unaligned reads in the Tophat2 alignment results was used as anchor sequences to be realigned onto the genome using Bowtie2 for the detection of circRNAs. After aligning the anchor sequence of each sample to the reference genome, we combined the alignment results of all samples to identify circRNAs using find_circ. Then, the highly confident circRNAs were filtered on the basis of the following criteria: (1) breakpoints = 1; (2) anchor_overlap ≤ 2 ; (3) edit ≤ 2 ; (4) n_uniq > 2 and n_uniq $>$ samples and n_uniq $>$ int (1/2 samples); (5) best_qual_A > 35 or best_qual_B > 35 ; (6) circRNA is less than 100 k in length. The expression level of circRNAs was estimated by transcript per million.

2.4. Analysis of Differentially Expressed Transcripts

The mRNAs, lncRNAs, and circRNAs expressed differentially between the high-fecundity group and low-fecundity group were identified by DESeq, and the conditions for screening differentially expressed genes were as follows: expression difference multiplicity $|\log_2 \text{ fold change}| > 1$, significance $p\text{-value} < 0.05$.

2.5. Analysis of lncRNA-Regulated Target Genes

The function of lncRNAs is primarily achieved by acting on target genes through cis- and trans-actions. The cis-acting target gene prediction suggested that the function of lncRNA was related to protein-coding genes close to its coordinates. The basic principle of trans-action target gene prediction was that the function of lncRNA was not related to the position relationship of the coding gene but to the protein-coding gene it co-expresses. Therefore, we searched for genes encoded by the nearest protein of the lncRNA gene and concluded that the protein-coding gene we found may be its corresponding cis-regulatory

target gene [24]. Given the small number of samples, lncRNA trans-regulatory function prediction cannot be made.

2.6. Protein–Protein Interaction (PPI) Network Construction

The STRING database (<https://cn.string-db.org>, accessed on 9 November 2022) was used to predict the potential interactions among proteins translated from mRNAs, and a confidence score ≥ 0.4 was selected. The PPI network was visualized through Cytoscape (v3.8.0, <http://www.cytoscape.org/>, accessed on 15 November 2022).

2.7. GO and KEGG Pathway Analysis

Functional analysis of differential genes was performed using the GO and KEGG enrichment analysis tools of Omicshare Tools (<https://www.omicshare.com/tools>, accessed on 9 October 2022) from Gene Denovo Biotechnology Co. Ltd. (Guangzhou, China) $p < 0.05$ was considered significant.

2.8. Quantitative Real-Time PCR (qRT-PCR) Validation

We randomly selected two DE mRNAs, two DE lncRNAs, and two DE circRNAs for qRT-PCR to verify the reliability of the RNA-seq data. The RT Reagent Kit with gDNA Eraser (Takara, Dalian, China) was used for total RNA reverse transcription to synthesize cDNA, according to the manufacturer's instructions. PowerUp SYBR Green Master Mix (Life ABI, Austin, TX, USA) and QuantStudio 5 (Life ABI, Austin, TX, USA) were used to perform qRT-PCR. Melting curve analyses confirmed that all primers were specific for their respective transcript. *Capra hircus* β -actin were used as internal controls, and the results were calculated using the $2^{-\Delta\Delta C_t}$ method [25]. All the primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The values were expressed as means \pm SD. The primer sequences are shown in Table S1.

3. Results

3.1. Results of Sequencing and Characteristics of Transcripts

In total, seven hypothalamic tissue samples obtained from high- and low-fecundity Leizhou goats were subjected to Illumina sequencing after rRNA depletion, which led to the generation of approximately 103.30 million raw reads per sample. After quality control, approximately 102.30 million clean reads per sample were obtained, accounting for $99.03\% \pm 0.28\%$ of raw reads in each library. The Q30 ratio was $>91\%$, and no GC bias was observed, indicating that sequencing clean data were qualified. In the alignment of all clean reads, $87.28\% \pm 1.02\%$ of the clean reads was perfectly mapped to the reference genome of *chx*, including $97.84\% \pm 0.33\%$ uniquely mapped, indicating that sequencing data had an excellent performance and high credibility for downstream analysis results (Table S2). After assembly, a total of 20,194 mRNAs, 3895 lncRNAs, and 37,655 circRNAs were identified in the hypothalamus of Leizhou goats among seven samples (Table S3).

3.2. Differential Transcription Expression Profile

In the hypothalamus, as an important organ of the HPO axis in female mammals, the differential expression of its genes or transcripts may affect the fecundity of Leizhou goats. Therefore, we screened DE mRNAs, DE lncRNAs, and DE circRNAs from RNA-seq data using $p \leq 0.05$ and $|\log_2 \text{fold change}| \geq 1$ as screening conditions. A total of 153 DE mRNAs, 9 DE lncRNAs, and 97 circRNAs were identified and compared between the high- and low-fecundity group. The volcano plot showed that a total of 42 mRNAs, 7 lncRNAs, and 54 circRNAs were significantly upregulated, whereas 111 mRNAs, 2 lncRNAs, and 43 circRNAs were significantly downregulated in the high-fertility group of Leizhou goats compared with the low-fertility group of Leizhou goats (Table S4, Figure 1). These differentially expressed transcripts are valuable for further studies to examine the reproductive performance of Leizhou goats.

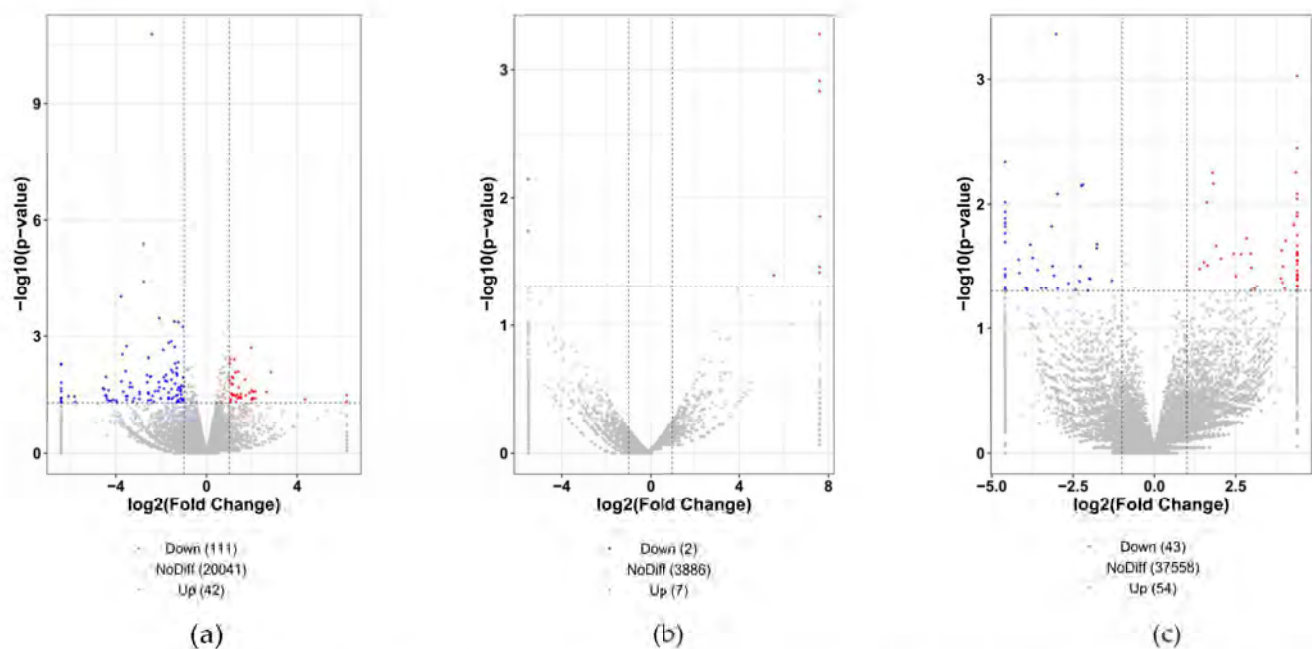


Figure 1. Analysis of differentially expressed mRNAs, lncRNAs, and circRNAs. Volcano plot of DE mRNAs (a), DE lncRNAs (b), and DE circRNAs (c). Red and blue indicate the upregulated and downregulated expression levels, respectively.

3.3. GO and KEGG Enrichment Analysis of DE mRNAs

We performed GO and KEGG enrichment analyses on 153 DE mRNAs to predict the potential biological function of these DE mRNAs. KEGG enrichment analysis showed that these DE mRNAs were significantly enriched to eight pathways, and the three most significant pathways included African trypanosomiasis, natural killer cell-mediated cytotoxicity, and ABC transporters. These signal transduction systems may play a role in the reproductive process, which requires further investigation (Table S5, Figure 2a,b). In GO terms, in the order of the number of enriched genes, the most evident correlation: with biological processes (BP) were cellular process, single-organism process, biological regulation, and regulation of BP; with molecular function (MF) were binding and catalytic activity; and with cellular components (CC) were cell and cell part (Table S6, Figure 2c). Notably, nine genes were enriched into the reproductive process, namely, dpy-19-like 2 (*DPY19L2*), cilia and flagella-associated protein 157 (*CFAP157*), cytokine signaling 3 (*SOC3*), transcription factor 21 (*TCF21*), keratin 8 (*KRT8*), retinoic acid 6 (*STRA6*), natriuretic peptide precursor-A (*NPPA*), LIM homeobox 3 (*LHX3*), and serpin family F member 1 (*SERPINF1*). These genes may be involved in physiological activities related to reproduction, which are worth studying.

3.4. Protein–Protein Interaction Network of DE mRNAs

In investigating the important role of protein interactions in high- and low-fecundity Leizhou goats, we performed PPI network analysis on differential mRNA using String (Figure 3). A total of 140 proteins were enriched in these differential mRNA. After removing the proteins that did not interact with one another, 77 proteins were left to form a complex functional network. Based on the PPI diagram, periostin (*POSTN*, degree = 8), coagulation factor II (*F2*, degree = 7), microfibril-associated protein 5 (*MFAP5*, degree = 7), and decorin (*DCN*, degree = 6) were strongly associated with other proteins (Table S7). These central proteins may play an important function in the regulatory role of the hypothalamus in Leizhou goats.

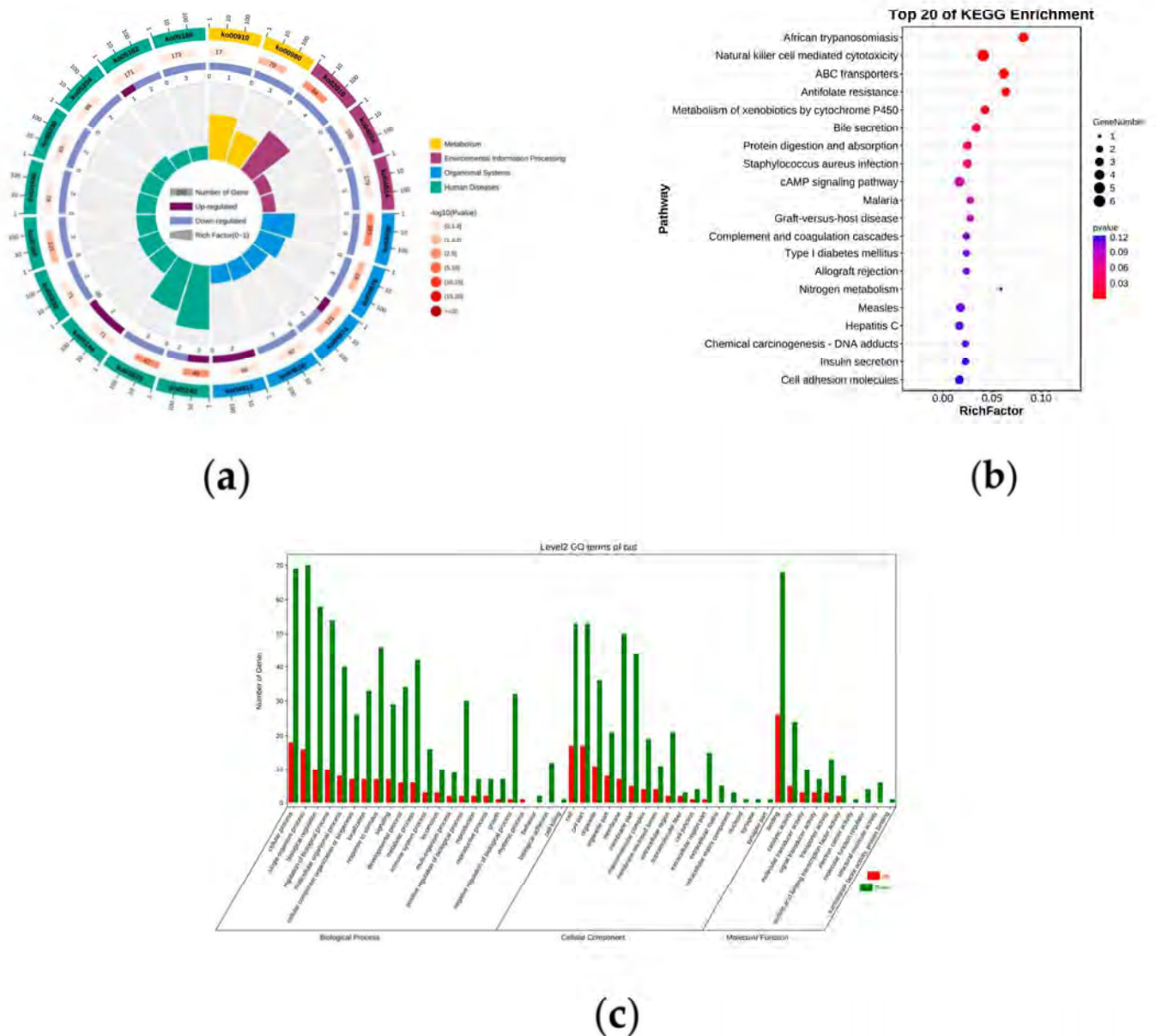


Figure 2. KEGG and GO pathway annotations of DE mRNAs. The enrichment circle map (a) and enrichment bubble map (b) of the top 20 KEGG terms of DE mRNA. The first lap indicates the top 20 KEGG terms, and the number of genes corresponds to the outer lap. The second lap indicates the number of genes in the genome background and Q values for the enrichment of the upregulated genes for the specified BP. The third lap indicates the ratio of the upregulated genes (deep purple) and downregulated genes (light purple). The fourth lap indicates the enrichment factor of each KEGG term. Enrichment factor represents the ratio between the differentially expressed genes and all annotated genes enriched in the pathway. Bubble scale represents the number of different genes; the depth of bubble color represents *p* value. (c) GO annotation of up- and down-regulated genes in BP, CC, and MFs. The abscissa and ordinate represent the GO terms and number of enriched genes, respectively.

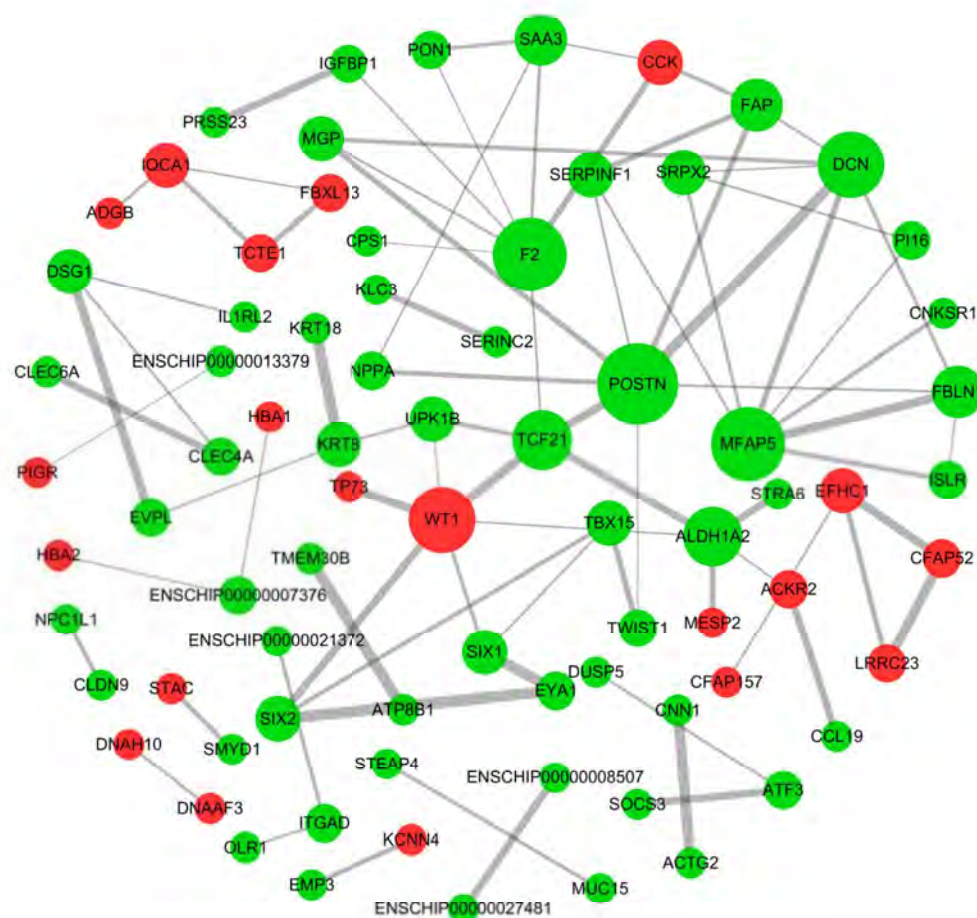


Figure 3. Protein–protein interaction network of DE mRNAs. The nodes in the network represent proteins, and the width of the lines in the nodes indicates the interaction between two proteins. Red nodes, upregulated mRNA; green nodes, downregulated mRNA. The wider the line, the stronger the interaction between the two proteins.

3.5. Functional and Pathway Enrichment Analyses of DE lncRNAs

lncRNA plays an important role in many physiological activities and diseases. lncRNAs can exert regulatory effects through cis-action and trans-actions. The prediction of cis-acting target genes suggests that the function of lncRNA is related to the protein-coding genes close to its coordinates. Here, we constructed a DE lncRNA–mRNA network for DE lncRNAs and their possible cis-regulatory target genes to explore the function of DE lncRNAs (Table S8, Figure 4a). In exploring the effect of DE lncRNAs in the hypothalamus on the fecundity of Leizhou goats, we analyzed the target genes of DE lncRNAs by KEGG and GO enrichment analyses to predict the potential function of DE lncRNAs. In KEGG enrichment analysis (Table S9, Figure 4b), the DE lncRNAs were enriched to a total of 11 pathways, of which six pathways were significantly correlated: antifolate resistance, folate biosynthesis, retrograde endocannabinoid signaling, oxidative phosphorylation, and non-alcoholic fatty liver disease. GO enrichment analysis showed that most of the BP of the target genes of the DE lncRNAs were enriched into metabolic and cellular processes, and most of the MF of the DE lncRNAs were binding; furthermore, most of the CC of the DE lncRNAs were cell and cell part (Table S10, Figure 4c).

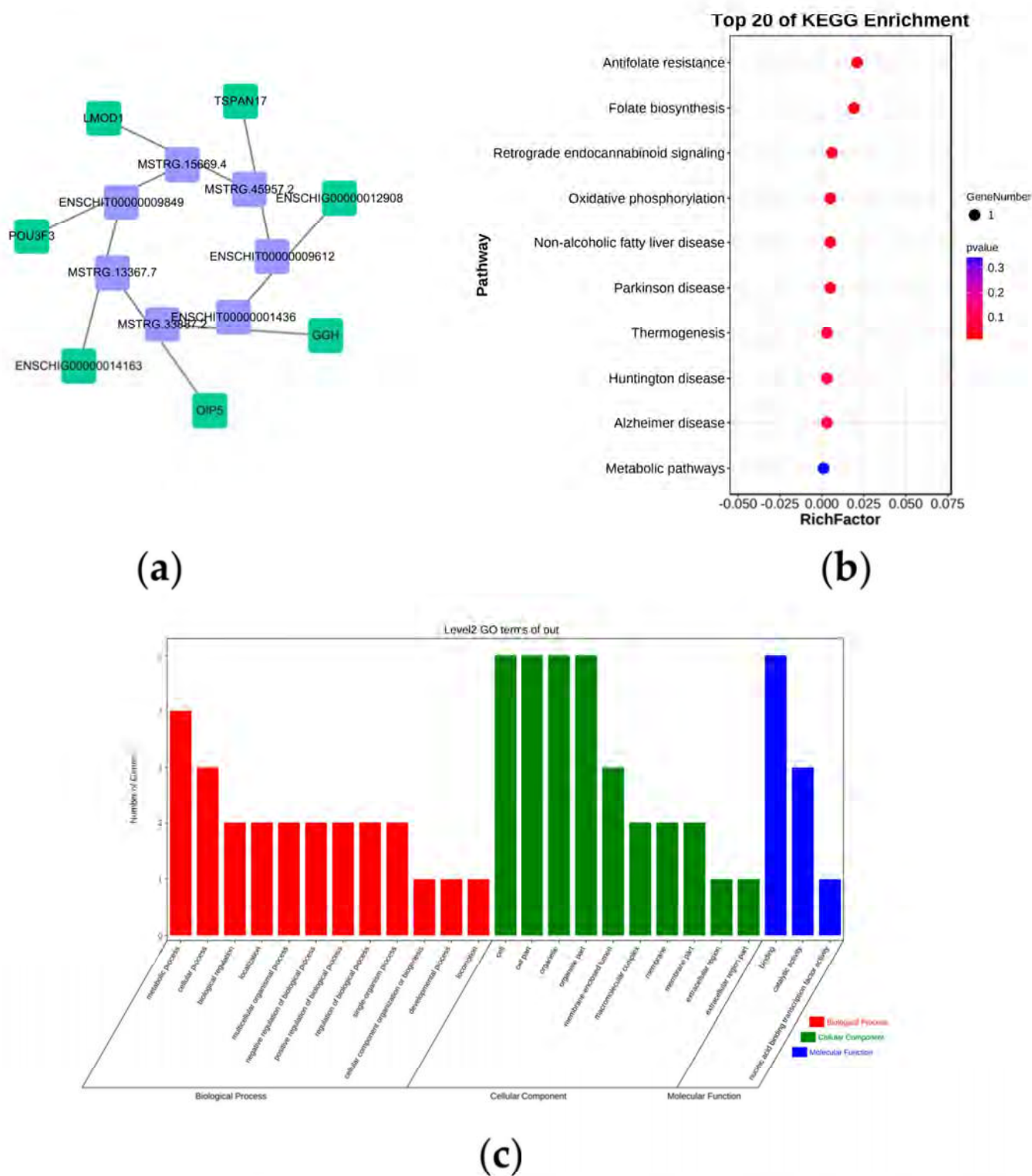


Figure 4. Functional identification of DE lncRNAs. (a) DE lncRNA–mRNA network map. Purple represents DE lncRNA; green represents DE lncRNA’s cis-regulatory target genes. (b) KEGG pathway analyses of target genes of DE lncRNAs. (c) GO analysis of target genes of DE lncRNAs.

3.7. Sequencing Data Validation

In verifying the RNA-seq results, we randomly selected several mRNAs, lncRNAs, and circRNAs for expression-level detection. The expression patterns of these genes were determined using qRT-PCR and compared with RNA-seq results. The results showed that the RNA-seq and qRT-PCR results exhibited similar expression patterns (Figure 6), indicating that the data generated by RNA-seq are reliable.

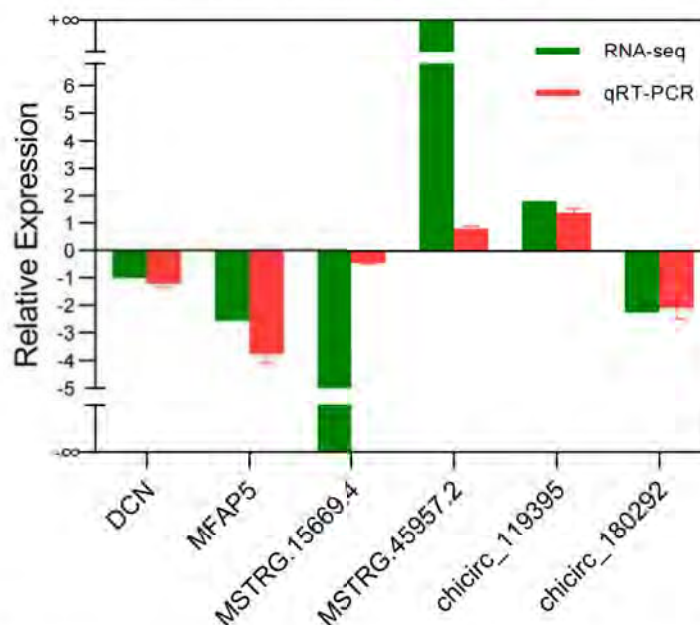


Figure 6. Comparison between RNA-seq and qRT-PCR results. The vertical axis shows the mean of fold change (\log_2 scale) of each RNA measured by qRT-PCR and sequencing.

4. Discussion

As an important economic livestock, goats provide meat, milk, and fur that greatly enrich human life, and they are particularly important to tropical agricultural systems, sustainable agricultural development, pastoralist poverty reduction, and marginal grazing [26,27]. As an indigenous goat breed in China, the Leizhou goat has excellent reproductive performance. The hypothalamus is the center of the endocrine system and the nervous system because of its ability to receive many nerve impulses that regulate reproduction, growth, and energy metabolism in the animal organism. Therefore, we used RNA-seq to identify DE mRNAs, DE lncRNAs, and DE circRNAs in the hypothalamus of high- and low-fertility Leizhou goats and predicted their functions. Our study will expand the molecular mechanism in the hypothalamus that regulates litter size in goats.

4.1. SOCS3 in the Hypothalamus May Affect the Fertility of Leizhou Goats

Animal traits are regulated by gene expression. Litter size is a quantitative character controlled by minor effects and polygenes. With the development of sequencing technology, many genes associated with lambing number were gradually being uncovered, such as bone morphogenetic protein receptor type 1B (*BMPRI1B*) [28,29], growth differentiation factor 9 (*GDF9*) [30,31], and bone morphogenetic protein 15 (*BMP15*) [32]. These genes affected the number of lambs by influencing the ovulation rate of follicles and embryo survival. At present, no studies have been conducted on the differentially expressed genes in the hypothalamus of high- and low-fertility Leizhou goats. A total of 20,194 mRNAs were identified in the hypothalamus of high- and low-fertility Leizhou goats, of which the expression of 153 mRNAs was significantly different. These DE mRNAs were significantly enriched into the immune system, membrane trafficking, digestive system, tissue, and organ development. Furthermore, some of the signaling pathways (prolactin signaling

pathway and Jak-STAT signaling pathway) enriched by these differential genes were similar to the results of a previous study [15]. Therefore, the prolactin signaling pathway and Jak-STAT signaling pathway in the follicular hypothalamus may influence litter size in goats. Remarkably, we found that SOCS3 was enriched into multiple signaling pathways associated with reproduction, including the prolactin signaling pathway and JAK-STAT signaling pathway. SOCS3 belongs to the SOCS family. The study found that the SOCS family of proteins included negative-feedback inhibitors of signaling induced by cytokines that act via the Jak-STAT pathway [33]. The Jak-STAT signaling pathway was the central node of cell function. SOCS3 could directly and specifically bind to JAK or its receptor to inhibit the kinase activity of JAK, thereby inhibiting the Jak-STAT signaling pathway [34]. The Jak-STAT signaling pathway has important functions in GnRH neurons [35]. Therefore, SOCS3 may be able to influence the function of GnRH neurons by regulating the Jak-STAT signaling pathway. SOCS3 may also be involved in regulating leptin signal transduction to affect the reproductive function of the hypothalamus. Leptin, a key metabolic messenger in the neuroendocrine reproductive axis, coordinates fertility by acting on neurons in the preoptic region of the hypothalamus and inducing the synthesis of the freely diffusible volume-based transmitter NO through the activation of neuronal NO synthase (nNOS) in these neurons [36]. As a negative feedback signal inhibitor, SOCS3 can affect JAK and STAT expression to regulate the activity of leptin receptor b, thereby affecting the activity of the leptin signaling pathway [37,38]. Alterations in the activity of the leptin signaling pathways can affect the secretion of proopiomelanocortin (POMC) and Kisspeptin, thereby affecting GnRH secretion, thereby regulating animal reproductive function [39,40]. In addition, a previous study has found that prolactin can affect the reproductive ability of female rats by regulating kisspeptin neurons in the arcuate nucleus [41]. We hypothesize that SOCS3 may regulate the secretion of reproduction-related hormones by affecting the activity of multiple pathways, such as the JAK-STAT signaling pathway, leptin signaling pathway, and prolactin signaling pathway, which may be a factor contributing to the difference in reproductive capacity of Leizhou goats.

4.2. Pivot Protein Function Analysis in High- and Low-Fecundity Leizhou Goats

Among PPI networks, the pivot proteins are encoding products from vital mRNAs, which may play important regulatory roles in the hypothalamus. *POSTN* is a cell-associated protein that participates in cell migration and proliferation, tumorigenesis, and inflammation responses, and it plays an important role in TGF- β signaling [42]. Many genes of the TGF- β family have been found to affect the litter size of sheep, such as *BMP15* [43] and *GDF9* [44]. In addition, *POSTN* regulates spermatogonia proliferation through the Wnt/ β -catenin signal pathway, which may serve as a cytokine for male infertility treatment [45]. Therefore, *POSTN* may play a role in regulating the reproductive capacity of Leizhou goats by determining cell fate or influencing certain signaling pathways in the hypothalamus. *MFAP5* is an extracellular matrix glycoprotein, which has been proven to be involved in the signal transduction of microfiber assembly, elastic formation, and cell survival. It plays an important role in the occurrence of breast cancer [46], ovarian cancer [47], and cervical cancer [48]. As a member of the small leucine-rich proteoglycan family of proteins, *DCN* determines cell fate by mediating various cellular processes such as autophagy, inflammation, cell cycle, and apoptosis [49–51]. These pivot proteins affect cell survival, but their role in the hypothalamus have not yet been reported. We hypothesize that they may regulate the HPO axis by regulating the activity of some hypothalamic neurons to affect the secretion of reproduction-related hormones. The effect of these central proteins on lambing numbers of Leizhou goats deserves further exploration.

4.3. Functional Analysis of DE lncRNAs in High- and Low-Fecundity Leizhou Goats

Gene expression is regulated by ncRNAs such as lncRNAs and circRNAs. Based on the Encyclopedia of DNA Elements transcriptome project, only about 1.2% of the genome can encode proteins, whereas about 80% of the genome is actively transcribed into various ncRNAs [52]. These ncRNAs are involved in a wide range of BPs, such as cell survival, enzyme activity, and disease development. lncRNA and circRNA, as important non-coding RNA family members, have been shown to play a role in the secretion of hypothalamic GnRH. For example, circRNAs such as oar_circ_0018794 and oar_circ_0008291 in sheep may be involved in reproduction by affecting hypothalamic GnRH activity or the expression of key genes [53]. A total of 9 DE lncRNAs and 97 DE circRNAs were identified in high- and low-fecundity Leizhou goats. These nine DE lncRNAs were significantly enriched for antifolate resistance, folate biosynthesis, and retrograde endocannabinoid signaling. Studies have shown that retrograde endocannabinoid signaling is involved in regulating fertility, which reduces GABAergic afferent drive onto GnRH neurons via the activation of presynaptic CB1 receptors [54]. Folate is an important cofactor for DNA synthesis, repair, methylation modification, and other related enzymes necessary for normal cell growth and replication [55]. The appropriate folate concentration promotes G protein subunit α Q (GANQ) promoter methylation, thereby affecting GnRH secretion [56]. In addition, different folate intake during pregnancy affects the development and function of two neuron populations in rats after birth, hypothalamic neuropeptide Y, and opioid melanin (POMC) [57]. Mammals cannot synthesize folate, and they depend on supplementation to maintain normal levels. Therefore, folate absorption and metabolism may affect the function of the hypothalamus. GGH can affect cell proliferation, and intracellular folate metabolism is a ubiquitously expressed intracellular enzyme. Based on our sequencing results, GGH is a cis-regulatory target gene of lncRNA (MSTRG.33887.2). We hypothesized that lncRNAs (MSTRG.33887.2) may regulate GGH expression through cis-regulatory effects to influence hypothalamic folate metabolism. Moreover, these DE lncRNAs were significantly enriched during the development of glomeruli and adrenal glands in GO terms. Glucocorticoids secreted by the adrenal glands can directly regulate the secretion of GnRH in the hypothalamus to influence animal reproduction, although the mechanism of this regulatory effect remains unclear [58]. Furthermore, glucocorticoids secreted by the adrenal glands can indirectly regulate hypothalamic and pituitary reproductive functions by affecting the activity of KISS1 and GnRH neurons [59]. lncRNA has a wide range of biological functions, and it plays an important role in epigenetic modification, transcription, and post-transcriptional regulation. However, given the complexity of its action mechanism, all of its action mechanisms have not been fully understood, which is worthy of further study.

4.4. Functional Analysis of DE circRNAs in High- and Low-Fecundity Leizhou Goats

circRNAs are a class of non-coding RNA that can regulate the expression of host genes by competitively splicing pre-RNA with host genes or binding to U1snRNP (U1 small nuclear ribonucleoprotein) through RNA–RNA interaction and then further interacting with the PolII transcriptional complex to enhance host gene expression [60]. Therefore, studying the function of the host genes of circRNAs is important to understand the biological function of circRNAs. Ryanodine receptor 2 (RYR2) is the host gene of chicirc_098002, which play a critical role in regulating insulin secretion and glucose metabolism homeostasis [61]. In vitro tests have shown that a high-glucose concentration (450 mg/dL) environment led to irreversible cellular damage in GnRH-secreting neurons, which may cause dysfunction of the hypothalamic GnRH pulse generator [62]. chicirc_098002 was downregulated in highly fertile Leizhou goats, and it may regulate RYR2 to influence hypothalamic glucose concentration to regulate GnRH secretion. cAMP positively regulated GnRH secretion, and its mechanism of action may stimulate GnRH release by increasing cAMP through depolarization of neurons initiated by increased cation conductance and cAMP-gated cation channels [63,64]. Phosphodiesterase 10A (PDE10A) is the host gene for chicirc_053531,

which encodes a protein that belongs to the cyclic nucleotide phosphodiesterase family responsible for the hydrolysis of cAMP and cGMP into the inactive forms of 5'-AMP and 5'-GMP, respectively [65]. Therefore, we hypothesize that *PDE10A* may affect GnRH secretion by altering cAMP levels. Our study found that the host gene glutamate metabotropic receptor 1 (*GRM1*) of *chicirc_072583* was enriched in multiple reproduction-related pathways, such as glutamatergic synapse, retrograde endocannabinoid signaling, and estrogen signaling pathway. γ -Aminobutyric acid and glutamate play an important role in the control of GnRH neuronal excitability. The co-culture of GnRH neurons and astrocytes can increase *GRM1* expression and GnRH secretion, indicating that *GRM1* may play a role in the secretion of GnRH [66]. In addition, *GRM1* may serve as a potential molecular marker for controlling seasonal reproduction and litter size in sheep, which is similar to our findings. Therefore, these DE circRNAs may play an important role in regulating the reproductive activity of females.

5. Conclusions

Our study provides the first network of DE lncRNAs, mRNAs, DE circRNAs, and mRNAs from the follicular phase in high- and low-fecundity Leizhou goats. In addition, we identified several DE mRNAs (*SOCS3*, *POSTN*, *MFAP5*, and *DCN*), DE lncRNAs (lncRNA [MSTRG.33887.2]), and DE circRNAs (*chicirc_098002*, *chicirc_053531*, and *chicirc_072583*) from the RNA-seq data of high- and low-fecundity Leizhou goats. These DE mRNAs, DE lncRNAs, and DE circRNAs may play an important role in the regulation of the hypothalamus during the follicular phase, which was worthy of further investigation into the reproductive regulatory mechanism of lambing in goats in the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/genes14020444/s1>, Table S1: Primer sequences used for qRT-PCR; Table S2: Summary of the transcriptome sequencing data obtained in this study; Table S3: CircRNAs information detected in sequence data of hypothalamic transcriptome of Leizhou Goat; Table S4: Expression information the different expression gene; Table S5: The KEGG pathway annotations of DE mRNAs; Table S6: The GO pathway annotations of DE mRNAs; Table S7: Protein information in protein-protein interaction network; Table S8: The DE lncRNAs and their possible cis-regulatory target genes; Table S9: The KEGG pathway annotations of target genes of DE lncRNAs; Table S10: The GO pathway annotations of target genes of DE lncRNAs; Table S11: The DE circRNAs and their host genes; Table S12: The KEGG pathway annotations of host genes of DE circRNAs; Table S13: The GO pathway annotations of host genes of DE circRNAs.

Author Contributions: Conceptualization, G.L. and S.D.; methodology, Y.L.; validation, D.L.; formal analysis, C.Y.; resources, M.D.; data curation, B.H.; writing—original draft preparation, S.D.; writing—review and editing, Y.G., M.D., B.S. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of Guangdong Province (2021A1515010636) and the Modern Agricultural Industry Technology System of Guangdong Province (2022KJ127).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Zhu, M.; Zhang, H.; Yang, H.; Zhao, Z.; Blair, H.T.; Zhai, M.; Yu, Q.; Wu, P.; Fang, C.; Xie, M. Polymorphisms and association of GRM1, GNAQ and HCRTR1 genes with seasonal reproduction and litter size in three sheep breeds. *Reprod. Domest. Anim.* **2022**, *57*, 532–540. [\[CrossRef\]](#)
- van Wettere, W.H.; Herde, P.; Hughes, P.E. Supplementing sow gestation diets with betaine during summer increases litter size of sows with greater numbers of parities. *Anim. Reprod. Sci.* **2012**, *132*, 44–49. [\[CrossRef\]](#)
- Esmaili-Fard, S.M.; Gholizadeh, M.; Hafezian, S.H.; Abdollahi-Arpanahi, R. Genome-wide association study and pathway analysis identify NTRK2 as a novel candidate gene for litter size in sheep. *PLoS ONE* **2021**, *16*, e0244408. [\[CrossRef\]](#)
- Vashi, Y.; Magotra, A.; Kalita, D.; Banik, S.; Sahoo, N.R.; Gupta, S.K.; Naskar, S. Evaluation of candidate genes related to litter traits in Indian pig breeds. *Reprod. Domest. Anim.* **2021**, *56*, 577–585. [\[CrossRef\]](#)
- Chen, X.; Wang, D.; Xiang, H.; Dun, W.; Brahi, D.O.H.; Yin, T.; Zhao, X. Mitochondrial DNA T7719G in tRNA-Lys gene affects litter size in Small-tailed Han sheep. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 31. [\[CrossRef\]](#)
- Ye, J.; Zhao, X.; Xue, H.; Zou, X.; Liu, G.; Deng, M.; Sun, B.; Guo, Y.; Liu, D.; Li, Y. RNA-Seq Reveals miRNA and mRNA Co-regulate Muscle Differentiation in Fetal Leizhou Goats. *Front. Vet. Sci.* **2022**, *9*, 829769. [\[CrossRef\]](#)
- Zhao, Z.Q.; Wang, L.J.; Sun, X.W.; Zhang, J.J.; Zhao, Y.J.; Na, R.S.; Zhang, J.H. Transcriptome analysis of the Capra hircus ovary. *PLoS ONE* **2015**, *10*, e0121586. [\[CrossRef\]](#)
- Mikhael, S.; Punjala-Patel, A.; Gavrilova-Jordan, L. Hypothalamic-Pituitary-Ovarian Axis Disorders Impacting Female Fertility. *Biomedicine* **2019**, *7*, 5. [\[CrossRef\]](#)
- Clarke, I.J. Interface between metabolic balance and reproduction in ruminants: Focus on the hypothalamus and pituitary. *Horm. Behav.* **2014**, *66*, 15–40. [\[CrossRef\]](#)
- Stincic, T.L.; Kelly, M.J. Estrogenic regulation of reproduction and energy homeostasis by a triumvirate of hypothalamic arcuate neurons. *J. Neuroendocrinol.* **2022**, *34*, e13145. [\[CrossRef\]](#)
- Prevot, V.; Dehouck, B.; Sharif, A.; Ciofi, P.; Giacobini, P.; Clasadonte, J. The Versatile Tanycyte: A Hypothalamic Integrator of Reproduction and Energy Metabolism. *Endocr. Rev.* **2018**, *39*, 333–368. [\[CrossRef\]](#)
- Simonneaux, V. A Kiss to drive rhythms in reproduction. *Eur. J. Neurosci.* **2020**, *51*, 509–530. [\[CrossRef\]](#)
- Cech, T.R.; Steitz, J.A. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* **2014**, *157*, 77–94. [\[CrossRef\]](#)
- Palazzo, A.F.; Koonin, E.V. Functional Long Non-coding RNAs Evolve from Junk Transcripts. *Cell* **2020**, *183*, 1151–1161. [\[CrossRef\]](#)
- Liang, C.; Han, M.; Zhou, Z.; Liu, Y.; He, X.; Jiang, Y.; Ouyang, Y.; Hong, Q.; Chu, M. Hypothalamic Transcriptome Analysis Reveals the Crucial MicroRNAs and mRNAs Affecting Litter Size in Goats. *Front. Vet. Sci.* **2021**, *8*, 747100. [\[CrossRef\]](#)
- Memczak, S.; Jens, M.; Elefsinioti, A.; Torti, F.; Krueger, J.; Rybak, A.; Maier, L.; Mackowiak, S.D.; Gregersen, L.H.; Munschauer, M.; et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* **2013**, *495*, 333–338. [\[CrossRef\]](#)
- Ashwal-Fluss, R.; Meyer, M.; Pamudurti, N.R.; Ivanov, A.; Bartok, O.; Hanan, M.; Evtantal, N.; Memczak, S.; Rajewsky, N.; Kadener, S. circRNA biogenesis competes with pre-mRNA splicing. *Mol. Cell* **2014**, *56*, 55–66. [\[CrossRef\]](#)
- Vo, J.N.; Cieslik, M.; Zhang, Y.; Shukla, S.; Xiao, L.; Zhang, Y.; Wu, Y.M.; Dhanasekaran, S.M.; Engelke, C.G.; Cao, X.; et al. The Landscape of Circular RNA in Cancer. *Cell* **2019**, *176*, 869–881.e813. [\[CrossRef\]](#)
- Kopp, F.; Mendell, J.T. Functional Classification and Experimental Dissection of Long Noncoding RNAs. *Cell* **2018**, *172*, 393–407. [\[CrossRef\]](#)
- Bouckenheimer, J.; Fauque, P.; Lecellier, C.H.; Bruno, C.; Commes, T.; Lemaître, J.M.; De Vos, J.; Assou, S. Differential long non-coding RNA expression profiles in human oocytes and cumulus cells. *Sci. Rep.* **2018**, *8*, 2202. [\[CrossRef\]](#)
- Wang, C.; Tan, S.; Liu, W.R.; Lei, Q.; Qiao, W.; Wu, Y.; Liu, X.; Cheng, W.; Wei, Y.Q.; Peng, Y.; et al. RNA-Seq profiling of circular RNA in human lung adenocarcinoma and squamous cell carcinoma. *Mol. Cancer* **2019**, *18*, 134. [\[CrossRef\]](#)
- Perera, B.M.; Bongso, T.A.; Abeynaik, P. Oestrus synchronisation in goats using cloprostenol. *Vet. Rec.* **1978**, *102*, 314. [\[CrossRef\]](#)
- Alavez Ramírez, A.; Arroyo Ledezma, J.; Montes Pérez, R.; Zamora Bustillos, R.; Navarrete Sierra, L.F.; Magaña Sevilla, H. Short communication: Estrus synchronization using progestogens or cloprostenol in tropical hair sheep. *Trop. Anim. Health Prod.* **2014**, *46*, 1515–1518. [\[CrossRef\]](#)
- Yan, S.; Yue, Y.; Sun, M.; Chen, Y.; Wang, X.; Qian, H. Comparative Transcriptome Analysis Reveals Relationship among mRNAs, lncRNAs, and circRNAs of Slow Transit Constipation. *Biomed Res. Int.* **2021**, *2021*, 6672899. [\[CrossRef\]](#)
- Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2^{−ΔΔCT} Method. *Methods* **2001**, *25*, 402–408. [\[CrossRef\]](#)
- Fatet, A.; Pellicer-Rubio, M.-T.; Leboeuf, B. Reproductive cycle of goats. *Anim. Reprod. Sci.* **2011**, *124*, 211–219. [\[CrossRef\]](#)
- Muriuki, C.; Bush, S.J.; Salavati, M.; McCulloch, M.E.B.; Lisowski, Z.M.; Agaba, M.; Djikeng, A.; Hume, D.A.; Clark, E.L. A Mini-Atlas of Gene Expression for the Domestic Goat (*Capra hircus*). *Front. Genet.* **2019**, *10*, 1080. [\[CrossRef\]](#)
- Shimasaki, S.; Moore, R.K.; Otsuka, F.; Erickson, G.F. The bone morphogenetic protein system in mammalian reproduction. *Endocr. Rev.* **2004**, *25*, 72–101. [\[CrossRef\]](#)
- Wen, Y.L.; Guo, X.F.; Ma, L.; Zhang, X.S.; Zhang, J.L.; Zhao, S.G.; Chu, M.X. The expression and mutation of BMPR1B and its association with litter size in small-tail Han sheep (*Ovis aries*). *Arch. Anim. Breed.* **2021**, *64*, 211–221. [\[CrossRef\]](#)
- Silva, J.R.; van den Hurk, R.; van Tol, H.T.; Roelen, B.A.; Figueiredo, J.R. Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. *Mol. Reprod. Dev.* **2005**, *70*, 11–19. [\[CrossRef\]](#)

31. Bi, Y.; Li, J.; Wang, X.; He, L.; Lan, K.; Qu, L.; Lan, X.; Song, X.; Pan, C. Two Novel Rare Strongly Linked Missense SNPs (P27R and A85G) Within the GDF9 Gene Were Significantly Associated With Litter Size in Shaanbei White Cashmere (SBWC) Goats. *Front. Vet. Sci.* **2020**, *7*, 406. [\[CrossRef\]](#)
32. Aboelhassan, D.M.; Darwish, A.M.; Ali, N.I.; Ghaly, I.S.; Farag, I.M. A study on mutation points of GDF9 gene and their association with prolificacy in Egyptian small ruminants. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 85. [\[CrossRef\]](#)
33. Liao, N.P.D.; Laktyushin, A.; Lucet, I.S.; Murphy, J.M.; Yao, S.; Whitlock, E.; Callaghan, K.; Nicola, N.A.; Kershaw, N.J.; Babon, J.J. The molecular basis of JAK/STAT inhibition by SOCS1. *Nat. Commun.* **2018**, *9*, 1558. [\[CrossRef\]](#)
34. Hu, X.; Li, J.; Fu, M.; Zhao, X.; Wang, W. The JAK/STAT signaling pathway: From bench to clinic. *Signal Transduct. Target. Ther.* **2021**, *6*, 402. [\[CrossRef\]](#)
35. Ko, E.K.; Chorch, L.P.; Sullivan, M.E.; Cameron, R.S.; Layman, L.C. JAK/STAT signaling pathway gene expression is reduced following Nelf knockdown in GnRH neurons. *Mol. Cell. Endocrinol.* **2018**, *470*, 151–159. [\[CrossRef\]](#)
36. Bellefontaine, N.; Chachlaki, K.; Parkash, J.; Vanacker, C.; Colledge, W.; d'Anglemont de Tassigny, X.; Garthwaite, J.; Bouret, S.G.; Prevot, V. Leptin-dependent neuronal NO signaling in the preoptic hypothalamus facilitates reproduction. *J. Clin. Invest.* **2014**, *124*, 2550–2559. [\[CrossRef\]](#)
37. Robertson, S.A.; Leininger, G.M.; Myers, M.G. Molecular and neural mediators of leptin action. *Physiol. Behav.* **2008**, *94*, 637–642. [\[CrossRef\]](#)
38. Inagaki-Ohara, K.; Mayuzumi, H.; Kato, S.; Minokoshi, Y.; Otsubo, T.; Kawamura, Y.I.; Dohi, T.; Matsuzaki, G.; Yoshimura, A. Enhancement of leptin receptor signaling by SOCS3 deficiency induces development of gastric tumors in mice. *Oncogene* **2014**, *33*, 74–84. [\[CrossRef\]](#)
39. Guzmán, A.; Hernández-Coronado, C.G.; Rosales-Torres, A.M.; Hernández-Medrano, J.H. Leptin regulates neuropeptides associated with food intake and GnRH secretion. *Ann. D'endocrinologie* **2019**, *80*, 38–46. [\[CrossRef\]](#)
40. Sobrino, V.; Avendaño, M.S.; Perdices-López, C.; Jimenez-Puyet, M.; Tena-Sempere, M. Kisspeptins and the neuroendocrine control of reproduction: Recent progress and new frontiers in kisspeptin research. *Front. Neuroendocrinol.* **2022**, *65*, 100977. [\[CrossRef\]](#)
41. Araujo-Lopes, R.; Crampton, J.R.; Aquino, N.S.; Miranda, R.M.; Kokay, I.C.; Reis, A.M.; Franci, C.R.; Grattan, D.R.; Szawka, R.E. Prolactin regulates kisspeptin neurons in the arcuate nucleus to suppress LH secretion in female rats. *Endocrinology* **2014**, *155*, 1010–1020. [\[CrossRef\]](#)
42. Landry, N.M.; Cohen, S.; Dixon, I.M.C. Periostin in cardiovascular disease and development: A tale of two distinct roles. *Basic Res. Cardiol.* **2018**, *113*, 1. [\[CrossRef\]](#)
43. Monniaux, D. Driving folliculogenesis by the oocyte-somatic cell dialog: Lessons from genetic models. *Theriogenology* **2016**, *86*, 41–53. [\[CrossRef\]](#)
44. Christoforou, E.R.; Pitman, J.L. Intrafollicular growth differentiation factor 9: Bone morphogenetic 15 ratio determines litter size in mammals. *Biol. Reprod.* **2019**, *100*, 1333–1343. [\[CrossRef\]](#)
45. Li, C.; Cheng, D.; Xu, P.; Nie, H.; Zhang, T.; Pang, X. POSTN Promotes the Proliferation of Spermatogonial Cells by Activating the Wnt/ β -Catenin Signaling Pathway. *Reprod. Sci.* **2021**, *28*, 2906–2915. [\[CrossRef\]](#)
46. Chen, Z.; Yan, X.; Li, K.; Ling, Y.; Kang, H. Stromal fibroblast-derived MFAP5 promotes the invasion and migration of breast cancer cells via Notch1/slug signaling. *Clin. Transl. Oncol. Off. Publ. Fed. Span. Oncol. Soc. Natl. Cancer Inst. Mex.* **2020**, *22*, 522–531. [\[CrossRef\]](#)
47. Leung, C.S.; Yeung, T.L.; Yip, K.P.; Pradeep, S.; Balasubramanian, L.; Liu, J.; Wong, K.K.; Mangala, L.S.; Armaiz-Pena, G.N.; Lopez-Berestein, G.; et al. Calcium-dependent FAK/CREB/TNNC1 signalling mediates the effect of stromal MFAP5 on ovarian cancer metastatic potential. *Nat. Commun.* **2014**, *5*, 5092. [\[CrossRef\]](#)
48. Li, Q.; Zhang, Y.; Jiang, Q. MFAP5 suppression inhibits migration/invasion, regulates cell cycle and induces apoptosis via promoting ROS production in cervical cancer. *Biochem. Biophys. Res. Commun.* **2018**, *507*, 51–58. [\[CrossRef\]](#)
49. Neill, T.; Iozzo, R.V. The Role of Decorin Proteoglycan in Mitophagy. *Cancers* **2022**, *14*, 804. [\[CrossRef\]](#)
50. Diehl, V.; Huber, L.S.; Trebicka, J.; Wygrecka, M.; Iozzo, R.V.; Schaefer, L. The Role of Decorin and Biglycan Signaling in Tumorigenesis. *Front. Oncol.* **2021**, *11*, 801801. [\[CrossRef\]](#)
51. Liu, J.; Zhu, S.; Zeng, L.; Li, J.; Klionsky, D.J.; Kroemer, G.; Jiang, J.; Tang, D.; Kang, R. DCN released from ferroptotic cells ignites AGER-dependent immune responses. *Autophagy* **2022**, *18*, 2036–2049. [\[CrossRef\]](#)
52. An integrated encyclopedia of DNA elements in the human genome. *Nature* **2012**, *489*, 57–74. [\[CrossRef\]](#)
53. Zhang, Z.; Tang, J.; He, X.; Zhu, M.; Gan, S.; Guo, X.; Zhang, X.; Zhang, J.; Hu, W.; Chu, M. Comparative Transcriptomics Identify Key Hypothalamic Circular RNAs that Participate in Sheep (*Ovis aries*) Reproduction. *Animals* **2019**, *9*, 557. [\[CrossRef\]](#)
54. Farkas, I.; Kalló, I.; Deli, L.; Vida, B.; Hrabovszky, E.; Fekete, C.; Moenter, S.M.; Watanabe, M.; Liposits, Z. Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. *Endocrinology* **2010**, *151*, 5818–5829. [\[CrossRef\]](#)
55. Wang, S.; Chen, Y.; Fang, H.; Xu, Y.; Ding, M.; Ma, C.; Lin, Y.; Cui, Z.; Sun, H.; Niu, Q.; et al. A γ -glutamyl hydrolase lacking the signal peptide confers susceptibility to folates/antifolates in acute lymphoblastic leukemia cells. *FEBS Lett.* **2022**, *596*, 437–448. [\[CrossRef\]](#)
56. Zhai, M.; Zhao, Z.; Yang, M.; Liang, Y.; Liang, H.; Xie, Y.; Han, J. The effect of GNAQ methylation on GnRH secretion in sheep hypothalamic neurons. *J. Cell. Biochem.* **2019**, *120*, 19396–19405. [\[CrossRef\]](#)

57. Yang, N.V.; Pannia, E.; Chatterjee, D.; Kubant, R.; Ho, M.; Hammoud, R.; Pausova, Z.; Anderson, G.H. Gestational folic acid content alters the development and function of hypothalamic food intake regulating neurons in Wistar rat offspring post-weaning. *Nutr. Neurosci.* **2020**, *23*, 149–160. [\[CrossRef\]](#)
58. Du, X.; He, X.; Liu, Q.; Di, R.; Liu, Q.; Chu, M. Comparative Transcriptomics Reveals the Key lncRNA and mRNA of Sunite Sheep Adrenal Gland Affecting Seasonal Reproduction. *Front. Vet. Sci.* **2022**, *9*, 816241. [\[CrossRef\]](#)
59. Whirledge, S.; Cidlowski, J.A. Glucocorticoids and Reproduction: Traffic Control on the Road to Reproduction. *Trends Endocrinol. Metab.* **2017**, *28*, 399–415. [\[CrossRef\]](#)
60. Ebbesen, K.K.; Kjems, J.; Hansen, T.B. Circular RNAs: Identification, biogenesis and function. *Biochim. Biophys. Acta* **2016**, *1859*, 163–168. [\[CrossRef\]](#)
61. Santulli, G.; Pagano, G.; Sardu, C.; Xie, W.; Reiken, S.; D’Ascia, S.L.; Cannone, M.; Marziliano, N.; Trimarco, B.; Guise, T.A.; et al. Calcium release channel RyR2 regulates insulin release and glucose homeostasis. *J. Clin. Investig.* **2015**, *125*, 1968–1978. [\[CrossRef\]](#)
62. Pal, L.; Chu, H.P.; Shu, J.; Topalli, I.; Santoro, N.; Karkanias, G. In vitro evidence of glucose-induced toxicity in GnRH secreting neurons: High glucose concentrations influence GnRH secretion, impair cell viability, and induce apoptosis in the GT1-1 neuronal cell line. *Fertil. Steril.* **2007**, *88*, 1143–1149. [\[CrossRef\]](#)
63. Frattarelli, J.L.; Krsmanovic, L.Z.; Catt, K.J. The relationship between pulsatile GnRH secretion and cAMP production in immortalized GnRH neurons. *Am. J. Physiol. Endocrinol. Metab.* **2011**, *300*, E1022–E1030. [\[CrossRef\]](#)
64. Vitalis, E.A.; Costantin, J.L.; Tsai, P.S.; Sakakibara, H.; Paruthiyil, S.; Iiri, T.; Martini, J.F.; Taga, M.; Choi, A.L.; Charles, A.C.; et al. Role of the cAMP signaling pathway in the regulation of gonadotropin-releasing hormone secretion in GT1 cells. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 1861–1866. [\[CrossRef\]](#)
65. Amin, H.S.; Parikh, P.K.; Ghatge, M.D. Medicinal chemistry strategies for the development of phosphodiesterase 10A (PDE10A) inhibitors—An update of recent progress. *Eur. J. Med. Chem.* **2021**, *214*, 113155. [\[CrossRef\]](#)
66. Ojeda, S.R.; Lomniczi, A.; Sandau, U. Contribution of glial-neuronal interactions to the neuroendocrine control of female puberty. *Eur. J. Neurosci.* **2010**, *32*, 2003–2010. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.



Identification and Comparative Analysis of Long Non-coding RNAs in High- and Low-Fecundity Goat Ovaries During Estrus

Yaokun Li¹, Xiangping Xu¹, Ming Deng¹, Xian Zou², Zhifeng Zhao¹, Sixiu Huang¹, Dewu Liu^{1*} and Guangbin Liu^{1*}

OPEN ACCESS

Edited by:

Rui Su,
Inner Mongolia Agricultural University,
China

Reviewed by:

Ran Di,
Institute of Animal Sciences, Chinese
Academy of Agricultural Sciences,
China
Zhibin Ji,
Shandong Agricultural University,
China
Jiangjiang Zhu,
Southwest Minzu University, China
Adeyemi Adenaike,
Federal University of Agriculture,
Abeokuta, Nigeria

*Correspondence:

Dewu Liu
dwlw@scau.edu.cn
Guangbin Liu
gblw@scau.edu.cn

Specialty section:

This article was submitted to
Livestock Genomics,
a section of the journal
Frontiers in Genetics

Received: 31 December 2020

Accepted: 06 May 2021

Published: 25 June 2021

Citation:

Li Y, Xu X, Deng M, Zou X,
Zhao Z, Huang S, Liu D and Liu G
(2021) Identification and Comparative
Analysis of Long Non-coding RNAs
in High- and Low-Fecundity Goat
Ovaries During Estrus.
Front. Genet. 12:648158.
doi: 10.3389/fgene.2021.648158

¹ Guangdong Laboratory for Lingnan Modern Agriculture, College of Animal Science, South China Agricultural University, Guangzhou, China, ² State Key Laboratory of Livestock and Poultry Breeding, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China

The ovary is the most important reproductive organ in goats and directly affects the fecundity. Long non-coding RNAs (lncRNAs) are involved in the biological process of oocyte maturation. However, in the context of reproduction in goats, few studies have explored the regulation of lncRNAs. Therefore, we herein used the ovaries of high and low fecundity Leizhou black goats to identify differentially expressed lncRNAs (DElncRNAs) by high-throughput RNA sequencing; moreover, we analyzed the target genes of lncRNAs by functional annotation to explore the role of DElncRNAs in ovarian development. Twenty DElncRNAs were identified, of which six were significantly upregulated and 14 were significantly downregulated in high fecundity goats. Gene Ontology analyses suggested that MSTRG.3782 positively influences the expression of the corresponding gene *API5*, exerting regulative effects on the development of follicles, through which litter size might show variations. The target gene *KRR1* of ENSCHIT00000001883 is significantly enriched in cell components, and ENSCHIT00000001883 may regulate cell growth and thus affect follicular development. Further, as per Kyoto Encyclopedia of Genes and Genomes pathway analyses, MSTRG.2938 was found to be significantly enriched, and we speculate that MSTRG.2938 could regulate ribosomal biogenesis in the pre-snoRNP complex as well as cell transformation in eukaryotes. Quantitative real-time PCR results were consistent with sequencing data. To conclude, our research results indicate that some lncRNAs play a key role in regulating follicle development and cell growth during goat's ovarian development.

Keywords: long non-coding RNA, litter size, goats, reproduction, fertility, high-throughput nucleotide sequencing

INTRODUCTION

Litter size is influenced not only by nutrition levels and environment but also by inheritance (Cui et al., 2009). The ovary is the most important organ for the normal reproductive function of goats. It secretes estrogen to maintain sexual characteristics and cyclical reproductive activity; further, oocytes and ovulation have a major impact on the fertility of goats (Barnett et al., 2006;

Zhao et al., 2015). Studies have shown that the ovulation rate of goats is linked to high productivity (Pramod et al., 2013). lncRNAs play a chief role in reproduction-related processes in animals, but very limited information is available on the functions of lncRNAs in goats. In particular, in the context of reproduction in goats, few studies have explored the regulation of lncRNAs (Xing et al., 2014). Long non-coding RNAs are non-coding RNA transcripts of >200 nucleotides in length; they have a complex structure and lack the ability to code proteins (Jarroux et al., 2017). They can regulate gene expression and protein function to perform biological functions. Studies have reported that lncRNAs can regulate reproductive processes, such as ovarian development and maturation in female animals (Li et al., 2015; Ling et al., 2017; Liu et al., 2020). Therefore, it is crucial to study their role by exploring the function of key target genes.

High-throughput RNA sequencing and functional analyses have been used to elucidate the reproductive function of lncRNAs that were identified to be differentially expressed between the ovaries of multiparous and uniparous Anhui white goats; TCONS_00136407, TCONS_00146968, and TCONS_00320849, for example, were suggested to participate in oocyte meiosis (Ling et al., 2017). Using the same method to study the function of differentially expressed lncRNAs (DElncRNAs) in Chuanzhong black goats, ENSCHIT00000005909 and ENSCHIT00000005910 were suggested to regulate the viability and proliferation of keratinocyte-derived cells by influencing *IL1R2* (interleukin 1 receptor type II) thereby affecting ovarian function (Bouckenheimer et al., 2018). Leizhou black goat is a special local goat breed in southern China, which shows excellent adaptability to the living circumstance with high humidity and high temperature, and using high-throughput sequencing and bioinformatics analysis can help us to explore the novel functional DElncRNAs in the ovaries of goats.

Litter size is one of the most important economic traits in goat production, determining the benefit of farming enterprises. To provide a theoretical basis for goat breeding and improve the production efficiency of goat industry, it is vital to conduct in-depth research on the mechanisms regulating litter size. We herein screened DElncRNAs between the ovaries of high and low fecundity Leizhou black goats and predicted the target genes of DElncRNAs. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were used to analyze the function of target genes. Our results not only enrich the transcriptomic data of the goat ovary but also provide a theoretical basis for combining molecular breeding and conventional breeding technologies.

MATERIALS AND METHODS

Ethics Statement

All study protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals at the South China Agricultural University (permit no.: SYXK-2014-0136). Further,

all experiments were performed in accordance with the guidelines of the South China Agricultural University.

Animals and Sample Collection

Seven healthy female Leizhou black goats (age, 3.5–4.5 years) were divided into high and low fecundity groups. The litter size of high-fecundity group ($n = 3$) and low-fecundity group ($n = 4$) were more than one and only one, respectively. Meanwhile, all of the samples in this study were from goats with three parity records of litter size. The female goats were injected with 0.1 mg cloprostenol to induce estrus (Perera et al., 1978; Martemucci and D'Alessandro, 2011; Fierro et al., 2013). The goats were kept under observation to determine whether they were in heat (bleating, searching for the male goat, frequent urination, hyperemia, edema, contraction of the vulva, and vaginal mucus discharge). The basis of estrus was the female goat shaking their tail, standing, and accepting to mate with the male goat (Taylor, 1978; Mekuriaw et al., 2016). The ovaries were collected within 24 h of estrus. The selected goats were killed and dissected, and both whole ovaries from each goat were collected immediately. The intact ovaries were collected and washed with 75% alcohol thrice. Then they were soaked into phosphate buffered saline. After the collection of the ovary, the ligaments and attached tissues were trimmed off under surgical anatomy microscope, ovarian follicles were isolated from the ovary, and the isolated ovarian tissue was frozen in liquid nitrogen and stored at -80°C .

Total RNA Isolation, cDNA Library Construction, and Transcriptome Sequencing

After thoroughly grinding the ovarian tissue, total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, United States). NanoDrop ND-2000 was used to measure RNA concentration (Thermo Science, Wilmington, DE, United States). RNA integrity was assessed by denaturing agarose gel electrophoresis. Further, the cDNA library was constructed using 3 μl of total RNA from each sample, and double-terminal sequencing was performed on the HiSeq X-TEN sequencing platform by Shanghai Parsons Biotech Co., Ltd.

Quality Control of Raw Sequences

We used Cutadapt to remove reads with an average quality score below Q20. The Q20 value referred to the error probability of 1% for the identified bases in the process of base recognition. The reference genome index (GCF_001704415.1_ARS1_genomic¹) was established by Bowtie 2, and the filtered reads were compared with the reference genome using TopHat 2. If the mismatch between the reads and the reference genome sequence was within 2, we considered the alignment to be successful (Kim et al., 2013).

Assembly and Novel lncRNA Prediction

According to the TopHat 2 results, StringTie was used for transcript assembly, and candidate lncRNAs were then selected

¹<http://www.ensembl.org/>

based on the splicing results and structural features of lncRNAs. The screening conditions to identify lncRNAs were as follows: (1) transcripts with low expression levels, low credible single exon transcripts, and exon numbers < 2 were filtered out and (2) transcripts < 200 bp in length were excluded (Trapnell et al., 2010; Cabili et al., 2011). Moreover, Coding-Non-Coding-Index v2 (Barnett et al., 2006), Coding Potential Calculator (0.9-r2; Sun et al., 2013), Pfam Scan v1.3 (Punta et al., 2012), and phylogenetic codon substitution frequency (v20121028; Lin et al., 2011) were used for coding potential analyses. Transcripts without coding potential comprised the candidate set of lncRNAs. lncRNA expression at the transcription level was analyzed with StringTie. DESeq was used to analyze the expression of lncRNAs; the screening conditions were $|\text{Log2FoldChange}| > 1$ and $P < 0.05$ (Love et al., 2014). The ggplot 2 software package was used to construct a volcano map of DElncRNAs, and the pheatmap software package was used to perform clustering according to the expression level of same lncRNAs in different samples and that of different lncRNAs in the same sample. Distance was calculated with the Euclidean method and clustering was performed using hierarchical agglomerative clustering (Wang et al., 2010).

Target Gene Prediction

To explore the functions of lncRNAs, we predicted the target genes of DElncRNAs. Because the reliability of the analysis results is not high when the sample number is small, the function of trans-regulation can not be predicted. We searched the genes 100 kb downstream and upstream of lncRNAs and analyzed their functions.

GO and KEGG Pathway Analyses for Target Genes of DElncRNAs

GO analysis was performed with the predicted target genes using DAVID². Furthermore, we used the KEGG database to analyze the potential functions of these genes in pathways³ (Dennis et al., 2003; Han et al., 2012). A hypergeometric test was applied to discover the significant enrichment of GO terms and KEGG pathways so as to determine the main biological functions of differentially expressed genes (Wang et al., 2020; Huang et al., 2007). $P < 0.05$ indicated statistical significance.

Quantitative Real-Time PCR (qRT-PCR) for DElncRNAs

Total RNA (1 µg) was first reverse-transcribed using an RT Reagent Kit with gDNA Eraser (Takara, Dalian, China), according to manufacturer instructions. qRT-PCR was performed on a StepOnePlus Real-Time PCR System (Life Technologies, United States), as per the standard protocol, using TB Green Fast qPCR Mix (Takara, Dalian, China). Primer Premier 5 used in primer design. Capra hircus β-actin served as the endogenous control for mRNA and lncRNA expression analyses.

²<https://david.ncifcrf.gov>

³<http://www.genome.jp/kegg/>

RESULTS

Sequencing Data Quality Control

The raw reads from the high and low fecundity groups were analyzed for quality control before further analyses. The Q30 value for each sample exceeded 93% (Table 1). Within the mapped reads, >85% of total reads were mapped to the reference genome without any mismatch (Table 2), indicating that the sequencing data was of high quality and suitable for subsequent analyses.

Screening and Validation of DElncRNAs

Of 4,462 lncRNAs, 20 were differentially expressed between the high and low fecundity groups. Compared with the low fecundity group, six lncRNAs were upregulated and 14 were downregulated in the high fecundity group ($P < 0.05$; Figure 1A). From the heatmap analysis, the expression level of the same lncRNA in the same group was essentially the same, indicating that there was little difference between the samples in the same group. Four and three samples belonging to the low and high fecundity groups, respectively, were clustered together, indicating that lncRNA expression patterns in the groups were different (Figure 1B). Six DElncRNAs were randomly selected for qRT-PCR to verify the reliability of RNA sequencing data. qRT-PCR results were fundamentally consistent with sequencing results, confirming that the sequencing data had high reliability (Figure 1C).

GO Analyses for Target Genes of lncRNAs

GO Analyses for Target Genes of All lncRNAs

The 4,462 lncRNAs corresponded to 2,870 genes, of which DElncRNAs corresponded to 19 target genes. To explore the biological function of lncRNAs involved in regulating litter size, we performed GO and KEGG pathway analysis to identify the functions of target genes. GO analyses revealed diverse biological functions, such as positive regulation of transcription from RNA polymerase II promoter, patterning of blood vessels, and palate development, and multiple target genes were involved, such as *CTNNA1* (encoding catenin beta-1), *WNT5A*, and *EDN1* (endothelin-1). Transcription factor complex, nucleus,

TABLE 1 | Quality control of RNA-seq data.

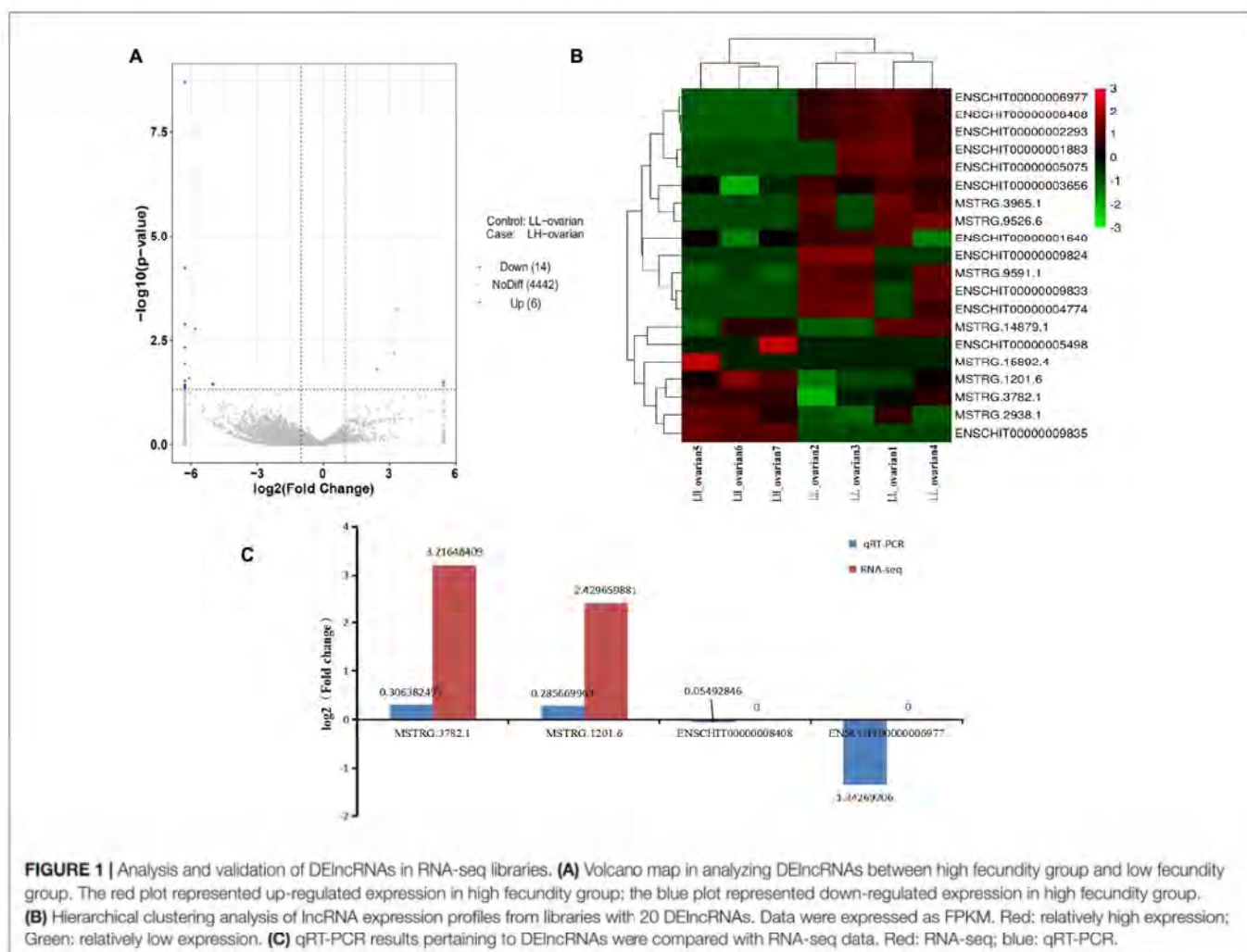
Sample	Clean reads (bp)	Clean reads (%)	Q30 (bp)	Q30 (%)
LL_ovarian1	102,568,128	99.71	14,469,811,737	93.78
LL_ovarian2	105,050,222	99.76	14,876,252,498	94.18
LL_ovarian3	101,304,042	99.66	14,193,808,086	93.09
LL_ovarian4	106,886,182	99.74	15,093,669,895	93.89
LH_ovarian1	101,039,510	99.51	14,381,870,205	94.43
LH_ovarian2	104,636,964	99.36	14,844,532,299	93.97
LH_ovarian3	101,631,476	99.59	14,245,966,824	93.07

Q30 value represents the error probability of 0.1% for the identified bases in the process of base recognition; LL_ovarian1–LL_ovarian4, low fecundity goats; LH_ovarian1–LH_ovarian3, high fecundity goats.

TABLE 2 | Statistics of the mapping result.

Sample	Total-mapped(bp)	Multi-mapped(bp)	Uniquely-mapped(bp)
LL_ovarian1	87,722,258(85.53%)	3,401,453(3.88%)	84,320,805(96.12%)
LL_ovarian2	94,045,775(89.52%)	2,863,389(3.04%)	91,182,386(96.96%)
LL_ovarian3	88,004,353(86.87%)	2,413,301(2.74%)	85,591,052(97.26%)
LL_ovarian4	95,760,551(89.59%)	2,719,333(2.84%)	93,041,218(97.16%)
LH_ovarian1	90,985,423(90.05%)	2,200,033(2.42%)	88,785,390(97.58%)
LH_ovarian2	93,646,857(89.50%)	2,601,986(2.78%)	91,044,871(97.22%)
LH_ovarian3	89,517,832(88.08%)	2,404,052(2.69%)	87,113,780(97.31%)

Multi-mapped, the total number of sequences aligned to multiple positions; Uniquely mapped number of sequences with unique alignment positions on the reference sequence.



and integral component of plasma membrane were the top three terms significantly enriched in the cellular component, whereas transcriptional repressor activity, RNA polymerase II core promoter proximal region sequence-specific binding, sequence-specific DNA binding and RNA polymerase II core promoter proximal region sequence-specific DNA binding were the top three terms significantly enriched in the molecular function ($P < 0.05$; Table 3 and Figure 2A). *ZNF536* and *SALL1* (sal-like 1) are noted to be involved in these functions.

GO Analyses for Target Genes of DElncRNAs

GO analysis revealed that 47 terms were significantly enriched between the high and low fecundity groups; the target genes involved were *IER2* (immediate early response protein 2), *TBXT*, *API5* (encoding apoptosis inhibitor 5), *KRR1*, *ARRDC4* (arrestin domain containing 4), *NOP56* (encoding nucleolar protein 56), and *OIP5* (encoding OPA-interacting protein 5) ($P < 0.05$). The target gene *API5* of MSTRG.3782 participated in 14 GO terms, including nuclear lumen, negative regulation

TABLE 3 | Top 10 significantly enriched Gene Ontology (GO) terms of target genes of all long non-coding RNAs (lncRNAs).

GO ID	GO name	Observed gene count	P
Molecular function			
GO:0001078	Transcriptional repressor activity, RNA polymerase II core promoter proximal region sequence-specific binding	25	8.11923E-09
GO:0043565	Sequence-specific DNA binding	50	2.81848E-07
GO:0000978	RNA polymerase II core promoter proximal region sequence-specific DNA binding	44	6.27029E-07
GO:0003682	Chromatin binding	44	7.63455E-06
GO:0001077	Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	32	1.22538E-05
GO:0003700	Transcription factor activity, sequence-specific DNA binding	55	5.63408E-05
GO:0044212	Transcription regulatory region DNA binding	21	0.000261987
GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	10	0.001142393
GO:0005249	Voltage-gated potassium channel activity	10	0.001653743
GO:0003705	Transcription factor activity, RNA polymerase II distal enhancer sequence-specific binding	7	0.001653743
Biological process			
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	79	4.46E-09
GO:0001569	Patterning of blood vessels	13	1.33E-07
GO:0060021	Palate development	20	6.08E-07
GO:0051965	Positive regulation of synapse assembly	16	1.60E-06
GO:0090090	Negative regulation of canonical Wnt signaling pathway	20	6.79359E-06
GO:0045665	Negative regulation of neuron differentiation	14	1.19474E-05
GO:0007411	Axon guidance	20	1.20265E-05
GO:0042493	Response to drug	18	1.83209E-05
GO:0042733	Embryonic digit morphogenesis	14	3.44706E-05
GO:0042475	Odontogenesis of dentin-containing tooth	13	3.71271E-05
Cellular component			
GO:0005667	Transcription factor complex	32	2.15852E-07
GO:0005634	Nucleus	232	6.64207E-07
GO:0005887	Integral component of plasma membrane	74	0.003866795
GO:0005615	Extracellular space	81	0.005325092
GO:0030424	Axon	16	0.006989932
GO:0005794	Golgi apparatus	50	0.007490282
GO:0005783	Endoplasmic reticulum	45	0.022593322
GO:0016592	Mediator complex	7	0.022693577
GO:0009897	External side of plasma membrane	20	0.022811136
GO:0071944	Cell periphery	5	0.031220242

of fibroblast apoptotic process, and regulation of fibroblast apoptotic process. The target gene *NOP56* of MSTRG.2938 participated in 13 GO terms, including nuclear lumen, histone methyltransferase binding, and pre-snoRNP complex. Further, we classified the function of the target genes into the three major GO categories of biological process, cellular component, and molecular function. Positive regulation of transcription from RNA polymerase II promoter involved in myocardial precursor cell differentiation, positive regulation of transcription from RNA polymerase II promoter involved in heart development, regulation of transcription from RNA polymerase II promoter involved in myocardial precursor cell differentiation were the top three abundant terms in the biological process category ($P < 0.05$). In the cellular component category, nuclear lumen, pre-snoRNP complex, and membrane-enclosed lumen were the top three abundant terms, whereas in the molecular function category, histone methyltransferase binding, protein binding, bridging involved in substrate recognition for ubiquitination, and snoRNA binding were the top three abundant terms ($P < 0.05$; Table 4 and Figure 2B).

KEGG Pathway Analyses for Target Genes of lncRNAs

KEGG Pathway Analyses for Target Genes of All lncRNAs

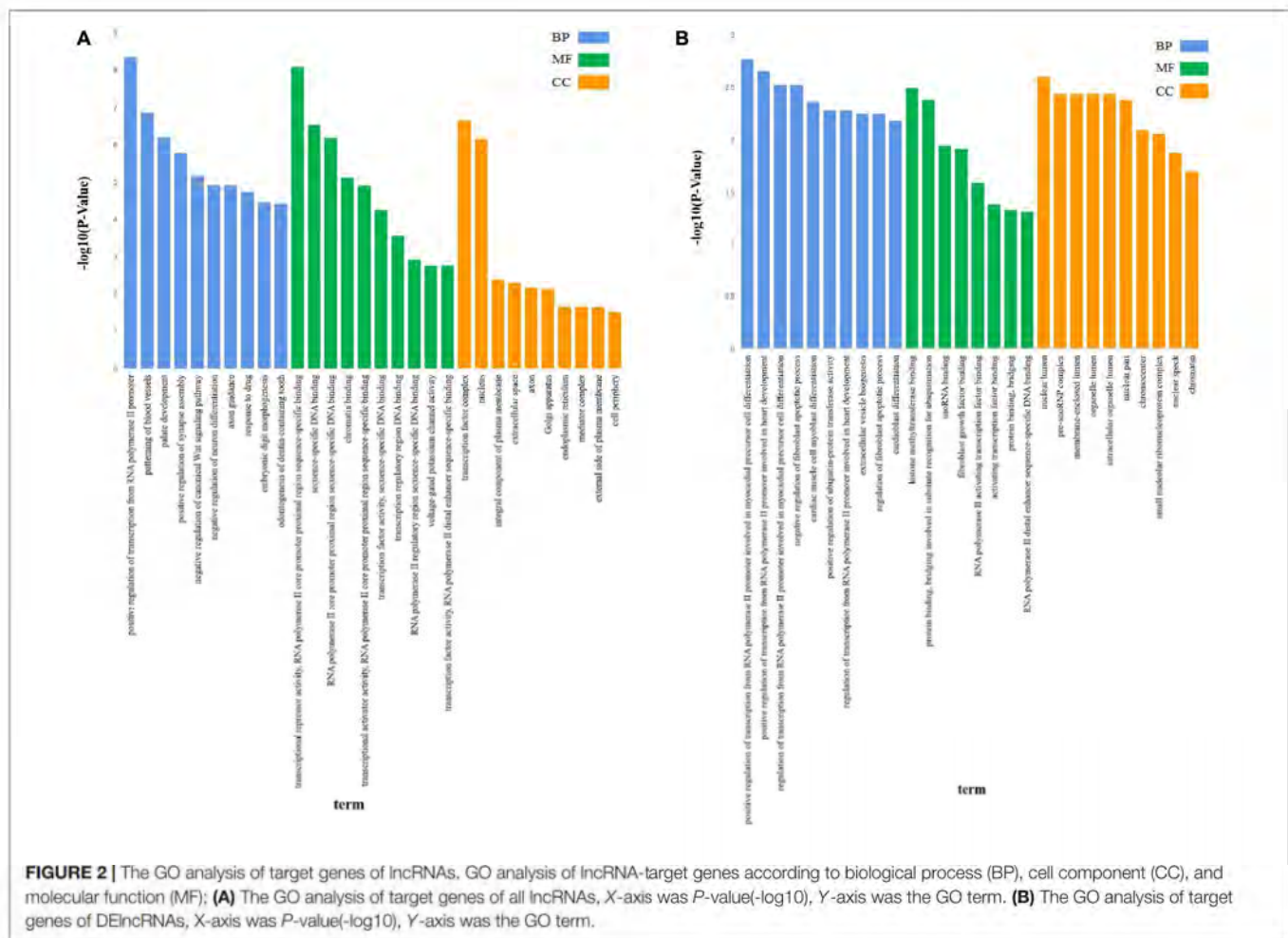
As per KEGG pathway analysis, 20 pathways were significantly enriched ($P < 0.05$). The top 10 pathways were primarily associated with transforming growth factor-beta (TGF- β), signaling pathways regulating pluripotency of stem cells, pathways in cancer, basal cell carcinoma, Wnt signaling pathway, HTLV-I infection, neuroactive ligand-receptor interaction, proteoglycans in cancer, Hippo signaling pathway, and transcriptional misregulation in cancer (Table 5 and Figure 3A). The target genes included *CTNNB1*, *WNT5A*, and *TGF- β 2*, among others. The target gene *WNT5A* of ENSCHIG00000000774 was involved in seven signaling pathways, such as the Wnt signaling pathway, basal cell carcinoma, and HTLV-I infection.

KEGG Pathway Analyses for Target Genes of DElncRNAs

According to KEGG pathway enrichment analyses, the target genes involved ribosomal biogenesis in eukaryotes and olfactory transduction pathways, of which only the former showed significant enrichment ($P < 0.05$). The target gene *NOP56* of MSTRG.2938 was involved in this pathway (Table 6 and Figure 3B).

DISCUSSION

The elucidation of mechanisms regulating litter size can provide a theoretical basis for breeding technologies in goats. Therefore, in this study, we used the ovaries of high and low fecundity Leizhou black goats to identify DElncRNAs by high-throughput RNA sequencing; moreover, we analyzed the target genes of lncRNAs to explore the role of DElncRNAs in ovarian development.



We herein identified enriched terms and signaling pathways; subsequently, we analyzed them as well as pertinent target genes involved in the regulation of reproduction. Fibroblasts are the main cellular component of loose connective tissue (Cai et al., 2012; Yeung et al., 2013). Carcinoma-associated fibroblasts evidently regulate the development of epithelial ovarian cancer by affecting the proliferation, apoptosis, migration, and invasive activity of ovarian cancer cells (Zhang et al., 2011). Fibroblast growth factor (FGFs) is involved in follicular development and follicular atresia (Costa et al., 2009; Miyoshi et al., 2010; Asgari et al., 2015; Coticchio et al., 2015). The expression of FGFs related to follicular development changes with the development of follicles. FGFs need to bind to distinct receptors to physiologically function (Basu et al., 2014). Apoptosis inhibitor 5, which is encoded by *API5*, is involved in regulating the cell cycle. Further, it promotes DNA synthesis and cell cycle G1/S transition, and regulates cell growth, proliferation, and apoptosis (Garcia-Jove Navarro et al., 2013). *API5* plays an important role in the termination of diapause and early embryonic development of *Artemia sinica* (Zhang et al., 2017). Through GO analysis, we found that the target gene *API5* of MSTRG.3782 was involved in the regulation of fibroblast apoptotic process and FGF binding. MSTRG.3782 was significantly upregulated in the high fecundity

group. Accordingly, we hypothesized that lncRNA participates in cell growth, thereby affecting follicular development.

The nucleus is the main repository of genetic information in eukaryotic cells, and the site of DNA replication and transcription; it consequently controls genetic and metabolic activities (Lynch and Marinov, 2017). Chromosomes are the most important structures in the nucleus and carry hereditary information (Zetterström, 2008). A study found that OPA-interacting protein 5 (*OIP5*) was enriched in centrosomes during the G1 phase of the cell cycle and mediated the regulation of cell division (Naetar et al., 2007). In addition, *OIP5* reportedly has a fundamental role in maintaining the structure and function of centrosomes/centromeres (Fujita et al., 2007). *KRR1* encodes proteins present in early 90 S precursor particles of the small ribosomal subunit, and its locus has been implied to contribute to the development of polycystic ovary syndrome (Gromadka and Rytka, 2000; Zheng et al., 2014; Pau et al., 2017). In this study, we found that the target gene *OIP5* of MSTRG.1201 and the target gene *KRR1* of ENSCHIT00000001883 were significantly enriched in the cellular component of nuclear lumen, chromatin, organelle lumen, intracellular organelle lumen, among others. In the high fecundity group, MSTRG.1201 was significantly upregulated and ENSCHIT00000001883 was significantly

TABLE 4 | Top 10 significantly enriched Gene Ontology (GO) terms of target genes of differentially expressed long non-coding RNAs (DElncRNAs).

GO ID	GO name	Genes	P
Molecular function			
GO:1990226	Histone methyltransferase binding	NOP56	0.0032
GO:1990756	Protein binding, bridging involved in substrate recognition for ubiquitination	ARRDC4	0.0041
GO:0030515	snoRNA binding	NOP56	0.0113
GO:0017134	Fibroblast growth factor binding	API5	0.0121
GO:0001102	RNA polymerase II activating transcription factor binding	TBXT	0.0257
GO:0033613	Activating transcription factor binding	TBXT	0.0415
GO:0030674	Protein binding, bridging	ARRDC4	0.047
GO:0000980	RNA polymerase II distal enhancer sequence-specific DNA binding	TBXT	0.0486
GO:0060090	Molecular adaptor activity	ARRDC4	0.0563
GO:0001158	Enhancer sequence-specific DNA binding	TBXT	0.0602
Biological process			
GO:0003257	Positive regulation of transcription from RNA polymerase II promoter involved in myocardial precursor cell differentiation	TBXT	0.0017
GO:1901228	Positive regulation of transcription from RNA polymerase II promoter involved in heart development	TBXT	0.0022
GO:0003256	Regulation of transcription from RNA polymerase II promoter involved in myocardial precursor cell differentiation	TBXT	0.003
GO:2000270	Negative regulation of fibroblast apoptotic process	API5	0.003
GO:0060379	Cardiac muscle cell myoblast differentiation	TBXT	0.0043
GO:0051443	Positive regulation of ubiquitin-protein transferase activity	ARRDC4	0.0052
GO:1901213	Regulation of transcription from RNA polymerase II promoter involved in heart development	TBXT	0.0052
GO:0140112	Extracellular vesicle biogenesis	ARRDC4	0.0056
GO:2000269	Regulation of fibroblast apoptotic process	API5	0.0056
GO:0010002	Cardioblast differentiation	TBXT	0.0065
Cellular component			
GO:0031981	Nuclear lumen	IER2, TBXT, API5, KRR1, NOP56, OIP5	0.0025
GO:0070761	Pre-snoRNP complex	NOP56	0.0036
GO:0031974	Membrane-enclosed lumen	IER2, TBXT, API5, KRR1, NOP56, OIP5	0.0036
GO:0043233	Organelle lumen	IER2, TBXT, API5, KRR1, NOP56, OIP5	0.0036
GO:0070013	Intracellular organelle lumen	IER2, TBXT, API5, KRR1, NOP56, OIP5	0.0036
GO:0044428	Nuclear part	IER2, TBXT, API5, KRR1, NOP56, OIP5	0.0041
GO:0010369	Chromocenter	OIP5	0.008
GO:0005732	Small nucleolar ribonucleoprotein complex	NOP56	0.0087
GO:0016607	Nuclear speck	API5, OIP5	0.0131
GO:0000785	Chromatin	TBXT, OIP5	0.0202

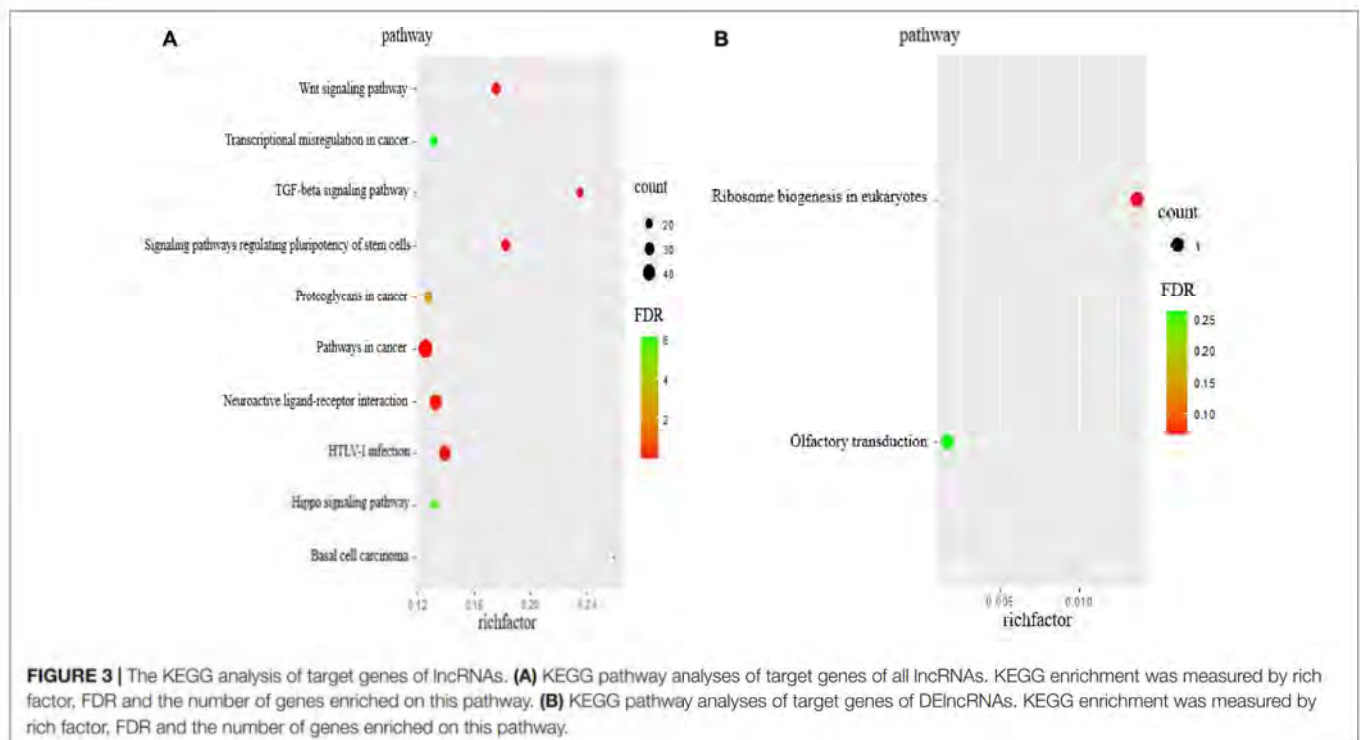
TABLE 5 | Top 10 significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of target genes of all long non-coding RNAs.

KEGG pathway	Number of genes	P
TGF-beta signaling pathway	19	3.48E-06
Signaling pathways regulating pluripotency of stem cells	25	7.62E-06
Pathways in cancer	49	1.28E-05
Basal cell carcinoma	14	3.25E-05
Wnt signaling pathway	23	3.61E-05
HTLV-I infection	34	4.89E-05
Neuroactive ligand-receptor interaction	35	9.47E-05
Proteoglycans in cancer	25	0.00199537
Hippo signaling pathway	20	0.004450684
Transcriptional misregulation in cancer	20	0.004786637

downregulated. Therefore, we believe that MSTRG.1201 and ENSCHIT00000001883 affect follicular development by regulating cell division.

Mature snoRNP particles are composed of a series of small nucleolar RNA and core proteins. snoRNPs regulate the processing and modification of pre-rRNA and play an important role in ribosomal biogenesis (Richard and Kiss, 2006). Nucleolar protein 56 (encoded by *Nop56*) is involved in the synthesis of snoRNP as a core protein (Lykke-Andersen et al., 2018). Furthermore, an increase in the ribosome biosynthesis rate can promote the expression of the proto-oncogene *C-myc* and enhance the proliferative ability of cancer cells (Tomczak et al., 2015). *C-myc* encodes a transcription factor with a direct role in controlling translation (Ruggero, 2009). *Nol5a/Nop56* may be a critical gene involved in Myc-mediated oncogenic transformation (Cowling et al., 2014). According to our GO and KEGG pathway analyses, MSTRG.2938 was significantly upregulated in the high fecundity group, and its target gene *NOP56* was involved in ribosomal biogenesis in eukaryotes and the pre-snoRNP complex. We thus speculate that MSTRG.2938 regulates ribosomal biogenesis in the pre-snoRNP complex as well as cell transformation in eukaryotes. However, the specific mechanism of regulation of each lncRNA remains to be further investigated.

According to GO analyses, the target genes [Guanine Nucleotide Binding Protein, alpha 13 (*GNA13*), Mothers against decapentaplegic homolog 2 (*SMAD2*), and Fibronectin Leucine Rich Transmembrane Protein 2 (*FLRT2*)] of all lncRNAs were mainly enriched in positive regulation of transcription from RNA polymerase II promoters, patterning of blood vessels, palate development, and positive regulation of synapse assembly. RNA polymerase II plays a pivotal role in the transcription of protein-encoding genes in all eukaryotic cells (Bernecky et al., 2016). *GNA13* participates in regulating cell movement and developmental angiogenesis (Offermanns et al., 1997). Moreover, *SMAD2* overexpression has been reported to repair secondary cleft palate by increasing apoptosis of medial edge epithelial cells in the TGF- β 3 pathway (Miyazono et al., 2018). We thus report that these genes play a major role in maintaining the healthy growth of goats.



The Wnt signaling pathway and its downstream effectors not only regulate physiological processes such as cell growth and differentiation, cell migration, and genetic material stability but are also important for cancer progression, including for regulating tumor growth, cell senescence, and cell death. The Wnt/ β -catenin signaling pathway is involved in various important processes, such as the regulation of embryo development, cell proliferation, and cell migration (Nusse and Clevers, 2017; Peng et al., 2017; Steinhart and Angers, 2018). β -Catenin is an essential structural component of cadherin-based adherens junctions and is a key component of Wnt/ β -catenin signal transduction. Aberrant expression of *CTNNB1* and *WNT5A* has been observed to affect cell proliferation and lead to cancer occurrence. A mutation in *CTNNB1* is one of the many causes of β -catenin degradation (Kim et al., 2018). The Wnt/*CTNNB1* pathway is a pivotal signaling pathway that regulates steroid production (Abedini et al., 2016). *WNT5A* is a highly evolved conservative non-classical Wnt ligand, which is required for normal ovarian follicle development (Abedini et al., 2016). *WNT5A* is differentially expressed during the development of mouse follicles, and it can significantly inhibit steroid production

in atretic follicles (Lapointe and Boerboom, 2011; Abedini et al., 2016; Kumawat and Gosens, 2016). By blocking the function of FSH (follicle-stimulating hormone) and luteinizing protein, *WNT5A* can induce the down-regulation of *CTNNB1* and cAMP-response element binding protein (CREB), thus affecting follicle development and gonadotropin reactivity (Abedini et al., 2015). We found that the target gene *CTNNB1* of ENSCHIG00000000641 is one of the two signaling pathway members of Wnt and proteoglycans in cancer. ENSCHIG00000000641 was downregulated in the high fecundity group. Further, the target gene *WNT5A* of ENSCHIG00000000774 participated in the negative regulation of canonical Wnt signaling pathway terms and the Wnt signaling pathway. ENSCHIG00000000774 was also downregulated in the high fecundity group. Thus, we believe that ENSCHIG00000000641 and ENSCHIG00000000774 affect follicular development by regulating cell proliferation and steroid production, respectively.

The TGF- β superfamily participates in many physiological activities in mammals via autocrine and paracrine pathways, and TGF- β is mainly produced locally in the ovary. It has been reported that *TGF- β 1* can promote the growth of mice follicles. Both TGF- β and activin A have proliferative action and cytodifferentiative action on granulosa cells (Liu et al., 1999). As a member of the transforming growth factor β family, TGF- β 2 also plays an important role in the growth and development of follicles. TGF- β 2 is located in follicular membrane cells and luteal cells and regulates the production of inhibitors and activins in granulosa cells and luteal cells (Knight and Glistler, 2003). *Smad2/Smad3* are key molecules in the TGF- β /Smad signaling pathway that regulate ovarian

TABLE 6 | Top significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of target genes of differentially expressed long non-coding RNAs.

KEGG pathway	Number of genes	P
Ribosome biogenesis in eukaryotes	1	0.033754056
Olfactory transduction	1	0.261408061

growth and development and maintain ovarian function (Coutts et al., 2008; AlMegbel and Shuler, 2020). *Smad2* and *Smad3* can maintain normal fertility in women, and further support the *Smad2/3* pathway in the ovary to participate in the regulation of signals produced by oocytes, which plays an important role in the coordination of ovulation (Li et al., 2008). In this study, we found that *TGF- β 2*, *TGF- β 2R2*, and *Smad2* participated in the *TGF- β* signaling pathway, and they were regulated by ENSCHIG00000000886, ENSCHIG00000000609, and ENSCHIG00000002761, respectively. We accordingly speculate that these lncRNAs regulate follicle development, but the specific mechanism needs to be further studied.

To conclude, we found that target genes of all lncRNAs were mainly involved in protein transcription and played a role in maintaining the healthy growth of animals. In addition, the *TGF- β* and *Wnt* signaling pathways were found to be related to reproduction in animals. Based on functional analyses of target genes of DElncRNAs, fibroblast apoptotic process, FGF binding, pre-snoRNP complex, and ribosomal biogenesis in eukaryotes were associated with reproduction in goats. Our data improves the current understanding of the transcriptome of goats and provides valuable information for functional genomics resources and biological studies; moreover, we believe that our results are of great significance for in-depth studies of candidate lncRNAs in breeding techniques.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: PRJNA728366).

ETHICS STATEMENT

The animal study was reviewed and approved by all study protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals at the South China Agricultural University (permit no. SYXK-2014-0136). Further, all experiments were performed in accordance with the guidelines of the South China Agricultural University.

AUTHOR CONTRIBUTIONS

YL: conceptualization, methodology, writing-reviewing, and editing. XX: data curation, writing-original draft

REFERENCES

- Abedini, A., Zamberlam, G., Boerboom, D., and Price, C. A. (2015). Non-canonical WNT5A is a potential regulator of granulosa cell function in cattle. *Mol. Cell Endocrinol.* 403, 39–45. doi: 10.1016/j.mce.2015.01.017
- Abedini, A., Zamberlam, G., Lapointe, E., Tourigny, C., Boyer, A., Paquet, M., et al. (2016). WNT5a is required for normal ovarian follicle development and antagonizes gonadotropin responsiveness in granulosa cells by suppressing canonical WNT signaling. *FASEB J.* 30, 1534–1547. doi: 10.1096/fj.15-280313

preparation, software, and validation. MD: conceptualization. DL: visualization and investigation. GL: supervision. XZ: investigation. ZZ: investigation. All authors contributed to the article and approved the submitted version.

FUNDING

The Modern Agricultural Industrial Technology System of Guangdong Province (2019KJ127), the Guangdong Provincial Promotion Project of Modern Seed Industry, Guangdong Provincial Promotion Project on Preservation and Utilization of Local Breed of Livestock and Poultry, and Natural Science Foundation of Guangdong Province (2019B1515210017), and Natural Science Foundation of Guangdong Province, Regulation of miR-128 and miR-450 on proliferation and apoptosis of goat follicular granulosa cells (2021A1515010636).

ACKNOWLEDGMENTS

We would like to thank WENS Company for helping us with performing experiments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fgene.2021.648158/full#supplementary-material>

Supplementary File 1 | The DElncRNAs in the ovary of high fecundity goats and low fecundity goats. (XLSX)

Supplementary File 2 | lncRNA primers sequences used for the qRT-PCR. (XLSX)

Supplementary File 3 | Protein-coding genes detected 100-kb upstream and downstream of the DElncRNAs. (XLSX)

Supplementary File 4 | GO enrichment analysis of target genes co-located with the all lncRNAs. (XLSX)

Supplementary File 5 | GO enrichment analysis of target genes co-located with the DElncRNAs. (XLSX)

Supplementary File 6 | KEGG enrichment analysis of target genes co-located with the all lncRNAs. (XLSX)

Supplementary File 7 | KEGG enrichment analysis of target genes co-located with the DElncRNAs. (XLSX)

Supplementary File 8 | Sequences of the DElncRNAs. (DOC)

- AlMegbel, A. M., and Shuler, C. F. (2020). SMAD2 overexpression rescues the *TGF- β 3* null mutant mice cleft palate by increased apoptosis. *Differ. Res. Biol. Diversity* 111, 60–69. doi: 10.1016/j.diff.2019.10.001
- Asgari, F., Valojerdi, M. R., Ebrahimi, B., and Fatehi, R. (2015). Three dimensional in vitro culture of preantral follicles following slow-freezing and vitrification of mouse ovarian tissue. *Cryobiology* 71, 529–536. doi: 10.1016/j.cryobiol.2015.11.001
- Barnett, K. R., Schilling, C., Greenfield, C. R., Tomic, D., and Flaws, J. A. (2006). Ovarian follicle development and transgenic mouse models. *Hum. Reprod. Update* 12, 537–555. doi: 10.1093/humupd/dml022

- Basu, M., Mukhopadhyay, S., Chatterjee, U., and Roy, S. S. (2014). FGF16 promotes invasive behavior of SKOV-3 ovarian cancer cells through activation of mitogen-activated protein kinase (MAPK) signaling pathway. *J. Biol. Chem.* 289, 1415–1428.
- Bernecky, C., Herzog, F., Baumeister, W., Plitzko, J. M., and Cramer, P. (2016). Structure of transcribing mammalian RNA polymerase II. *Nature* 529, 551–554. doi: 10.1038/nature16482
- Bouckenheimer, J., Fauque, P., Lecellier, C. H., Bruno, C., Commes, T., Lemaître, J. M., et al. (2018). Differential long non-coding RNA expression profiles in human oocytes and cumulus cells. *Sci. Rep.* 8:2202. doi: 10.1038/s41598-018-20727-0
- Cabilli, M. N., Trapnell, C., Goff, L., Koziol, M., Tazon-Vega, B., Regev, A., et al. (2011). Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* 25, 1915–1927. doi: 10.1101/gad.17446611
- Cai, J., Tang, H., Xu, L., Wang, X., Yang, C., Ruan, S., et al. (2012). Fibroblasts in omentum activated by tumor cells promote ovarian cancer growth, adhesion and invasiveness. *Carcinogenesis* 33, 20–29. doi: 10.1093/carcin/bgr230
- Costa, I., Teixeira, N., Ripamonte, P., Guerra, D., Price, C., Buratini, J., et al. (2009). Fgf1 mRNA in (bovine)antral follicles and corpora lutea. *Anim. Reprod.* 6, 409–415.
- Coticchio, G., Dal Canto, M., Mignini Renzini, M., Guglielmo, M. C., Brambillasca, F., Turchi, D., et al. (2015). Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. *Hum. Reprod. Update* 21, 427–454. doi: 10.1093/humupd/dmv011
- Coutts, S. M., Childs, A. J., Fulton, N., Collins, C., Bayne, R. A., McNeilly, A. S., et al. (2008). Activin signals via SMAD2/3 between germ and somatic cells in the human fetal ovary and regulates kit ligand expression. *Dev. Biol.* 314, 189–199. doi: 10.1016/j.ydbio.2007.11.026
- Cowling, V. H., Turner, S. A., and Cole, M. D. (2014). Burkitt's lymphoma-associated c-Myc mutations converge on a dramatically altered target gene response and implicate Nf5a/Nop56 in oncogenesis. *Oncogene* 33, 3519–3527. doi: 10.1038/ncr.2013.338
- Cui, H. X., Zhao, S. M., Cheng, M. L., Guo, L., Ye, R. Q., Liu, W. Q., et al. (2009). Cloning and expression levels of genes relating to the ovulation rate of the Yunling black goat. *Biol. Reprod.* 80, 219–226. doi: 10.1095/biolreprod.108.069021
- Dennis, G. Jr., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., et al. (2003). DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol.* 4:3.
- Fierro, S., Gil, J., Viñoles, C., and Olivera-Muzante, J. (2013). The use of prostaglandins in controlling estrous cycle of the ewe: a review. *Theriogenology* 79, 399–408. doi: 10.1016/j.theriogenology.2012.10.022
- Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., Kokubo, A., Obuse, C., et al. (2007). Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. *Dev. Cell* 12, 17–30. doi: 10.1016/j.devcel.2006.11.002
- García-Jové Navarro, M., Basset, C., Arcondéguy, T., Touriol, C., Perez, G., Prats, H., et al. (2013). Api5 contributes to E2F1 control of the G1/S cell cycle phase transition. *PLoS One* 8:e71443. doi: 10.1371/journal.pone.0071443
- Gromadka, R., and Rytka, J. (2000). The KRR1 gene encodes a protein required for 18S rRNA synthesis and 40S ribosomal subunit assembly in *Saccharomyces cerevisiae*. *Acta Biochim. Pol.* 47, 993–1005.
- Han, L., Zhang, K., Shi, Z., Zhang, J., Zhu, J., Zhu, S., et al. (2012). LncRNA profile of glioblastoma reveals the potential role of lncRNAs in contributing to glioblastoma pathogenesis. *Int. J. Oncol.* 40, 2004–2012. doi: 10.3892/ijo.2012.1413
- Huang, D. W., Sherman, B. T., Tan, Q., Kir, J., Liu, D., Bryant, D., et al. (2007). DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res.* 35, W169–W175. doi: 10.1093/nar/gkm415
- Jarroux, J., Morillon, A., and Pinskaya, M. (2017). History, discovery, and classification of lncRNAs. *Adv. Exp. Med. Biol.* 1008, 1–46. doi: 10.1007/978-981-10-5203-3_1
- Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S. L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* 14:R36. doi: 10.1186/gb-2013-14-4-r36
- Kim, G., Kurnit, K. C., Djordjevic, B., Singh, C., Munsell, M. F., Wang, W. L., et al. (2018). Nuclear β -catenin localization and mutation of the CTNNB1 gene: a context-dependent association. *Modern Pathol.* 31, 1553–1559. doi: 10.1038/s41379-018-0080-0
- Knight, P. G., and Glistler, C. (2003). Local roles of TGF-beta superfamily members in the control of ovarian follicle development. *Anim. Reprod. Sci.* 78, 165–183. doi: 10.1016/s0378-4320(03)00089-7
- Kumawat, K., and Gosens, R. (2016). WNT-5A: signaling and functions in health and disease. *Cell. Mol. Life Sci. CMLS* 73, 567–587. doi: 10.1007/s00018-015-2076-y
- Lapointe, E., and Boerboom, D. (2011). WNT signaling and the regulation of ovarian steroidogenesis. *Front. Biosci.* 3:276–285.
- Li, J., Cao, Y., Xu, X., Xiang, H., Zhang, Z., Chen, B., et al. (2015). Increased new lncRNA-mRNA gene pair levels in human cumulus cells correlate with oocyte maturation and embryo development. *Reproductive Sci.* 22, 1008–1014. doi: 10.1177/1933719115570911
- Li, Q., Pangas, S. A., Jorge, C. J., Graff, J. M., Weinstein, M., and Matzuk, M. M. (2008). Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. *Mol. Cell. Biol.* 28, 7001–7011. doi: 10.1128/MCB.00732-08
- Lin, M. F., Jungreis, I., and Kellis, M. (2011). PhyloCSF: a comparative genomics method to distinguish protein coding and non-coding regions. *Bioinformatics* 27, i275–i282. doi: 10.1093/bioinformatics/btr209
- Ling, Y., Xu, L., Zhu, L., Sui, M., Zheng, Q., Li, W., et al. (2017). Identification and analysis of differentially expressed long non-coding RNAs between multiparous and uniparous goat (*Capra hircus*) ovaries. *PLoS One* 12:e0183163. doi: 10.1371/journal.pone.0183163
- Liu, G., Liu, S., Xing, G., and Wang, F. (2020). lncRNA PVT1/MicroRNA-17-5p/PTEN axis regulates secretion of E2 and P4, proliferation, and apoptosis of ovarian granulosa cells in PCOS. *Mol. Therapy. Nucleic Acids* 20, 205–216. doi: 10.1016/j.omtn.2020.02.007
- Liu, X., Andoh, K., Abe, Y., Kobayashi, J., Yamada, K., Mizunuma, H., et al. (1999). A comparative study on transforming growth factor-beta and activin A for preantral follicles from adult, immature, and diethylstilbestrol-primed immature mice. *Endocrinology* 140, 2480–2485. doi: 10.1210/endo.140.6.6827
- Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8
- Lykke-Andersen, S., Ardal, B. K., Hollensen, A. K., Damgaard, C. K., and Jensen, T. H. (2018). Box C/D snoRNP autoregulation by a cis-acting snoRNA in the NOP56 Pre-mRNA. *Mol. Cell* 72, 99–111.e5. doi: 10.1016/j.molcel.2018.08.017
- Lynch, M., and Marinov, G. K. (2017). Membranes, energetics, and evolution across the prokaryote-eukaryote divide. *eLife* 6:e20437. doi: 10.7554/eLife.20437
- Martemucci, G., and D'Alessandro, A. G. (2011). Induction/synchronization of oestrus and ovulation in dairy goats with different short term treatments and fixed time intrauterine or exocervical insemination system. *Anim. Reprod. Sci.* 126, 187–194. doi: 10.1016/j.anireprosci.2011.05.011
- Mekuriaw, Z., Assefa, H., Tegegne, A., and Muluneh, D. (2016). Estrus response and fertility of Menz and crossbred ewes to single prostaglandin injection protocol. *Trop. Animal Health Prod.* 48, 53–57. doi: 10.1007/s11250-015-0919-z
- Miyazono, K. I., Moriwaki, S., Ito, T., Kurisaki, A., Asashima, M., and Tanokura, M. (2018). Hydrophobic patches on SMAD2 and SMAD3 determine selective binding to cofactors. *Sci. Signal.* 11:eaao7227. doi: 10.1126/scisignal.aao7227
- Miyoshi, T., Otsuka, F., Yamashita, M., Inagaki, K., Nakamura, E., Tsukamoto, N., et al. (2010). Functional relationship between fibroblast growth factor-8 and bone morphogenetic proteins in regulating steroidogenesis by rat granulosa cells. *Mol. Cell. Endocrinol.* 325, 84–92. doi: 10.1016/j.mce.2010.04.012
- Naetar, N., Hutter, S., Dorner, D., Dechat, T., Korbei, B., Gotzmann, J., et al. (2007). LAP2alpha-binding protein LINT-25 is a novel chromatin-associated protein involved in cell cycle exit. *J. Cell Sci.* 120(Pt 5), 737–747. doi: 10.1242/jcs.03390
- Nusse, R., and Clevers, H. (2017). Wnt/ β -Catenin signaling, disease, and emerging therapeutic modalities. *Cell* 169, 985–999. doi: 10.1016/j.cell.2017.05.016
- Offermanns, S., Mancino, V., Revel, J. P., and Simon, M. I. (1997). Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency. *Science* 275, 533–536. doi: 10.1126/science.275.5299.533

- Pau, C. T., Mosbrugger, T., Saxena, R., and Welt, C. K. (2017). Phenotype and tissue expression as a function of genetic risk in polycystic ovary syndrome. *PLoS One* 12:e0168870. doi: 10.1371/journal.pone.0168870
- Peng, Y., Zhang, X., Feng, X., Fan, X., and Jin, Z. (2017). The crosstalk between microRNAs and the Wnt/ β -catenin signaling pathway in cancer. *Oncotarget* 8, 14089–14106. doi: 10.18632/oncotarget.12923
- Perera, B. M., Bongso, T. A., and Abeynaike, P. (1978). Oestrus synchronisation in goats using cloprostenol. *Vet. Record* 102:314. doi: 10.1136/vr.102.14.314-a
- Pramod, R. K., Sharma, S. K., Singhi, A., Pan, S., and Mitra, A. (2013). Differential ovarian morphometry and follicular expression of BMP15, GDF9 and BMPRII influence the prolificacy in goat. *Reprod. Domest. Anim.* 48, 803–809. doi: 10.1111/rda.12165
- Punta, M., Coggill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., et al. (2012). The Pfam protein families database. *Nucleic Acids Res.* 40, D290–D301. doi: 10.1093/nar/gkr1065
- Richard, P., and Kiss, T. (2006). Integrating snoRNP assembly with mRNA biogenesis. *EMBO Rep.* 7, 590–592. doi: 10.1038/sj.embor.7400715
- Ruggero, D. (2009). The role of Myc-induced protein synthesis in cancer. *Cancer Res.* 69, 8839–8843. doi: 10.1158/0008-5472.CAN-09-1970
- Steinhart, Z., and Angers, S. (2018). Wnt signaling in development and tissue homeostasis. *Development* 145:dev146589. doi: 10.1242/dev.146589
- Sun, L., Luo, H., Bu, D., Zhao, G., Yu, K., Zhang, C., et al. (2013). Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. *Nucleic Acids Res.* 41:e166. doi: 10.1093/nar/gkt646
- Taylor D. J. (1978). Oestrus synchronisation in goats. *Vet. Rec.* 102:390.
- Tomczak, K., Czerwińska, P., and Wiznerowicz, M. (2015). The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary Oncol.* 19, A68–A77. doi: 10.5114/wo.2014.47136
- Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28, 511–515. doi: 10.1038/nbt.1621
- Wang, H., Nie, X., Li, X., Fang, Y., Wang, D., Wang, W., et al. (2020). Bioinformatics analysis and high-throughput sequencing to identify differentially expressed genes in nebulin gene (NEB) mutations mice. *Med. Sci. Monitor* 26:e922953. doi: 10.12659/MSM.922953
- Wang, L., Feng, Z., Wang, X., Wang, X., and Zhang, X. (2010). DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 26, 136–138. doi: 10.1093/bioinformatics/btp612
- Xing, Z., Lin, A., Li, C., Liang, K., Wang, S., Liu, Y., et al. (2014). lncRNA directs cooperative epigenetic regulation downstream of chemokine signals. *Cell* 159, 1110–1125. doi: 10.1016/j.cell.2014.10.013
- Yeung, T. L., Leung, C. S., Wong, K. K., Samimi, G., Thompson, M. S., Liu, J., et al. (2013). TGF- β modulates ovarian cancer invasion by upregulating CAF-derived versican in the tumor microenvironment. *Cancer Res.* 73, 5016–5028. doi: 10.1158/0008-5472.CAN-13-0023
- Zetterström, R. (2008). The discovery of the structure and function of chromosomes: the basis of cytogenetics. *Acta Paediatrica* 97, 673–676. doi: 10.1111/j.1651-2227.2008.00755.x
- Zhang, S., Yao, F., Jing, T., Zhang, M., Zhao, W., Zou, X., et al. (2017). Cloning, expression pattern, and potential role of apoptosis inhibitor 5 in the termination of embryonic diapause and early embryo development of *Artemia sinica*. *Gene* 628, 170–179. doi: 10.1016/j.gene.2017.07.021
- Zhang, Y., Tang, H., Cai, J., Zhang, T., Guo, J., Feng, D., et al. (2011). Ovarian cancer-associated fibroblasts contribute to epithelial ovarian carcinoma metastasis by promoting angiogenesis, lymphangiogenesis and tumor cell invasion. *Cancer Lett.* 303, 47–55. doi: 10.1016/j.canlet.2011.01.011
- Zhao, Z. Q., Wang, L. J., Sun, X. W., Zhang, J. J., Zhao, Y. J., Na, R. S., et al. (2015). Transcriptome analysis of the *Capra hircus* ovary. *PLoS One* 10:e0121586. doi: 10.1371/journal.pone.0121586
- Zheng, S., Lan, P., Liu, X., and Ye, K. (2014). Interaction between ribosome assembly factors Krr1 and Faf1 is essential for formation of small ribosomal subunit in yeast. *J. Biol. Chem.* 289, 22692–22703. doi: 10.1074/jbc.M114.584490

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Li, Xu, Deng, Zou, Zhao, Huang, Liu and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



华南农业大学

学报

JOURNAL OF SOUTH CHINA AGRICULTURAL UNIVERSITY

ISSN 1001-411X
CN 44-1110/S
CODEN HNDXBH

《中文核心期刊要目总览》核心期刊
《中国科学引文数据库(CSCD)》来源期刊
RCCSE中国核心学术期刊

中国·广州
GUANGZHOU CHINA



2016

9月 第37卷 第5期
Sep. Vol.37 No.5

华南农业大学学报 第 37 卷 第 5 期 2016 年 9 月 目 次

养殖鱼类对干啤酒糟和发酵啤酒糟饲料的表观消化率	李小霞, 柳碧薇, 雷小婷, 胡文锋, 潘 庆(1)
猪 BMSCs 成脂分化中细胞膜钙离子通道、钙敏感受体及成脂定向相关基因表达研究	
..... 叶晶晶, 张枫琳, 艾 玮, 朱晓彤, 束 刚, 王丽娜, 高 萍, 江青艳, 王松波(7)	
G418 处理核供体细胞对猪克隆胚胎体外发育效率的影响	李嘉琪, 李紫聪, 吴珍芳, 刘德武(13)
湖羊和川中黑山羊在华南地区适应性研究	周多恩, 刘德武, 廖迎新, 柳广斌(19)
广西普通野生稻遗传多样性中心的确定与核心种质构建	
..... 薛艳霞, 梁燕理, 冯 璇, 黄金艳, 刘 芳, 覃宝祥, 邱永福, 李容柏(24)	
转 <i>cry1Ab13</i> 基因玉米新种质的创制	
..... 陈沼汀, 董昭旭, 刘 双, 孙国旭, 李 晖, 魏晓禹, 孙 苏, 关淑艳(31)	
大豆根际溶磷菌分离鉴定及溶磷过程中有机酸的分泌	
..... 杨美英, 王春红, 武志海, 岳胜天, 付 丽, 刘晶晶, 孙合美(38)	
基于高光谱植被指数的马铃薯叶片叶绿素含量估测模型	
..... 何彩莲, 郑顺林, 周少猛, 张 琴, 袁继超, 胡建军(45)	
吉林省临江硅藻土及其尾矿对钾的吸附性能	高 将, 赵兰坡, 荣立杰, 姜亦梅, 刘胜楠, 李 昕(50)
温度和寄主对柚木虱生长发育的影响	吴丰年, 陈燕玲, 黄家权, 邓晓玲, 岑伊静(57)
亚洲柑橘木虱 2 株高致病力病原真菌菌株的筛选	
..... 代晓彦, 李翌菡, 许炜明, 沈祖乐, 黄 振, 吴建辉, 任顺祥, 邱宝利(62)	
海南尖峰岭檀香心材比例、精油含量和成分的分布特征	刘小金, 徐大平, 杨曾奖, 张宁南(66)
池杉叶片和球果挥发油化学成分分析及抗细菌活性	
..... 单体江, 唐祥佑, 刘 易, 王 伟, 陈 璇, 段志豪, 伍慧雄, 王 军(72)	
20 种真菌对白木香挥发油成分的影响	王东光, 张宁南, 杨曾奖, 刘小金, 洪 舟, 徐大平(77)
14 个香椿种源生长节律的观测与分析	周祥斌, 周 玮, 林 玮, 周 鹏, 陈晓阳(84)
16 年生大叶相思高效芽诱导、增殖体系研究	王 鸿, 黄烈健, 胡 峰(91)
柑橘机器人夹持损伤有限元预测及试验验证	陈 燕, 谈建豪, 蒋志林, 李嘉威, 邹湘军, 王佳盛(98)
CO ₂ 充入量对小型热泵干燥机性能的影响	杨道龙, 李娟玲, 姬长英, 何艳东(103)
基于响应曲面法的窝眼轮式三七精密排种器性能试验	胡子武, 马文鹏, 邢金龙, 赖庆辉(109)

期刊基本参数: CN44-1110/S* 1959* b* A4* 116* zh* P* ¥15.00* 1600* 19* 2016-09

封面设计: 林 敏



周多恩, 刘德武, 廖迎新, 等. 湖羊和川中黑山羊在华南地区适应性研究[J]. 华南农业大学学报 2016 37(5):19-23.

湖羊和川中黑山羊在华南地区适应性研究

周多恩, 刘德武, 廖迎新, 柳广斌

(华南农业大学 动物科学学院, 广东 广州 510642)

摘要:【目的】研究山羊和绵羊引进品种在华南地区适应性问题。【方法】以外省引进的湖羊及川中黑山羊为研究对象, 统计分析湖羊和川中黑山羊生长、繁殖性能的变化, 及与环境温湿度的关系。【结果】湖羊和川中黑山羊在引种到华南地区的1年内, 其部分生长、繁殖性能有不同程度的下降($P < 0.01$)。湖羊的产羔率与环境温度、湿度显著相关($P < 0.05$)。湖羊和川中黑山羊的羔羊死亡率与温度显著相关($P < 0.05$)。【结论】从外省引进的湖羊及川中黑山羊在华南地区饲养, 短期内会出现一定的适应性问题, 湖羊母羊的繁殖率容易受到高温高湿天气的影响, 而川中黑山羊羔羊的当月死亡率在华南地区的冬季会明显上升, 因此在管理上要注意湖羊夏季防暑及川中黑山羊的冬季羔羊保温。

关键词: 湖羊; 川中黑山羊; 华南地区; 适应性

中图分类号: S826.92

文献标志码: A

文章编号: 1001-411X(2016)05-0019-05

Study on adaptabilities of Hu sheep and Chuanzhong black goat in Southern China

ZHOU Duo'en, LIU Dewu, LIAO Yingxin, LIU Guangbin

(College of Animal Science, South China Agricultural University, Guangzhou 510642, China)

Abstract 【Objective】In order to study the adaptabilities of introduced varieties of sheep and goat in Southern China. 【Method】Hu sheep and Chuanzhong black goats were introduced from other provinces into Southern China. Their growth and reproduction performances and the relationship between growth, reproduction performances and ambient temperature and humidity were statistically analyzed. 【Result】Within one year of introduction, certain growth and reproduction performances of Hu sheep and Chuanzhong black goats declined in different degrees ($P < 0.01$). Lambing percentage of Hu sheep was significantly correlated with ambient temperature and humidity ($P < 0.05$), and the mortality rates of Hu sheep and Chuanzhong black goats were significantly correlated with temperature ($P < 0.05$). 【Conclusion】There are adaptation problems of Hu sheep and Chuanzhong black goats within short period of introduction into Southern China from other provinces. The reproduction rate of female Hu sheep is negatively affected by the high temperature and humidity climate, and the monthly mortality rate of Chuanzhong black goat lambs largely rises in winter. Therefore, we should pay attention to the heatstroke prevention of Hu sheep in summer and heat preservation of Chuanzhong black goats in winter during management.

Key words: Hu sheep; Chuanzhong black goat; Southern China; suitability

收稿日期: 2016-01-28 优先出版时间: 2016-07-05

优先出版网址: <http://www.cnki.net/kcms/detail/44.1110.s.20160705.1200.046.html>

作者简介: 周多恩(1990—), 男, 硕士研究生, E-mail: 1498173948@qq.com; 通信作者: 柳广斌(1982—), 男, 讲师, 博士, E-mail: gbliu@scau.edu.cn

基金项目: 广东省公益研究与能力建设项目(2015A020209122)

<http://xuebao.scau.edu.cn>

中国养羊史悠久,无论从其品种数量还是出栏量、存栏量来看,都是养羊大国^[1]。羊作为反刍动物,是最早被人类驯养的家畜之一^[2]。羊肉富含蛋白质和必需氨基酸,并且脂肪、胆固醇含量低,营养价值极高^[3]。改革开放后,随着经济体制的改革,中国养羊业的生产水平提高了很多,生产方式也有了较大的变化^[4]。特别是近年来,随着产业结构的调整和人民生活水平提高,人民的饮食习惯及膳食结构也在发生着不断的变化,羊肉越来越受到人们的喜爱。据统计,从2000年开始,中国羊肉的消费量和价格就呈现稳步上升的趋势。造成羊肉价格不断上涨的主要原因之一就是羊肉供需不平衡^[5]。此外,羊可以充分利用牧草、秸秆等粗饲料,使人类无法食用的农副产品变废为宝,有可持续发展的优势^[6]。在如今国家大力提倡发展节粮型畜牧业、减少环境压力、退耕还林、退耕还草的政策下,养羊业已进入高速发展的黄金时期。

养羊业虽然在中国得到了快速的发展,但是其发展具有明显的地域性,呈现“北达南缓”的现象^[7-8]。虽然南方地区养羊业发展缓慢,但其优势在于气候温暖,雨水充足,牧草产量高,且有丰富的农作物副产品和秸秆资源,这些可以成为养羊业良好的饲料来源^[9-10]。而目前存在的问题是南方肉羊品种单一、良种化程度低及饲养方式原始等^[11]。其中,南方地区当地品种无法满足现在养殖业高效生产的需要,因此需要从外省或北方地区引进优良的肉用品种。安徽、南京等地区引进湖羊后,湖羊在生产、繁殖和疫病等方面表现优异,促进了当地养羊业发展^[12-13]。由于不同地区的气候差异等原因,会造成羊的适应性问题,其生产性能也会发生变化,而目前很少有关于引进羊在华南地区饲养条件下适应性问题的研究报道。湖羊和川中黑山羊是目前国内适合集约化舍饲的优良肉用品种,引进这2个品种有利于南方养羊业的发展。因此本研究以从外省引进的湖羊及川中黑山羊为研究材料,通过对环境温湿度及羊群生产性能连续1年的测定,分析湖羊及川中黑山羊在华南地区饲养的生产性能变化,以及温度和湿度对这2种羊生长及繁殖性能的影响,为今后在南方集约化饲养优良肉羊品种提供参考依据。

1 材料与方法

1.1 试验地概况

广东省云浮市新兴县位于广东省中部偏西,地处东经111°57′37″~112°31′32″,北纬22°22′46″~22°50′36″。属亚热带季风气候区,年平均气温21.5℃,6—8月平均气温超过30℃,年均降雨量1663.7 mm。
<http://xuebao.scau.edu.cn>

1.2 试验材料

本研究所使用的湖羊和川中黑山羊分别引自上海和四川的省级种羊场,湖羊母羊899只,川中黑山羊母羊1050只,2014年8—10月引种。全年实行高床舍饲养,饲料主要包括青贮、干草及精料,通过TMR机混合后饲喂,每日饲喂2次,夏季采用风扇、喷雾和湿帘等降温措施,冬季采取保暖措施,配种方式为人工辅助本交。

1.3 试验方法

1.3.1 生产性能测定 试验时间为2015年1—12月。1年内连续记录引进湖羊及川中黑山羊的母羊配种数、母羊受胎数、母羊流产数、母羊产羔数、死胎数、羔羊死亡数、羔羊初生体质量、30日龄体质量等生产数据,并统计每月母羊繁殖性能及羔羊生长指标。配种受胎率:当月受胎的母羊占当月配种母羊的比率;流产率:当月流产母羊数占妊娠母羊的比率;产羔率:当月产羔总数(含死胎)占分娩母羊的比例;死胎率:当月死胎数占当月总产羔数的比率;羔羊死亡率:当月死亡的羔羊占当月羔羊存栏的比例;公(母)羔初生体质量:当月出生的公(母)羔的平均初生体质量;公(母)羔30日龄日增质量:当月出生的公(母)羔30日龄内的平均日增质量。

1.3.2 环境温湿度测定 温度和湿度由干湿球温湿度计进行测定,悬挂羊舍中部距地面1.5 m处,每日进行3次数据采集,并计算温湿指数(THI)。THI是温度和湿度相结合用于估计炎热程度的一个综合指标,计算公式为 $THI = 0.72(T_d + T_w) + 40.6$,其中 T_d 、 T_w 分别为干球指数和湿球指数^[14]。

1.4 统计分析

采用Excel软件进行数据整合统计,SPSS 20.0软件进行单样本 t 检验和偏相关性分析。

2 结果与分析

2.1 湖羊及川中黑山羊在华南地区饲养条件下部分生产性能变化

由表1可见,在引种后的1年内,湖羊所处环境的年均THI为72.73(最低为1月60.21,最高为6月81.73),羔羊死亡率平均为4.77%(最低为11月1.08%,最高为3月10.61%),公羔平均初生体质量为2.93 kg,母羔平均初生体质量为2.74 kg,公羔30日龄平均日增质量0.173 kg,母羔30日龄平均日增质量0.160 kg。据时乾^[15]研究显示,湖羊羔羊原产地公羔初生体质量3.1 kg,母羔初生体质量2.9 kg。本研究测定结果与原产地数据相比,湖羊公羔初生体质量较原产地有明显的下降($P < 0.01$),湖羊母羔

初生体质量差异不显著($P>0.05$),但初生体质量及日增质量在1年内有逐步上升的趋势。

由表2可见,川中黑山羊在引种后的1年内所处环境的年均THI为72.68(最低为1月60.21,最高为6月80.45),羔羊死亡率平均为10.90%(最低为11月1.65%,最高为1月32.95%),公羔平均初生体质量为2.60 kg,母羔平均初生体质量为2.34 kg,公羔30日龄平均日增质量0.124 kg,母羔30日

龄平均日增质量0.111 kg。据报道,川中黑山羊原产地公羔初生体质量2.75 kg,母羔初生体质量2.45 kg^[16]。本研究测定结果显示,川中黑山羊在引入华南地区的1年内,其公羔初生体质量较原产地也有较明显的下降($P<0.05$),母羔初生体质量差异不显著($P>0.05$),但与湖羊相似,羔羊的生长性能在1年内有逐步上升的趋势。

表1 湖羊在华南地区的全年生产性能¹⁾

Tab.1 Annual reproduction performances of Hu sheep in Southern China

月份	THI	羔羊生产性能					种母羊繁殖性能			
		羔羊死亡率/%	公羔初生体质量/kg	母羔初生体质量/kg	公羔30日龄日增质量/kg	母羔30日龄日增质量/kg	死胎率/%	产羔率	配种受胎率/%	流产率/%
1	60.21	10.31	2.85	2.36	0.159	0.140	0.00	1.53	75.00	—
2	64.16	7.91	2.72	2.32	0.141	0.120	1.10	1.63	81.55	1.45
3	67.82	10.61	2.75	2.76	0.143	0.144	0.00	1.61	83.97	0.67
4	70.65	3.20	2.70	2.13	0.157	0.125	0.00	1.33	65.44	0.29
5	76.61	2.97	3.13	2.88	0.217	0.180	0.00	1.55	87.64	0.13
6	81.73	2.29	2.97	2.81	0.169	0.178	1.30	1.75	97.22	0.58
7	81.36	5.73	2.83	2.67	0.180	0.165	1.05	1.89	85.00	0.75
8	80.34	2.24	2.79	2.86	0.176	0.179	3.27	1.93	91.67	1.65
9	78.98	3.49	3.05	2.82	0.160	0.152	7.25	1.95	78.74	0.99
10	75.57	4.83	3.14	2.66	0.196	0.181	7.09	1.88	93.46	0.23
11	71.67	1.08	3.09	3.10	0.203	0.198	8.77	1.89	81.94	0.00
12	63.65	2.58	3.14	3.47	31.75	2.22	...	0.16
平均值	72.73	4.77	2.93	2.74	0.173	0.160	5.13	1.76	83.78	0.63

1) THI 为温湿指数,“...”表示未测出;种母羊繁殖性能对应的 THI 12 月份为 62.87、平均值为 72.66。

表2 川中黑山羊在华南地区的全年生产性能¹⁾

Tab.2 Annual reproduction performances of Chuanzhong black goats in Southern China

月份	THI	羔羊生产性能					种母羊繁殖性能			
		羔羊死亡率/%	公羔初生体质量/kg	母羔初生体质量/kg	公羔30日龄日增质量/kg	母羔30日龄日增质量/kg	死胎率/%	产羔率	配种受胎率/%	流产率/%
1	60.21	32.95	2.34	2.19	0.124	0.111	0.78	1.62	84.15	1.11
2	64.16	12.16	2.71	2.69	0.117	0.104	1.62	1.85	74.29	0.46
3	67.82	22.19	2.45	2.36	0.120	0.103	6.00	1.95	82.30	0.44
4	71.03	10.51	2.28	1.97	0.097	0.098	17.65	1.90	75.89	0.00
5	76.06	8.68	2.56	2.34	0.127	0.140	4.39	1.75	77.05	0.32
6	80.45	9.05	2.45	2.24	0.129	0.101	3.13	1.81	89.10	2.49
7	80.17	6.49	2.74	2.12	0.121	0.106	1.16	1.88	87.66	2.00
8	80.42	4.26	2.74	2.24	0.127	0.104	4.03	1.84	83.59	1.53
9	79.01	9.76	2.65	2.24	0.128	0.110	2.96	1.95	80.43	1.07
10	74.73	8.91	2.57	2.40	0.132	0.113	2.94	1.87	83.23	0.51
11	71.37	1.65	2.70	2.53	0.141	0.126	2.19	1.93	80.12	0.65
12	66.71	4.17	2.95	2.72	6.64	1.99	...	0.31
平均值	72.68	10.90	2.60	2.34	0.124	0.111	4.46	1.86	81.62	0.91

1) THI 为温湿指数,“...”表示未测出;种母羊繁殖性能对应的 THI 12 月份为 63.57、平均值为 72.42。

<http://xuebao.scau.edu.cn>

2.2 湖羊和川中黑山羊种母羊在华南地区饲养条件下的繁殖性能变化

由表1可见,湖羊种母羊平均死胎率为5.13%(最高为12月31.75%),平均产羔率为1.76(最低为4月1.33,最高为12月2.22),平均配种受胎率为83.78%,平均流产率0.63%。时乾^[15]研究显示,湖羊原产地产羔率为2.29,与本研究测定的在华南地区所饲养湖羊的产羔率差异极显著($P < 0.01$)。

由表2可见,川中黑山羊种母羊的平均死胎率为4.46%(最高为4月17.65%),平均产羔率1.86(最低为1月1.62,最高为12月1.99),平均配种受胎率为81.62%,平均流产率0.91%。据报道,川中黑山羊原产地产羔率为2.41^[16],与本研究中测定的川中黑山羊的产羔率差异极显著($P < 0.01$)。

虽然湖羊和川中黑山羊在华南地区的产羔率与

原产地相比都有较为明显的下降,但数据显示,随着饲养时间的延长,产羔率有逐步上升的趋势。

2.3 湖羊和川中黑山羊生长和繁殖性能与环境湿度的相关性分析

将湖羊及川中黑山羊的生长和繁殖性能与THI、温度及相对湿度进行相关性分析(表3)。表3结果显示:湖羊的羔羊死亡率与环境温度呈显著负相关($P < 0.05$),湖羊母羔30日龄日增质量与THI呈显著正相关($P < 0.05$);川中黑山羊的羔羊死亡率则与THI和环境温度呈显著负相关($P < 0.05$),川中黑山羊的羔羊生长速度与环境温湿度没有明显的相关性;湖羊的产羔率与温度和相对湿度具有显著相关性($P < 0.05$),而川中黑山羊的母羊繁殖率与环境温湿度没有明显的相关性。

表3 湖羊、川中黑山羊生产性能与气候条件的相关系数¹⁾

Tab.3 Correlation coefficients between reproduction performances of Hu sheep and Chuanshong black goats and climatic conditions

品种	指标	羔羊生产性能					种母羊繁殖性能			
		月死亡率	公羔初生体质量	母羔初生体质量	公羔30日龄日增质量	母羔30日龄日增质量	死胎率	产羔率	配种受胎率	流产率
湖羊	THI	-0.550	0.184	0.145	0.451	0.613*	-0.265	0.198	0.570	0.170
	温度	-0.744*	0.374	0.591	0.465	0.618	0.268	0.705*	0.555	0.023
	相对湿度	0.366	0.208	0.563	-0.106	0.293	0.520	0.742*	0.439	0.096
川中黑山羊	THI	-0.617*	0.167	-0.404	0.252	0.034	-0.059	0.141	0.425	0.572
	温度	-0.732*	0.434	-0.353	0.305	0.042	-0.019	0.391	0.426	0.543
	相对湿度	-0.080	0.385	0.467	0.571	0.131	-0.333	0.524	0.050	-0.186

1) “*”表示在0.05水平上显著相关。

3 讨论与结论

3.1 湖羊和川中黑山羊在华南地区饲养的适应性问题

南方养羊业发展缓慢的一个重要因素与羊本身的生物学特性有关。羊属于耐寒不耐热的动物,其被毛比较丰富,可以起到良好的御寒作用,而皮肤汗腺并不发达,皮肤蒸发散热量小,主要通过呼吸来缓解体温的升高,因此耐热能力较差。华南地区年均气温较高,夏季高温高湿及冬季阴冷气候易引发外来引进羊种的适应问题。本研究通过从外省引进的湖羊及川中黑山羊在华南地区饲养过程中出现的生长和繁殖性能的变化,发现这2个品种在刚引种的1年内的部分生长及繁殖性能较原产地都有较为明显的下降($P < 0.01$),但在这一年的饲养过程中,2种羊的羔羊初生体质量、日增质量及母羊产羔数都随着饲养时间的延长而逐步上升,有恢复到原产地水平的趋势。说明湖羊和川中黑山羊在引种到华南地

区的初期的确存在较为明显的适应性问题,但随着饲养时间的延长,羊只可以逐步适应华南地区的气候环境。

3.2 湖羊和川中黑山羊在华南地区适应性问题的主要表现

通过生产性能与温湿度的关联分析,发现湖羊和川中黑山羊对温度和湿度适应性的表现并不相同。湖羊和川中黑山羊在华南地区的适应性问题主要表现在羔羊死亡率和繁殖性能的变化上。在羔羊死亡率方面,虽然2种羊都与环境温度变化呈负相关($P < 0.05$),但受到影响的程度并不相同。湖羊的羔羊死亡率在最高月(3月10.61%)比最低月(11月1.08%)高了9.53%,而川中黑山羊的羔羊死亡率在最高月(1月32.95%)比最低月(11月1.65%)则高了31.30%,因此川中黑山羊对华南地区冬季的阴冷天气更为敏感。在母羊繁殖性能方面,湖羊更容易受到温度和湿度的影响,与环境温、湿度呈显著正相关($P < 0.05$),但川中黑山羊的产羔率与温度和

<http://xuebao.scau.edu.cn>

湿度的相关性不大。

另一方面,发现湖羊在12月份分娩的死胎率最高,达到31.75%。羊的妊娠一般为5个月,而12月份分娩的羊实际上妊娠阶段为7—11月,这个时期恰好是南方最热的时候,因此很有可能是高温高湿天气对湖羊妊娠期间的生理状况造成了不利的影响,进而导致12月份分娩时的死胎率明显上升。恰恰相反的是,川中黑山羊的死胎率高峰出现在4月,达17.65%,而其妊娠期实际上为11月—翌年3月,这刚好是华南地区最为阴冷的一个阶段,湿冷天气可能对川中黑山羊的妊娠状况有一定的影响。这些数据也在一定程度上进一步说明了绵羊更容易受到高温高湿的影响,而山羊对湿冷更为敏感。

3.3 集约化养殖条件下改善引进羊种适应性问题

的方法

山羊的等热区为20~28℃,绵羊为21~25℃。当环境温度不在等热区时,羊就会发生一系列的生理生化变化来调节自身的体温,严重时会造成应激反应^[17-18]。虽然热、冷应激反应会对家畜的生产性能造成严重的影响^[19-20],但在生产中,依然可以通过一些方法来预防或者减少热、冷应激反应对家畜生产性能的影响。目前主要方法是采用物理法和营养调控法。物理法是根据THI来进行相关操作,当夏季THI超过设定阈值后,采用风扇、水帘、喷雾等方法降低舍内的环境温度。在冬季寒冷时期,当THI低于阈值后,对羊舍进行密闭和供暖处理来提高舍内温度。营养调控法是指通过饲料添加剂或者药物来暂时性地提高动物自身的耐热、耐寒能力,减少热、冷应激反应对其生产性能的影响^[21]。例如:热应激反应会使奶牛血液中的热应激蛋白70(HSP70)的表达水平显著提高,而在日粮中添加甜菜碱和酵母铬可有效降低热应激状态下奶牛血液中的HSP70水平,从而减少热应激反应对奶牛生产性能的影响^[22];冬季提高日粮营养水平,补充维生素C,可以提高鸡的抗冷应激能力,减少经济损失^[23-24]。

综上所述,从外省引进的湖羊及川中黑山羊在华南地区饲养均存在一定程度的适应性问题,短期内生产性能较原产地有所下降,但有逐渐恢复的趋势。其中湖羊母羊的繁殖率容易受到高温高湿天气的影响,而川中黑山羊羔羊的死亡率在华南地区的冬季会明显上升。因此在饲养管理方面,湖羊应注意夏季高温季节的防暑降温,而川中黑山羊应加强冬季低温高湿阶段的羔羊保温工作。

参考文献:

[1] 杨永萍. 我国养羊业发展现状及展望[J]. 甘肃畜牧兽

医 2014, 44(6): 4-6.

- [2] 何晓红, 马月辉. 21 世纪的中国及世界养羊业的现状和发展趋势[J]. 黑龙江畜牧兽医, 2005, 1(1): 1-4.
- [3] 张鹏, 王永. 我国肉羊产业发展的前景、问题及对策[J]. 中国畜牧杂志, 2011, 47(10): 15-18.
- [4] 李军, 李秉龙. 中国传统社会养羊业发展影响因素研究: 技术之外的探讨[J]. 古今农业, 2012(2): 25-34.
- [5] 刘春芳, 王济民. 中国肉羊产业发展现状及供需展望[J]. 农业展望, 2013, 9(6): 45-51.
- [6] 马友记, 李发弟. 中国养羊业现状与发展趋势分析[J]. 中国畜牧杂志, 2011, 47(14): 16-20.
- [7] 张金松, 郭明宝, 关龙, 等. 关于我国肉羊产业发展的几点思考[J]. 中国畜牧杂志, 2014, 50(4): 52-53.
- [8] 张莉, 杜立新, 李捷. 我国肉羊业现状及发展对策[J]. 国外畜牧学(草食家畜), 2014(3): 1-6.
- [9] 何兰花, 甘荫全. 广东省肉羊产业化发展存在的问题及对策[J]. 现代农业科技 2014(14): 296-297.
- [10] 张子军, 李秉龙. 中国南方肉羊产业及饲草资源现状分析[J]. 中国草食动物科学 2012, 32(2): 47-51.
- [11] 许丹宁, 田允波. 广东省养羊业的现状及发展对策[J]. 广东农业科学, 2009, 36(8): 328-330.
- [12] 王明海, 吴结革, 毛绍斌, 等. 湖羊引种的适应性观察[J]. 金陵科技学院学报 2007, 23(2): 103-105.
- [13] 周厚健. 湖羊养殖在皖南山区的适应性分析[J]. 安徽农学通报 2015, 21(17): 110-111.
- [14] 薛白, 王之盛, 李胜利, 等. 温湿度指数与奶牛生产性能的关系[J]. 中国畜牧兽医, 2010, 37(3): 153-157.
- [15] 时乾. 湖羊部分繁殖性状及其羔羊生长发育研究[D]. 南京: 南京农业大学, 2007.
- [16] 国家畜禽遗传资源委员会. 中国畜禽遗传资源志: 羊志[M]. 北京: 中国农业出版社, 2011: 351-353.
- [17] 刘莉莉, 初芹, 徐青, 等. 动物冷应激的研究进展[J]. 安徽农业科学, 2012, 40(16): 8937-8940.
- [18] 李红军, 田夏清, 徐美娟. 动物应激的研究进展[J]. 饲料研究, 2006(10): 6-11.
- [19] 李亚洁, 廖晓艳, 李利. 高温高湿环境热应激研究进展[J]. 护理研究, 2004, 18(17): 1514-1517.
- [20] 魏学良, 张家骅, 王豪举, 等. 高温环境对奶牛生理活动及生产性能的影响[J]. 中国农学通报, 2005, 21(5): 13-15.
- [21] 米玉玲. 寒冷应激高能饲料对笼养蛋鸭生化指标及生产性能影响的研究[D]. 哈尔滨: 东北农业大学, 2002.
- [22] 胡永灵, 叶世莉, 罗佳捷. 中草药制剂对热应激奶牛泌乳性能、抗氧化能力及免疫功能的影响[J]. 草业学报, 2015, 24(1): 132-140.
- [23] 张丽. 日粮甜菜碱对热应激奶牛产奶性能、血液生理生化指标及 HSP70 的影响[D]. 南京: 南京农业大学, 2012.
- [24] 花丽茹, 李喆, 闵向松. 鸡冷应激综合症的防治及展望[J]. 养殖技术顾问, 2010(1): 126-126.

【责任编辑 柴 焰】

<http://xuebao.scau.edu.cn>

华南农业大学学报

JOURNAL OF SOUTH CHINA AGRICULTURAL UNIVERSITY



华南农业大学学报

Huanan Nongye Daxue Xuebao

(双月刊, 1959年创刊, 1980年复刊)

第37卷 第5期 2016年9月

Journal of South China Agricultural University

(Bimonthly, Started 1959, Resumed 1980)

Vol.37 No.5 Sep. 2016

主管单位: 华南农业大学

主办单位: 华南农业大学

主 编: 陈晓阳

编辑出版: 华南农业大学学报编辑部
(广州五山, 邮政编码: 510642)

网 址: <http://xuebao.scau.edu.cn>

电子邮箱: journal@scau.edu.cn

电 话: (020)85280069/38746672

印刷单位: 广州汉鼎印务有限公司

发行订购: 华南农业大学学报编辑部

国外发行: 中国国际图书贸易总公司
(中国国际书店) 北京399信箱

Responsible Institution: South China Agricultural University

Sponsor: South China Agricultural University

Chief Editor: CHEN Xiaoyang

Edited and Published by Editorial Department,
Journal of South China Agricultural University

Address: Wushan, Guangzhou, 510642, China

Website: <http://xuebao.scau.edu.cn>

E-mail: journal@scau.edu.cn

Tel: (86-20)85280069/38746672

Distributed by China International Book Trading Corporation(P.O.Box 399 Beijing, China)

ISSN 1001-411X

CN 44-1110/S

国内外公开发行

国内定价: 15.00 元/册

ISSN 1001-411X



9 771001 411164

09 >

中国畜牧杂志

ISSN 0258-7033
CN 11-2083/S

中国畜牧杂志

CHINESE JOURNAL OF ANIMAL SCIENCE

主管：中国科学技术协会

主办：中国畜牧兽医学会

互动网站：www.boyar.cn

股票代码：300401



浙江花园生物高科股份有限公司
ZHEJIANG GARDEN BIOCHEMICAL HIGH-TECH CO.,LTD



全国中文核心期刊
中国科技核心期刊
中国期刊网入网期刊

中国学术期刊综合评价数据库来源期刊
中国学术期刊（网络版）来源期刊

2021. 2

《中国畜牧杂志》编委会

(以姓名笔画为序)

主任

吴雷信

副主任

阎议平 冯子明 杨议春

顾问

印遇龙 刘守仁 张子仪 张 沅
李德发 陈焕春 南志标

编委会成员

刁其玉 马月辉 王立贤 王 恬
王济民 王洪荣 王楚端 孔平涛
文 杰 尹靖东 冯定远 齐广海
乔玉峰 刘玉满 刘庆平 刘作华
刘国世 刘建新 刘剑峰 刘继军
曲鲁江 同之春 朱化彬 吕明斌
孙 皓 张万刚 张许科 张宏福
张胜利 张细权 张英杰 张英俊
张若寒 张越杰 李 奎 李保明
李学伟 李胜利 李 辉 陈代文
陈国宏 陈瑞生 杨 宁 杨红建
连正兴 邵彩梅 金立志 林 海
罗海玲 赵广永 赵书红 赵国琦
侯水生 侯永清 姚军虎 洪 平
贺 喜 秦玉昌 秦应和 秦贵信
敖长金 敖志刚 高玉鹏 曹兵海
曹建民 康相涛 曾申明 曾建国
董 延 蒋宗勇 储明星 韩彦明
葛 翔 魏仕彦 蔡辉显

主编

冯子明

常务副主编

赵 楠

副主编

刘国世 杨红建 曲鲁江 尹靖东
张万刚 李保明 刘玉满 曹建民

乔玉峰

编辑部主任

孔平涛

编辑部副主任

赵 楠

市场总监

朱晓娟

总策划

王必勇

责任编辑

李淑兰 赵 楠 郑本艳 周会会

综 述

牛至油的生物学功能及其在畜牧生产中的应用研究进展

.....胡 益, 张云露, 宋泽和, 张海涵, 田文奎, 贺 喜 (1)

陈化稻米的主要营养特性及其在畜禽生产中的应用的研究进展

.....丁 婧, 吕宏伟, 范晨宇, 宋泽和, 贺 喜 (6)

菌藻共生系统在生猪养殖污水处理中的应用及其互作机制的研究进展

.....孙 宏, 李国成, 王 新, 沈 琦, 姚晓红, 吴逸飞, 汤江武 (11)

尿肠球菌的特性及其在畜牧生产中的应用研究进展

.....白天天, 郭雪峰 (16)

益生凝结芽孢杆菌在肉禽养殖中的应用及作用机理研究进展

.....苗华彪, 马 瑜, 者国园, 黄连杨 (21)

肠道微生物及代谢产物对动物肠道免疫的研究进展

.....钟奇祺, 张海波, 李清风, 关玮琨, 廖晓鹏, 黎力之, 郭冬生 (28)

热应激对雄性哺乳动物精液品质的影响机制及热应激公猪的营养调控

.....汤加勇, 李瑞婷, 赵 华, 贾 刚 (34)

番茄红素的生理功能及其在动物生产中的应用进展

.....左炎云 (40)

可变剪接的表现遗传学调控机制及其在脂肪代谢中的作用研究进展

.....葛 鑫, 马海明, 何 俊, 徐 康, 张跃博 (46)

科学技术

遗传育种

不同日龄乌蒙凤鸡体尺与屠宰性能相关性主成分分析

.....莫光艇, 张 勇, 黄明捷, 王天松, 郭 勇, 陆 静, 骆科印 (54)

中国荷斯坦牛 *PIN1* 基因对产奶性状的遗传效应分析

.....林 珊, 杨宇泽, 刘 林, 李艳华, 韩 博, 许令娜, 张俊楠, 麻 柱, 张胜利, 孙东晓 (62)

高山美利奴羊主观评定性状与客观测量性状现状及两者相关性研究

.....李文辉, 李范文, 王喜军, 王丽娟 (68)

绵羊 *BTG2* 基因 *CDs* 区克隆、生物信息学分析及组织 mRNA 表达研究

.....高 一, 刘理想, 肖 成, 金海国, 马惠海, 薛佳佳, 魏 天, 曹 阳 (74)

无量山乌骨鸡肉质性状及养分含量的性别差异分析

.....杨丕才, 曹海月, 张忠新, 余仕亮, 王 群, 张开佳, 李则达, 王兴容, 尹兆正 (80)

繁殖生理

基于生物信息学方法的小鼠精子发生关键基因筛选

.....李 欣, 田 佳, 脱征军, 邵怀峰, 张伟新, 李委奇, 王 琨, 徐 寒, 温 万 (84)

AA 肉鸡和北京油鸡早期消化器官发育规律研究

.....刘兴波, 靳 曼, 宁 然, 冯子明, 聂 伟 (90)

不同运动方式和运动时间对小鼠睾酮分泌和精子生成的影响

.....张 妮, 魏易玲, 卢静怡, 王蕾蕾, 常成杰, 徐 畅, 袁业现, 尹 聪, 束 刚, 江青艳 (96)

农大 3 号蛋鸡与海兰褐蛋鸡的消化生理研究

.....郭晓瑞, 武 威, 陈 静, 王友利, 武玉钦, 袁建敏 (101)

siRNA 干扰耗牛 *Bmal1* 基因对颗粒细胞激素分泌的影响

.....赵明旺, 张 君, 徐尚荣, 黄 荣, 舒 适, 彭 巍, 张琳钦 (107)

营养饲料

花椒籽替代不同比例玉米对育肥猪生长性能和肉品质的影响

.....梁永飞, 浪双宝, 王鹏飞, 同尊强, 马艳萍, 张 博, 陈 勇, 祁光海, 林 忠 (112)

酵母发酵饲料对瘤胃菌群数量及多样性的影响

.....郝凌魁, 刘世雄, 李冬芳, 于春微, 李松建, 陈 友, 高 民, 胡红莲, 刘大程 (116)

全株玉米青贮和谷草干草不同比例组合对杜湖杂交肉羊增重、屠宰性能与育肥效益的影响

.....冯小和, 吴启超, 张振威, 王彦芦, 薄玉琨, 崔朝阳, 姜耀文, 李文娟, 王玮康, 杨红建 (125)

木薯渣与甜菜颗粒粕组合效应对奶牛瘤胃发酵特性的影响	郑宇慧, 张新雨, 李胜利 (130)
γ-聚谷氨酸对小尾寒羊瘤胃发酵及菌群结构的影响	曹琪娜, 张艳梅, 敖长金, 张腾龙, 张兴夫, 白 晨, 赵亚波, 齐景伟 (136)
发酵湿玉米纤维饲料和几种常用粗饲料营养价值分析	房新鹏, 冯冠智, 吕静仪, 张广宁, 张永根 (143)
不同添加剂对天然牧草青贮品质的影响	徐生阳, 王 柱 (148)
不同铜源对存储期间大豆油脂氧化酸败的影响	朱晓萍, 庄智威, 刘孝春, 李克标, 尚秀国 (152)
黑水虻幼虫干对肉鸡生产性能、血清生化指标及鸡肉养分含量的影响	赵 燕, 侯凤香, 郑远博, 林立培, 张慧慧, 李圣才, 金俊杰 (156)
谷氨酰胺对冬毛期水貂生长性能、血清生化指标及抗氧化性能的影响	李柯汝, 纪碧波, 孙建华, 徐燕红, 司志文, 李文立 (161)
动物健康	
蛋白酶和益生菌复合制剂对白羽肉鸡生长性能、养分利用率和粪便有害气体排放的影响	谢 谦, 杨 楣, 张海涵, 宋泽和, 赵丽芳, 郭宝林, 王 璐, 贺 喜 (165)
丁酸梭菌对哺乳母猪繁殖性能、免疫和抗氧化功能的影响	冯云龙, 王晶莹, 于 森, 刘 莹, 马 倩, 刁新平 (170)
日粮添加产朊假丝酵母对 15 月龄新疆褐牛杂交育肥增重性能的影响	赵明远, 杨开伦, 姚 刚, 陈如龙, 杨红建 (177)
人参多糖对脂多糖刺激小鼠巨噬细胞的免疫调控作用	陈广勇, 韩乾杰, 张玲玲, 李 慧, 杨彩梅 (182)
复合益生菌乳膏对泌乳母猪便秘及生产性能的影响	张 乐, 吴 昊, 孙晓杰, 杨宝良, 韦兴茹, 沈红霞, 李晟硕, 姜迎霞, 尹靖东 (187)
生物技术	
改良背主动脉注射法对鸡胚发育与转基因表达效率的影响	韦金鑫, 韦范芹, 徐小明, 邢青波, 肖艳宇, 许梦缘, 吴文德, 李恭贺, 郑喜邦 (191)
利用同源重组技术构建 Chordin 基因载体及其在成纤维细胞中表达量的研究	刘开东, 荣 恒, 贺建宁, 张梦瑶, 赵 明, 柳 楠 (196)
生产与管理	
放牧和圈养对柯乐猪的屠宰性能及肉质性能的影响	黄维江, 燕志宏, 杨 蒙, 杨 蓬, 杨仕钰, 张 芸 (201)
华南地区舍饲川中黑山羊与雷州山羊的生产繁殖性能及死亡状况研究	邓金戈, 宋佳杰, 刘德武, 孙宝丽, 李耀坤, 郭勇庆, 邓 铭, 柳广斌 (206)
简州大耳羊和肥羔型黑山羊不同肌肉的 pH 变化分析	王江林, 王 永, 林亚秋, 朱江江, 熊朝瑞, 俄木曲者 (209)
不同产次限位母猪瞳孔光反射与规律行为变化分析	于浪潮, 尹国安, 王 磊, 关立伟 (215)
保存温度、湿度和时间对鹌鹑种蛋孵化成绩的影响	史雪萍, 吕祥娟, 周小桐, 毕慧娟, 沈华跃, 王述柏 (219)
产业经济	
畜牧经济	
中国畜牧业经济形势分析及对策研究	韩 磊, 刘长全 (224)
行业调研	
青海省牦牛种业发展现状、存在问题及应对策略	骆正杰, 马进寿, 保广才, 王 芳, 李青云, 刘更寿, 殷满财, 李吉叶 (231)
白羽商品肉鸡不同生产组织形式分析	王 晓峰 (235)
通辽市扎鲁特旗生态肉羊养殖模式实施现状与效果调查分析	全宗永, 李喜利, 郑丽娜, 何 峰, 李振松, 甄莉丽, 李向林 (238)

目次

02

2021

第 57 卷 第 02 期

月刊

支持单位

四川省畜牧饲料有限公司
浙江花园生物高科股份有限公司
北京市华都峪口禽业有限责任公司

华南地区舍饲川中黑山羊与雷州山羊的生产繁殖性能及死亡状况研究

邱金戈, 宋佳杰, 刘德武, 孙宝丽, 李耀坤, 郭勇庆, 邓 铭, 柳广斌*

(华南农业大学动物科学学院, 广东广州 510642)

摘要: 试验旨在探究华南地区舍饲情况下川中黑山羊与雷州山羊的生产养殖情况。本研究于2016年5月—2018年1月对广东某规模化舍饲种羊场存栏的680只川中黑山羊及385只雷州山羊(均为2~3岁, 1~3胎次)的生产繁殖性能与1313只山羊的死亡状况进行跟踪测定, 收集数据并进行对比分析。结果显示: 在华南地区舍饲条件下川中黑山羊与雷州山羊羔羊的平均初生重分别为3.13 kg与2.11 kg, 平均断奶重分别为9.84 kg与8.54 kg; 川中黑山羊与雷州山羊母羊的平均产羔数分别为2.24只与1.76只; 肠炎、肺炎与体弱是导致2种山羊死亡的主要原因, 0~20日龄阶段是主要的死亡区间, 该阶段羔羊因肠炎与体弱的死亡率高达90%。本研究表明, 川中黑山羊的生产繁殖性能高于雷州山羊, 在舍饲条件下对初生羔羊肠炎及体弱的防控是饲养管理的重要工作。

关键词: 川中黑山羊; 雷州山羊; 生产性能; 繁殖性能; 死亡状况; 舍饲

中图分类号: S827.2

文献标识码: A

DOI 编号: 10.19556/j.0258-7033.20200304-03

羊肉作为一种健康的肉类食品, 越来越受到消费者青睐, 市场对羊肉的需求越来越大, 近年来养羊业得到快速的发展^[1-2]。华南地区主要以本地品种雷州山羊以及引种至该地区的川中黑山羊的饲养与消费为主, 雷州山羊主要分布于雷州半岛及海南省, 具有性成熟早、耐高温高湿、耐粗饲、肉质好等特点, 但由于缺乏系统的选育, 生产繁殖性能下降明显; 川中黑山羊原产于四川省, 属以产肉为主的地方遗传资源, 具有体型较大、繁殖性能突出、产肉性能优良、适应范围广、遗传性能稳定等特点^[3]。华南地区地势复杂, 几乎没有可供大面积放牧的优质草场, 这促使华南肉羊产业由小型农户的放牧养殖向规模化集约化的舍饲养殖发展, 以提高生产效率满足市场需求。本试验旨在研究

华南地区规模化舍饲条件下川中黑山羊及雷州山羊的生产繁殖性能及死亡情况, 揭示2种山羊在华南地区舍饲条件下存在的问题, 为华南地区舍饲山羊的养殖生产提供数据和参考。

1 材料与方法

1.1 试验场区概况 试验场区于调查期间存栏川中黑山羊能繁母羊约750只, 雷州山羊能繁母羊约390只。场区地处于广东省, 属亚热带季风气候。春秋两季昼夜温差大, 夏季高温多雨, 5—10月日均最高气温均高于30℃, 7、8月最高气温高达37℃, 冬季低温期短, 最低温度基本不低于0℃。春季夏季湿度较大, 尤其是春季“回南天”时湿度可达100%。

1.2 试验动物饲养管理 试验山羊饲养在可自由活动的负压式通风高床羊舍。日粮主要为干草、青贮及精料, 通过全混合日粮(TMR)机彻底混合后饲喂, 每日饲喂2次, 2种山羊饲喂的日粮及方式一致。羊舍夏季采用风扇、喷雾和湿帘等降温设备, 冬季采用保温灯等保暖设备。配种方式均为人工授精辅助交配, 羔羊在60日龄统一断奶。

1.3 试验数据来源 本研究于2016年5月—2018年1

收稿日期: 2020-03-04; 修回日期: 2020-04-06

资助项目: 广东省畜禽地方品种保护与开发利用提升工程; 广东省科技计划项目(2017A020208050); 广东省现代农业产业技术体系(2019KJ127); 阳江市科技计划项目(2018032)

作者简介: 邱金戈(1995-), 男, 广东人, 硕士研究生, 主要从事动物健康养殖与安全生产研究, E-mail: immmmmm9@163.com

*通讯作者: 柳广斌(1982-), 男, 辽宁人, 博士, 讲师, 主要从事草食动物遗传育种与繁殖研究, E-mail: gbliu@scau.edu.cn

月对试验场区存栏的 680 只川中黑山羊与 385 只雷州山羊能繁母羊的胎产羔数、羔羊的初生重与断奶重等生产繁殖数据以及期间 1 313 只山羊的死亡原因及死亡日龄等信息进行跟踪测定。采集到川中黑山羊数据 945 胎次, 雷州山羊数据 449 胎次, 在此基础上取母羊第 1 胎及第 2 胎的数据, 并剔除母羊流产的数据, 经筛选后得有效数据进行分析(表 1)。

表 1 本研究有效数据情况

品种	产羔数数据, 胎次	初生重数据, 只	断奶重数据, 只	死亡山羊数据, 只
川中黑山羊	892	1742	995	857
雷州山羊	410	630	295	456

1.4 统计分析 数据经 Excel 软件初步筛选整理, 利用 SPSS 22.0 统计软件进行单因素方差分析, 再利用 Tamhane's T2 模型进行差异显著性检验, 以 $P<0.05$ 作为差异显著性判断标准, 结果表示为平均值 \pm 标准误(或标准差)。

2 结果

2.1 川中黑山羊与雷州山羊的生产性能分析 如表 2 所示, 川中黑山羊第 1 胎和第 2 胎羔羊的平均初生重及断奶重与总平均值均高于雷州山羊羔羊($P<0.05$)。

2.2 川中黑山羊与雷州山羊的繁殖性能分析

2.2.1 川中黑山羊与雷州山羊的产羔数对比 如表 3 所示, 川中黑山羊第 1 胎和第 2 胎的平均产羔数与总平均值均高于雷州山羊($P<0.05$)。

2.2.2 川中黑山羊与雷州山羊的产羔类型群体分布 如表 4 所示, 川中黑山羊主要以产双羔与三羔为主, 且有产四羔的情况; 雷州山羊主要以产单羔与双羔为主, 本研究调查中雷州山羊无产四羔情况。川中黑山羊第 2 胎产双羔比例较第 1 胎低, 产三羔比例较第 1 胎高; 雷州山羊第 2 胎产单羔比例较第 1 胎低, 产双羔比例较第 1 胎高。

2.3 川中黑山羊与雷州山羊的死亡状况分析

2.3.1 舍饲下川中黑山羊与雷州山羊的死亡原因分布 如表 5 所示, 肠炎、肺炎与体弱是导致 2 种山羊在舍饲

条件下死亡的主要原因。

表 3 川中黑山羊与雷州山羊的产羔数

品种	产羔数, 只		
	第 1 胎	第 2 胎	总平均值
川中黑山羊	2.16 \pm 0.02 ^a (576)	2.36 \pm 0.03 ^a (316)	2.24 \pm 0.02 ^a
雷州山羊	1.71 \pm 0.03 ^b (239)	1.86 \pm 0.03 ^b (171)	1.76 \pm 0.02 ^b

表 4 川中黑山羊与雷州山羊的产羔类型群体分布 %

品种	第 1 胎				第 2 胎			
	单羔	双羔	三羔	四羔	单羔	双羔	三羔	四羔
川中黑山羊	17.53 (101)	67.01 (386)	14.24 (82)	1.22 (7)	13.61 (43)	57.28 (181)	27.22 (86)	1.90 (6)
雷州山羊	48.54 (116)	49.79 (119)	1.67 (4)	-	32.16 (55)	64.33 (110)	3.51 (6)	-

表 5 川中黑山羊与雷州山羊的死亡原因分布 %

品种	死亡原因								
	肠炎	肺炎	体弱	意外	产后感染	乳房炎	难产	生产瘫痪	其他
川中黑山羊	39.56 (339)	34.31 (294)	16.34 (140)	3.15 (27)	1.28 (11)	1.17 (10)	0.93 (7)	0.35 (3)	2.92 (25)
雷州山羊	34.43 (157)	34.65 (158)	20.18 (92)	2.41 (11)	0.88 (4)	1.54 (7)	0.44 (2)	-	5.48 (25)

2.3.2 舍饲下川中黑山羊与雷州山羊的死亡日龄分布 如表 6 所示, 舍饲下 2 种山羊死亡主要分布在 0~60 日龄。

表 6 川中黑山羊与雷州山羊的死亡日龄分布 %

品种	0~60 日龄	60~365 日龄	超过 365 日龄
川中黑山羊	59.74 (512)	28.12 (241)	12.14 (104)
雷州山羊	59.65 (272)	32.02 (146)	8.33 (38)

对哺乳期羔羊的死亡日龄作进一步分析发现, 舍饲下 2 种山羊哺乳期羔羊的死亡主要分布在 0~10 日龄和 10~20 日龄 2 个阶段(表 7)。随着日龄增长, 哺乳期羔羊的死亡数量大体呈下降趋势。

表 7 川中黑山羊与雷州山羊哺乳期羔羊的死亡日龄分布 %

品种	0~10 日龄	10~20 日龄	20~30 日龄	30~40 日龄	40~50 日龄	50~60 日龄
川中黑山羊	49.22 (252)	27.93 (143)	7.81 (40)	5.08 (26)	5.86 (30)	4.10 (21)
雷州山羊	41.18 (112)	27.94 (76)	9.93 (27)	8.46 (23)	6.25 (17)	6.25 (17)

2.3.3 舍饲下川中黑山羊与雷州山羊 0~20 日龄羔羊的死亡原因分布 如表 8 所示, 肠炎与体弱是导致 2 种山羊 0~20 日龄羔羊在舍饲条件下死亡的主要原因。

表 2 川中黑山羊与雷州山羊羔羊的初生重与断奶重

品种	初生重			断奶重		
	第 1 胎	第 2 胎	总平均值	第 1 胎	第 2 胎	总平均值
川中黑山羊	3.23 \pm 0.02 ^a (1097)	2.95 \pm 0.02 ^a (645)	3.13 \pm 0.01 ^a	9.83 \pm 0.09 ^a (739)	9.89 \pm 0.16 ^a (256)	9.84 \pm 0.08 ^a
雷州山羊	2.08 \pm 0.02 ^b (352)	2.16 \pm 0.02 ^b (278)	2.11 \pm 0.01 ^b	8.38 \pm 0.17 ^b (179)	8.80 \pm 0.20 ^b (116)	8.54 \pm 0.13 ^b

注: 括号中数据为样本量。同列数据肩标不同小写字母表示差异显著($P<0.05$), 含相同字母表示差异不显著($P>0.05$)。下表同。

表8 川中黑山羊与雷州山羊0~20日龄羔羊的死亡原因分布

品种	死亡原因					%
	肠炎	体弱	肺炎	意外	其他	
川中黑山羊	57.22(226)	33.92(134)	4.56(18)	4.05(16)	0.25(1)	
雷州山羊	48.40(91)	45.21(85)	4.26(8)	1.06(2)	1.06(2)	

3 讨论

3.1 舍饲下川中黑山羊与雷州山羊的生产性能对比分析 羔羊的初生重与断奶重是检验母羊生产性能高低的重要指标。本研究中川中黑山羊羔羊的平均初生重为 (3.13 ± 0.01) kg, 高于早期放牧下的2.37 kg(公羔)及2.28 kg(母羔)^[4], 亦高于早期引入华南地区舍饲的2.60 kg(公羔)及2.34 kg(母羔)^[5], 可见该品种在华南地区舍饲下可发挥其良好的生产性能, 适应性较好。川中黑山羊羔羊的平均断奶重为 (9.84 ± 0.08) kg, 低于早期放牧下的13.35 kg(公羔)及11.11 kg(母羔)^[4], 亦低于早期引入华南地区舍饲的10.15 kg(公羔), 略高于报道的9.64 kg(母羔)^[6], 这一情况可能是由于初生羔羊本身受该地区潮湿炎热的气候影响, 生长在一定程度上受阻, 也有可能是母羊受气候影响而导致其泌乳性能受阻, 进而影响初生羔羊的初期发育所致。因此, 在分娩后为泌乳母羊及初生羔羊提供一个适宜的温湿度环境十分重要。

本研究中雷州山羊羔羊的平均初生重为 (2.11 ± 0.01) kg, 高于早期舍饲下的1.96 kg^[7], 亦高于早期放牧下的1.62 kg(公羔)及1.53 kg(母羔)^[8]; 雷州山羊羔羊的平均断奶重为 (8.54 ± 0.13) kg, 高于早期舍饲下的4.73 kg^[7], 亦高于早期放牧下的5.74 kg(公羔)及5.48 kg(母羔)^[8], 这一情况可能得益于雷州山羊饲养管理环境的改善。冬末春初草料缺乏会导致放牧饲养的母羊体况下降, 初生羔羊体重小, 母羊泌乳性能下降抑制初生羔羊后期的生长发育^[8]。科学的舍饲管理可为妊娠母羊提供充足的营养, 为泌乳母羊及初生羔羊提供适宜的温湿度环境。因此, 在华南地区有必要对山羊的规模化舍饲管理模式进行推广。

本研究中, 川中黑山羊的初生重及断奶重均显著高于雷州山羊, 说明川中黑山羊的生产性能优于雷州山羊, 适合在华南地区扩大生产^[9-10]。

3.2 舍饲下川中黑山羊与雷州山羊的繁殖性能对比分析 本研究中川中黑山羊产羔数为2.24只, 低于早期舍饲

下的2.41只^[11], 说明引入至华南地区的川中黑山羊可能受当地气候、牧草类型或者饲养管理等因素影响, 产羔性能有所下降。但尽管如此, 川中黑山羊的产羔数仍显著高于雷州山羊, 且其第1胎及第2胎的产羔数均显著高于雷州山羊。本研究中, 川中黑山羊2个胎次的产羔数均能稳定达到2只以上, 且主要以产双羔与三羔为主, 而雷州山羊产羔数1.76只, 高于早期放牧下所的1.73只^[8], 但2个胎次的产羔数均低于2只, 主要以产单羔与双羔为主, 说明在全舍饲条件下川中黑山羊的繁殖性能优于雷州山羊, 华南地区本土的雷州山羊仍需进一步加强选育工作, 改善其繁殖性能。

3.3 舍饲下川中黑山羊与雷州山羊的死亡状况分析 本研究发现, 导致山羊舍饲条件下死亡的主要原因是肠炎、肺炎与体弱。对死亡案例按日龄进行分类统计后发现, 舍饲下初生20 d内的羔羊死亡率最高, 且主要是死于肠炎与体弱, 致死率高达90%。曹鹏等^[12]以及叶宝宏^[13]在调查舍饲山羊羔羊死亡状况时也发现, 肠炎和体弱是致使羔羊死亡的主要原因。缺乏科学的饲养管会导致母羊妊娠期及泌乳期营养不良, 从而导致羔羊无法获得充足的营养和免疫力, 易产弱羔。产后哺乳工作不到位, 羔羊无法从母乳中获得充足的母源抗体从而免疫力不足, 加之羊舍内饲养密度过大而空气浑浊, 舍内卫生控制不佳导致栏舍内细菌滋生, 饲养员不加以妥善处理, 都会导致羔羊易于患肠炎, 剧烈腹泻后脱水虚弱致死。因此, 舍饲下对初生羔羊肠炎及体弱的防控是饲养管理的关键。

4 结论

本试验结果表明, 华南地区本地品种雷州山羊的生产繁殖性能低于引进品种川中黑山羊, 应进一步加强雷州山羊的选育工作, 改善其生产繁殖性能; 舍饲条件下, 肠炎、肺炎与体弱是导致山羊死亡的主要原因, 而0~20日龄的羔羊最容易因肠炎与体弱而死亡。因此, 舍饲情况下要更加注重饲养管理, 尤其是对初生羔羊的饲养管理。

参考文献:

- [1] 李军, 金海. 2018年肉羊产业发展概况、未来趋势及对策建议[J]. 中国畜牧杂志, 2019, 55(3): 138-145.
- [2] 赵印, 杜立新, 刘强德. 2017年中国羊产业发展报告与发展预测[A]. 第十五届(2018)中国羊业发展大会论文集[C]. 北京: 中国畜牧业协会, 2018: 8-17.

(下转第214页)

- [17] Boukha A, Bonfatti V, Cecchinato A, *et al.* Genetic parameters of carcass and meat quality traits of double muscled Piemontese cattle[J]. *Meat Sci*, 2011, 89(1): 84-90.
- [18] 熊朝瑞, 范景胜, 陈天宝, 等. 肥羔型黑山羊新品种的产肉性能研究[J]. *四川畜牧兽医*, 2014(1): 29-31.
- [19] 俄木曲者, 熊朝瑞, 范景胜, 等. 肥羔型黑山羊新品种羔羊肉品质研究[J]. *黑龙江畜牧兽医*, 2016(1): 108-111.
- [20] 李秀定. 浅谈简州大耳羊品种资源的保护及利用[J]. *中国畜禽种业*, 2013, 9(4): 68-70.
- [21] 俄木曲者, 熊朝瑞, 范景胜, 等. 简州大耳羊产肉性能世代选育进展及肉品质研究[J]. *中国草食动物科学*, 2014(3): 9-12.
- [22] 贾雪晖, 王小斌, 靳辉, 等. 不同排酸方式对巴美肉羊肉品质的影响[J]. *肉类研究*, 2012, 26(5): 14-16.
- [23] 孙煥林, 张文举, 刘艳丰, 等. 影响羊肉品质因素的研究进展[J]. *饲料博览*, 2014(1): 8-12.
- [24] 陈韬, 董文明, 李兴楼, 等. 云南山羊肉质研究[J]. *畜牧与兽医*, 1999(5): 14-16.
- [25] 苏建军. 肌肉 pH 变化及其意义[J]. *肉类工业*, 1997(2): 43-45.
- [26] Jimenez S M, Salsi M S, Tiburzi M C, *et al.* Spoilage microflora in fresh chicken breast stored at 4°C: influence of packaging methods[J]. *J Appl Microbiol*, 1997, 83(5): 613-618.
- [27] Kakouri A, Nychas G J E. Storage of poultry meat under modified atmospheres or vacuum packs: Possible role of microbial metabolites as indicator of spoilage[J]. *J Appl Bacteriol*, 1994, 76(2): 163-172.
- [28] Larzul C, Lefaucheur L, Ecolan P, *et al.* Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs[J]. *J Anim Sci*, 1997, 75(12): 3126.
- [29] Gentry J G, Meglone J J, Miller M F, *et al.* Environmental effects on pig performance, meat quality, and muscle characteristics[J]. *J Anim Sci*, 2004, 82(1): 209-217.
- [30] 沈林圆, 郑梦月, 张顺华, 等. 猪屠宰后 pH 变化对肉品质的影响[J]. *猪业科学*, 2013(4): 114-115.
- [31] 王文浩. 不同品种及排酸成熟时间对南方黄牛肉色和色素物质含量的影响[J]. *江苏农业科学*, 2019, 47(18): 228-231.
- [32] 刘建伟. 波尔山羊肌肉组织学性状与肉品质理化性状的研究[D]. 保定: 河北农业大学, 2007.
- [33] Hunt M C, Hedrick H B. Chemical, Physical and sensory characteristics of bovine muscle from four quality groups[J]. *J Food Sci*, 1977, 42(3): 716-720.
- [34] Young O A, Reid D H, Scales G H. Effect of breed and ultimate pH on the odour and flavour of sheep meat[J]. *New Zeal J Agr Res*, 1993, 36(3): 363-370.

(责任编辑: 周会会)

(上接第 208 页)

- [3] 国家畜禽遗传资源委员会组编. 中国畜禽遗传资源志·羊志[M]. 北京: 中国农业出版社, 2011.
- [4] 许德贵, 张家明, 唐诗军, 等. 金堂黑山羊生产性能研究[J]. *四川畜牧兽医*, 2006(11): 25-26.
- [5] 周多恩, 刘德武, 廖迎新, 等. 湖羊和川中黑山羊在华南地区适应性研究[J]. *华南农业大学学报*, 2016, 37(5): 19-23.
- [6] 练志全, 柳广斌, 邹炯, 等. 华南地区舍饲川中黑山羊繁殖性能及疾病状况分析[J]. *中国畜牧兽医*, 2019, 46(6): 1695-1703.
- [7] 徐铁山, 王东劲, 夏万良, 等. 海南黑山羊早期生长发育规律研究[J]. *热带农业科学*, 2007(6): 27-28, 31.
- [8] 刘艳芬, 刘铀, 叶昌辉, 等. 雷州山羊繁殖性能及其影响因素的研究[J]. *四川畜牧兽医*, 2001(3): 18-20.
- [9] 何春, 范景胜, 杨世忠, 等. 川中黑山羊改良建昌黑山羊的效果观察[J]. *四川畜牧兽医*, 2019, 46(1): 28-30.
- [10] 黄磊, 徐刚毅, 陈彬, 等. 乐至黑山羊与营山黑山羊杂交改良效果初探[J]. *四川畜牧兽医*, 2010, 37(1): 25-26.
- [11] 文永照. 川中黑山羊(乐至型)繁殖性能研究[A]. 第九届中国羊业发展大会论文集[C]. 怀仁: 中国畜牧业协会, 2012: 106-110.
- [12] 曹鹏, 刘志东, 王延莉. 陕西省安塞县新生羔羊死因调查及防控对策[J]. *畜牧与饲料科学*, 2010, 31(5): 158-159.
- [13] 叶宝宏. 榆林市某羊场舍饲陕北白绒山羊羔羊死亡原因分析[J]. *黑龙江畜牧兽医*, 2015(16): 68-69.

(责任编辑: 周会会)

68

1953-2021



《中国畜牧杂志》创刊于1953年，是中国科学技术协会主管、中国畜牧兽医学学会主办、中国农业大学为学术支撑单位的学术期刊。期刊现已被全国中文核心期刊数据库、中国科技核心期刊数据库、中国学术期刊综合评价数据库、中国核心期刊（遴选）数据库、维普网等多家数据库收录。

2020年，《中国畜牧杂志》获得武汉大学中国科学评价研究中心（RCCSE）“权威学术期刊（A+）”评价，在全国“畜牧、兽医科学”类90本期刊中排名前三；在中国知网发布的《中国学术期刊影响因子年报》中，《中国畜牧杂志》影响力指数CI值为571.933，复合影响因子达到1.151。

《中国畜牧杂志》始终坚持以“内容为王”为宗旨，秉承客观、公正、科学的传播理念，以引导正确的科技方向为己任，近年来重点打造了综述、科学技术、产业经济、特别报道等传统栏目和遗传育种、繁殖生理、饲料营养、检测技术、食品安全、环境控制与动物福利、动物健康、科技应用、产业透视、畜牧经济等二级栏目，在畜牧、兽医科学类期刊中具有鲜明特色。

ISSN 0258-7033
CN11-2083/S

国内邮发代号：82-147

国外发行代号：SM452

定价：40.00元



微信订阅

ISSN 0258-7033
CN 11-2083/S

中国畜牧杂志

CHINESE JOURNAL OF ANIMAL SCIENCE

主管：中国科学技术协会

主办：中国畜牧兽医学会

互动网站：www.boyar.cn

**美瑞泰科**
MERITECH

减抗替抗——优选好力高

专有技术

20年应用

30年研发

高效抗菌

强抗氧化

提高采食



好力高 | 泰乐津

多数大集团企业使用

天然止痢草提取物

富吸旺/好力盾 DetoxinPro 霉菌毒素脱毒剂	好力甜/泰科甜 Tech-Sweet 饲用甜味剂	好力酸/泰科酸 Tech-Acid 复合酸化剂
美力生 Tech-Zyme 复合酶制剂	好力克/泰克霉 缓释性防霉剂	美力醇/泰克敏 Anti-Q 抗氧化剂

广州美瑞泰科生物工程有限公司

地址：广州国际生物岛螺旋四路1号研发A区405室
电话：020-84098830 传真：020-84098832
电邮：service@meritech.com.cn
网址：www.meritech.com.cn



官方微信



全国中文核心期刊
中国科技核心期刊
中国期刊网网刊

中国学术期刊综合评价数据库来源期刊
中国学术期刊（网络版）来源期刊

2020. 5

《中国畜牧杂志》编委会

(以姓名笔画为序)

主任

吴常信

副主任

阎汉平 禹子明 杨汉春

顾问

印遇龙 刘守仁 张子仪 张 兢
李德发 陈焕春 南志标

编委会成员

刁其玉 马月辉 王立贤 王 恬
王济民 王洪荣 王楚端 孔平涛
文 杰 尹靖东 冯定远 齐广海
乔玉峰 刘玉满 刘庆平 刘作华
刘国世 刘建新 刘剑锋 刘继军
曲鲁江 同之春 朱化彬 任 军
吕明斌 孙 皓 张万刚 张许科
张宏福 张胜利 张细权 张英杰
张英俊 张若寒 张越杰 李 奎
李保明 李学伟 李胜利 李 辉
陈代文 陈国宏 陈瑞生 杨 宁
杨红建 连正兴 邵彩梅 金立志
林 海 罗海玲 赵广永 赵书红
赵国琦 侯水生 侯永清 姚军虎
洪 平 贺 喜 泰玉昌 泰应和
泰贵信 敖长金 敖志刚 高玉鹏
曹兵海 曹建国 康相涛 曹申明
曹建国 董 延 蒋宗勇 储明星
韩彦明 葛 翔 谁仕彦 蔡辉益

主编

禹子明

常务副主任

韩长华

副主任

刘国世 杨红建 曲鲁江 尹靖东
张万刚 李保明 刘玉满 曹建民
乔玉峰

编辑部主任

孔平涛

编辑部副主任

赵 楠

市场总监

朱晓娟

总策划

王必勇

责任编辑

李蕊兰 赵 楠 郑永艳 周会会

综 述

哺乳动物 *Bcl-2L13* 基因的研究进展

刘理想, 高 一, 吕 阳, 薛佳佳, 吴 健, 肖 成, 张国梁 (1)

肌红蛋白和线粒体互作研究进展

罗 培, 赵伟杰, 王丽娜, 江青艳 (6)

动物源性食品中磺胺类药物和 β -受体激动剂残留的色谱和质谱检测技术研究进展

刁志祥, 王旭堂, 张培杨, 王 波, 谢恺舟 (12)

残次枣在畜禽饲料中的应用研究进展

周小玲, 蒋 慧, 陈 宁 (19)

经济学视角下禽畜养殖疫情研究综述

鹿傲飞, 李 乾, 王玉斌 (26)

外源性蛋白酶在饲料工业中的应用

谭 权, 赵 剑, 陶青燕, 穆玉云 (32)

奶牛生产寿命研究进展

唐 林, 王兴平, 马 云 (36)

科学技术

遗传育种

奶牛牛 *ACSL1* 基因组织表达分析及其对脂肪酸代谢相关基因表达的影响

梁莎莎, 鹿春英, 邓廷贤, 马小娅, 陆杏蓉, 段安琴, 梁贤威 (41)

mTOR 在不同毛色绵羊皮肤的表达与定位

渠国文, 孙美尉, 鹿全海 (46)

DNMTs 基因在苏博美利奴羊皮肤毛囊发育不同时期的表达分析

曾伟丹, 赵冰茹, 范 雪, 楚高洁, 徐新明, 付雪峰, 黄锡霞, 田可川 (50)

基于 RNA-seq 筛选不同饲养模式下肉鸡肌肉发育代谢相关差异基因

王佳洁, 沈中浩, 周晓龙, 杨松柏, 赵阿勇, 汪 涵 (54)

“京粉 6 号”粉壳蛋鸡配套系的培育

李光奇, 杨 宁, 孙 皓, 石凤英, 孙从俊, 李花妮, 赵丽红, 韩文朋, 王志强, 吴桂琴 (61)

新疆褐牛体型外貌线性评定方法的研究

刘建明, 杨光维, 李 静, 乃比江, 黄锡霞, 王雅春 (64)

性别因素对苏尼特羊背最长肌肌内脂肪沉积的影响及相关性研究

高 栋, 高爱琴, 冀 祥, 李 卿 (70)

不同哺喂模式乳鸽生长曲线分析与拟合研究

陈 丹, 谭会泽, 刘松柏, 彭运智, 邹 轶, 黎鸿彬, 吴徐俊, 刘 伟 (74)

广西麻鸡产蛋相关基因的 SNPs 检测及其与蛋用性能关联分析

李凤宁, 徐桂云, 张俊楠, 王喜琼, 李 毅, 韦凤英 (78)

BMP15 对绵羊卵丘细胞扩展相关基因及激素受体基因表达量的影响

赵 茜, 努日比娅姆·麦麦提托合提, 张 博, 阿尔曼·海热, 宋玉坤, 阿布力孜·吾斯曼 (83)

内蒙古白绒山羊不同部位肌肉脂肪酸的研究

刘志红, 谢遇春, 奈日乐, 米 璐, 马丽娜, 杨 峰, 王志新, 李金泉 (88)

繁殖生理

精胺对雌鹌卵巢生殖激素受体基因表达的影响

易治鑫, 李林祥, 徐 进, 李 苗, 姜冬梅 (92)

枸杞多糖对 17℃ 负压环境下猪精子保存质量的影响

王 硕, 赵家辉, 邹一诺, 高明山, 李雁冰, 黄大鹏, 魏国生, 李井春 (95)

牛血清蛋白及大豆卵磷脂对山羊精子保存效果的影响

.....刘文江,何玉强,周多恩,刘德武,孙宝丽,李耀坤,郭勇庆,邓 铭,柳广斌 (100)

Ube2s 在小鼠卵泡发育和黄体形成过程中的表达研究

.....吴 浩,于浩楠,倪 华 (106)

营养饲料

肉鸡基础饲料组成对豆粕中磷真消化率评定的影响

.....张 莉,蒲 劼,严鸿林,张宏福,刘静波 (112)

不同稻壳比例发酵型全混合日粮对海南黑山羊生长性能的影响

.....吕仁龙,张立冬,王 春,李 茂,唐 军,周汉林 (117)

阿朴酯和斑蝥黄对产蛋后期鸡生产性能、蛋品质、肝肾功能及抗氧化机能的影响

.....姬翔宇,杨继业,陈书明,杨 玉,卫 阳,任 娜,陈志敏 (122)

饲料添加铜对 1~21 日龄肉仔鸡生长性能和血清生化指标的影响

.....陈志勇,廖秀冬,张丽阳,吕 林,黄艳玲,罗绪刚 (127)

补喂色氨酸对伊犁马血浆 5-羟色胺、褪黑素含量及抗氧化能力的影响

.....张文杰,韩 明,王世昌,张仕琦,李晓斌,杨开伦 (132)

体外法研究添加不同比例葡萄籽对多浪羊瘤胃发酵及底物降解的影响

.....彭婉婉,许贵善,马忠杰,张哲藤,乌鹏颖 (136)

补充维生素 E 和硒对围产期奶牛分娩应激和繁殖性能的影响

.....陈 彪,柳巨雄 (142)

动物健康

中药复方制剂对雏鸡生产性能、肠道结构及微生物菌群的影响.....李姣清,

赖美辰,翁茁先,钟 鸣,李威娜,刘春燕,刘梦林,唐 玲,陈燕珊,吴文超,黄勋和 (147)

发酵扶正解毒口服液对蛋鸡生产性能、抗氧化功能及肠道组织结构的影响

.....史洪涛,乔宏兴,刘 松,张 嵩,边传周 (152)

生产与管理

运输应激对小鼠肠道的病理损伤及 Bcl-2 和 Bax 表达的影响

.....温宇婷,刘 萍,章志涛,张雯苑,梅 婷,彭瑞妮 (157)

延长肉类货架期的饲喂方案

.....付 瑶,王 俊,齐志国,路永强,郭江鹏,蒋林树 (163)

运输应激对安格斯牛血液生理生化指标的影响

..王莉梅,王德宝,赛音巴雅尔,王 峰,梁俊芳,王晓冬,康连和,郭天龙,翟 琇 (167)

环境控制

通风对异位发酵床处理肉鸭粪污效果的影响

.....晏 婷,朱志平,高理福,彭 华,李 冬,卢连水,骆乾亮 (171)

产业经济

畜牧经济

中国活鸡价格区域差异时空特征及其驱动因素研究

.....徐小华,王 琪,吴思平 (176)

行业调研

基于专利计量的全球设施畜牧业关键技术发展态势分析

.....任 妮,孙艺伟,李 果,陆学文 (183)

产业透视

2019 年广东省畜禽种业发展现状及未来展望

.....罗建民 (194)

目次

05
2020

第 56 卷 第 05 期

月刊

支持单位

四川省畜牧饲料有限公司
浙江花园生物高科股份有限公司
北京市华都峪口禽业有限责任公司

牛血清蛋白及大豆卵磷脂对山羊精子保存效果的影响

刘文江, 何玉强, 周多恩, 刘德武, 孙宝丽, 李耀坤, 郭勇庆, 邓 铭, 柳广斌*

(华南农业大学动物科学学院, 广东广州 510642)

摘 要: 本实验旨在研究在精液稀释液中添加牛血清蛋白(BSA)和大豆卵磷脂对山羊精液常温保存效果的影响, 并探讨常温保存山羊精液时 BSA 与大豆卵磷脂的最适浓度。配制 6 种稀释液, 比较其常温保存山羊精子的效果, 保存效果最好的一种作为基础稀释液。在优选稀释液中首先分别添加 0、1、2、3、4 g/L BSA, 在得到保存效果最佳的 BSA 浓度基础上分别添加 0、1、2、3 g/L 大豆卵磷脂, 检测两者在不同浓度条件下对山羊精子活力、质膜完整率、顶体完整率及保存时间的影响。结果表明: 基础稀释液中添加 3 g/L BSA 及 1 g/L 大豆卵磷脂的精子活力、质膜完整率及顶体完整率均显著高于其他浓度添加组, 且有效存活时间为 68.93 h, 总存活时间为 82.55 h, 均为最长。每 1 L 蒸馏水与葡萄糖 19 g、果糖 19 g、柠檬酸二钠 16 g、EDTA- Na_2 1.7 g、BSA 3 g、大豆卵磷脂 1 g、青霉素钠和硫酸链霉素各 100 万单位配制成的稀释液, 常温保存山羊精子效果较好, 适用于山羊人工授精。

关键词: 山羊; 牛血清蛋白; 大豆卵磷脂; 精液稀释液; 常温保存

中图分类号: S827.3

文献标识码: A

DOI 编号: 10.19556/j.0258-7033.20190620-06

人工授精技术在绵羊繁殖中的应用多于山羊, 主要是因为绵羊人工授精技术研发时间较长且技术成熟。山羊与绵羊的精子生理特性不同, 山羊精液中由尿道球腺分泌的卵黄凝固酶和 SBU III 分别会与绵羊用卵黄精液稀释液和乳汁稀释液发生毒害作用以及强烈抑制山羊精子活力的反应^[1], 而且添加动物源性蛋白也将提高感染风险^[2], 因此不能直接将绵羊人工授精技术用于山羊, 从而制约了山羊规模化养殖发展与优秀山羊品种的培育。

稀释液可扩大精液配种量并短期保存精液, 是影响山羊精子保存质量的关键所在^[3]。调整精液稀释液配方能增强其营养、缓冲、抗菌、抗氧化和保护等作用。牛血清蛋白(BSA)是绝大多数哺乳动物精子的获能物质,

可以阻止精子质膜粘连^[4]。一定浓度 BSA 能提高低温保存牛精子的生存能力^[5]。已有使用大豆卵磷脂等非动物源性物质替代卵黄在稀释液中作为其他动物精子保护物质的研究报道^[6], 但这些添加剂在山羊精液稀释液中的应用仍需优化。本研究拟利用 BSA 及大豆卵磷脂从精子活力、质膜完整率、顶体完整率及保存时间等指标评价并改良现有基础精液稀释液常温保存山羊精液的效果, 以期提供一种山羊常温精液稀释液, 为提高山羊配种效率及人工授精技术在山羊养殖业中的应用推广奠定基础。

1 材料与方法

1.1 实验动物 选取广东地区某种羊场健康且状况相似的成年川中黑山羊公羊 5 头。

1.2 实验试剂 水葡萄糖购于广州化学试剂厂; 果糖、柠檬酸钠、EDTA、碳酸氢钠、Hepes 均购于美国 Sigma-Aldrich 公司; BSA 购于北京 Solarbio 公司; 大豆卵磷脂购于生工生物工程股份有限公司; 青霉素、链霉素均购于圣旺药业股份有限公司; 恩诺沙星、诺氟沙星均购于华源药业有限公司; 庆大霉素购于大连兽药; 卵黄在市场购买; 精子快速染色试剂盒由南京建成公司生产。

收稿日期: 2019-06-20; 修回日期: 2019-07-18

资助项目: 广东省公益研究与能力建设项目(2017A020208050、2017B020201014)

作者简介: 刘文江(1994-), 男, 广东梅州人, 硕士研究生, 研究方向为动物健康养殖与安全生产, E-mail: liuwenjiticks@qq.com

* 通讯作者: 柳广斌(1982-), 男, 博士, 讲师, 主要研究方向为草食动物遗传育种、肉羊繁殖技术与生产管理、分子生物学及生物信息学, E-mail: gblu@scau.edu.cn

1.3 精液采集、检测 利用假阴道法采集其精液后立即检测,确保精液颜色和气味正常,精子活力达到0.7以上,密度在中等以上。检测合格后混合并按精液与精液稀释液 1:4 的比例稀释山羊精液,并从稀释时刻起每隔 12 h 对精子的活力、精子质膜完整率、顶体完整率、存活时间及存活指数进行检测。检测方法均按 GB 20557-2006 标准执行^[7]。将每份精液样品每次制备于 3 个玻片用于各指标检测,每次实验重复 3 次。

1.4 基础稀释液的筛选 参考文献^[8-10]选择 6 种精液稀释液配方作为候选基础配方,稀释液配方如表 1 所示。从精子活力、有效存活时间、总存活时间以及生存指数比较 6 种配方对山羊精子的保存效果,检测方法同 1.3。筛选效果最佳的一种作为本研究的基础配方。

1.5 精液的稀释方法 试验组首先在基础稀释液中分别添加 0、1、2、3、4 g/L BSA,筛选 BSA 最适添加浓度。再以所得最适 BSA 添加浓度为基础配制稀释液,各试验组分别添加 0、1、2、3 g/L 大豆卵磷脂,筛选最佳大豆卵磷脂浓度。检测项均同 1.4 并加上其对山羊精子质膜完整率和顶体完整率的影响,检测方法同 1.3。

1.6 统计分析 使用 WPS2019 软件对数据进行分组整理,数据的统计显著性检验使用 IBM SPSS Statistics 25 软件中的单因素方差分析, $P<0.05$ 为差异显著,并采用 Duncan's 方法对各组间平均数进行多重比较,结

果均以平均值±标准误表示。数据的可视化处理使用 GraphPad Prism 8 软件。

2 结果与分析

2.1 基础稀释液的筛选

2.1.1 精液稀释液成分的差异对山羊精子活力的影响 由图 1 可知,60 h 时,只有配方为 E 和 F 的精液稀释液中的精子仍然存活。最终结果显示 E 配方稀释液中的精子活力下降最少且速度最慢。

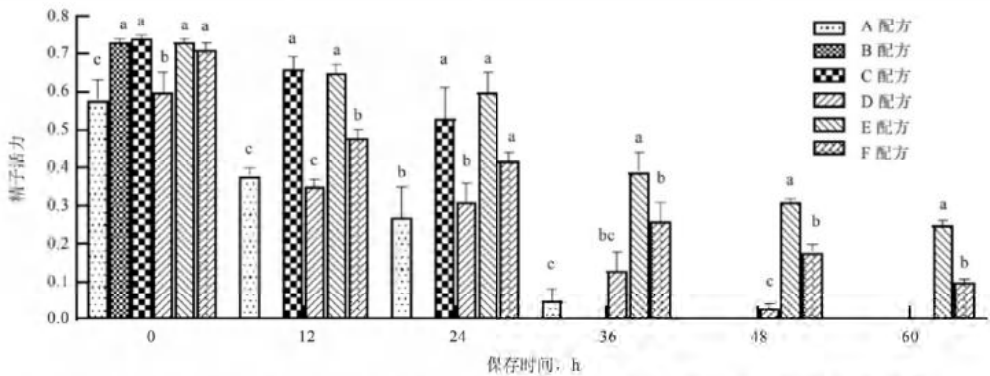
2.1.2 不同配方的精液稀释液对山羊精子保存时间的影响 由图 2 可知,E 配方稀释液组的有效存活时间、生存指数和总存活时间 3 项指标都显著高于其他配方组。总体来看,采用 E 配方的稀释液保存效果最优,因此选取 E 配方作为用于后续实验的基础稀释液。

2.2 BSA 对山羊精液保存效果的影响

2.2.1 BSA 对山羊精子活力和保存时间的影响 由图 3 可知,添加不同浓度 BSA 在 0 h 开始就对精子存活产生了影响,添加 BSA 可以提高精子的存活率($P<0.05$)。不添加 BSA 和添加 4 g/L BSA 组精液稀释液中的精子在 72 h 时全部死亡,而其他浓度梯度 BSA 组中的精子依然具有活力,其中 3 g/LBSA 组在各时间点的精子活力均高于其他组。由图 4 可得,稀释液添加 3 g/LBSA 时,精子的有效存活时间、总存活时间和生存指数都高于其

表 1 初选稀释液配方

配方	0.9%生理盐水,L	新鲜鸡蛋黄,L	葡萄糖,g	果糖,g	柠檬酸钠,g	EDTA,g	NaHCO ₃ ,g	Hepes,g	双蒸水,L	青霉素钠,万 IU	硫酸链霉素,g
A	1	-	-	-	-	-	-	-	-	100	1
B	0.8	0.2	-	-	-	-	-	-	-	100	1
C	-	0.2	30	-	14	-	-	-	0.8	100	1
D	-	-	46	-	4	1	-	-	1	100	1
E	-	-	19	19	16	1.7	-	-	1	100	1
F	-	-	22.5	22.5	9	2.4	1.2	9	1	100	1



同一时间肩标相同小写字母表示差异不显著 ($P>0.05$), 不同字母表示差异显著 ($P<0.05$)。图 3、5、6、7、9、10 同。

图 1 精液稀释液成分的差异对山羊精子活力的影响

他组 ($P<0.05$)。

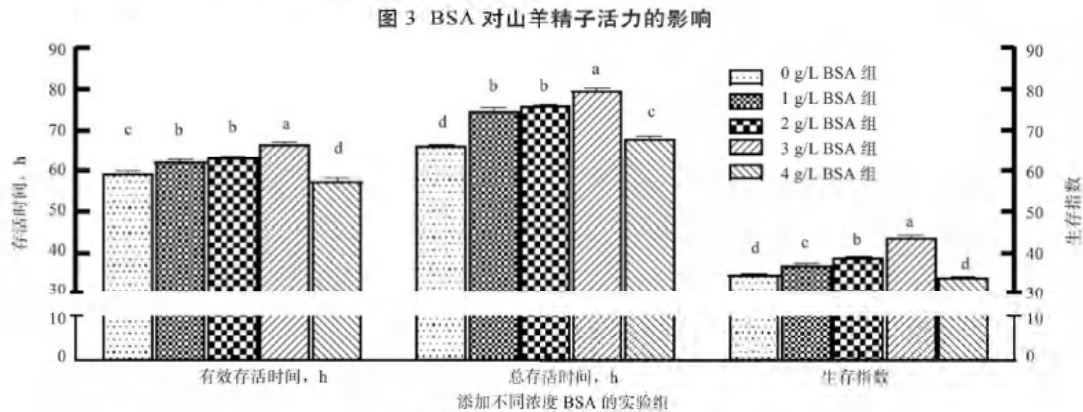
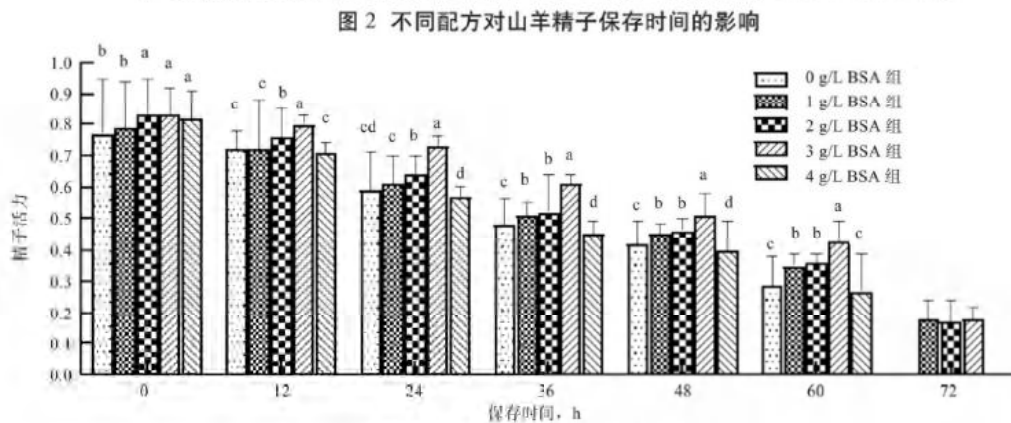
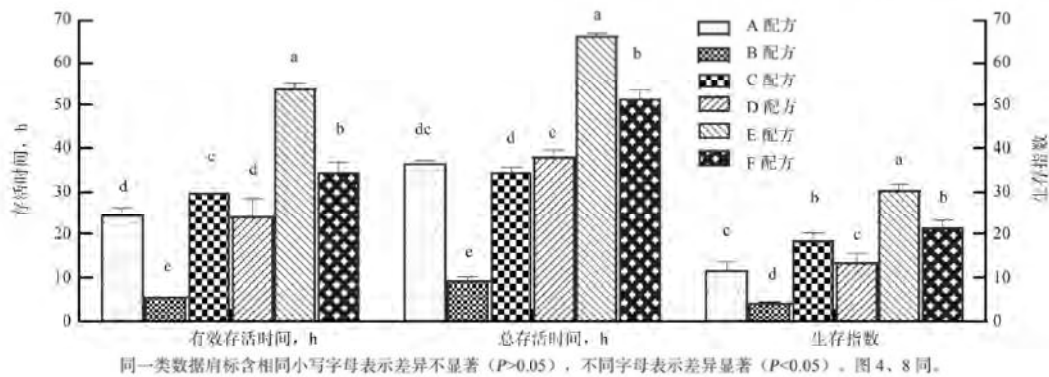
2.2.2 BSA 对山羊精子质膜完整率和顶体完整率的影响
由图 5 可得, 不添加 BSA 和添加 4 g/L BSA 组精液中的精子质膜在 72 h 时已经没有完整率, 而其他浓度梯度 BSA 组精液中的精子质膜依然具有完整率, 其中 3 g/L BSA 组在 0 h 之后各时间点的精子活率均高于其他组, 提高了山羊精子质膜的完整率 ($P<0.05$)。由图 6 可知, 0 h 时间点开始, 添加不同浓度 BSA 就对山羊精子顶体完整率产生了影响。1 g/L BSA 组精液稀释液中的精子在 12 h 时顶体完整率最高; 而在其他时间点, 3 g/L BSA 组的精子顶体完整率均高于其他组。从整体来看, 精液保存效果随着 BSA 浓度地增加呈先升后降的趋势,

其中以添加 3 g/L BSA 效果最佳。

2.3 大豆卵磷脂对山羊精液保存效果的影响

2.3.1 大豆卵磷脂对山羊精子活力和保存时间的影响
由图 7 可得, 从 0 h 时间点起, 添加不同浓度大豆卵磷脂即对精子的存活产生了影响。2、3 g/L 大豆卵磷脂组精液稀释液中的精子在 72 h 时全部死亡, 而其他实验组中的精子依然具有活率, 其中 1 g/L 大豆卵磷脂组在各时间点的精子活率均高于其他组。由图 8 可知, 1 g/L 大豆卵磷脂组被稀释的精子有效存活时间、总存活时间和生存指数均高于其他组 ($P<0.05$)。

2.3.2 大豆卵磷脂对山羊精子质膜完整率的影响 由图 9 可得, 2、3 g/L 大豆卵磷脂组精液中的精子在 72 h 时



全部死亡,且在其他时间点的精子质膜完整率低于另外两组 ($P<0.05$)。而其他浓度梯度大豆卵磷脂组精液中的精子依然具有质膜完整率,其中 1 g/L 大豆卵磷脂组在各保存时间测定点的精子质膜完整率都高于其他浓度组。由图 10 可得,添加了 1 g/L 大豆卵磷脂组在各时间点的精子顶体完整率均高于其他组。

3 讨论

由 6 种初选稀释液配方的对比实验结果可知,山羊精液使用配方 E 配制的稀释液保存效果最佳, F 次之。对比不同实验组可知,前两者除添加葡萄糖外,还等比例加

入了果糖,其余 4 种只加入单一种类的糖。曲宏伟等^[11]研究发现,在阿拉善型绒山羊精液稀释液中混合添加葡萄糖与蔗糖 2 种糖的实验组的精子保存效果显著优于添加单一蔗糖组;宣小龙等^[12]研究发现,按照接近于 1:1 比例添加 2 种不同的单糖作为夏洛莱绵羊精液稀释液营养剂效果比只加入葡萄糖的更优。蔗糖是非还原性二糖,是由一分子葡萄糖和一分子果糖在特定键位缩水而成,它被动物体摄入后在相关特定酶的作用下被分解成两分子葡萄糖参与供能或再被转化为肝糖原肌糖原储存在体内。本研究与已有发表的研究结果相同,可能是等比添加的葡萄糖和果糖在为精子供能方面优于添加单一的蔗

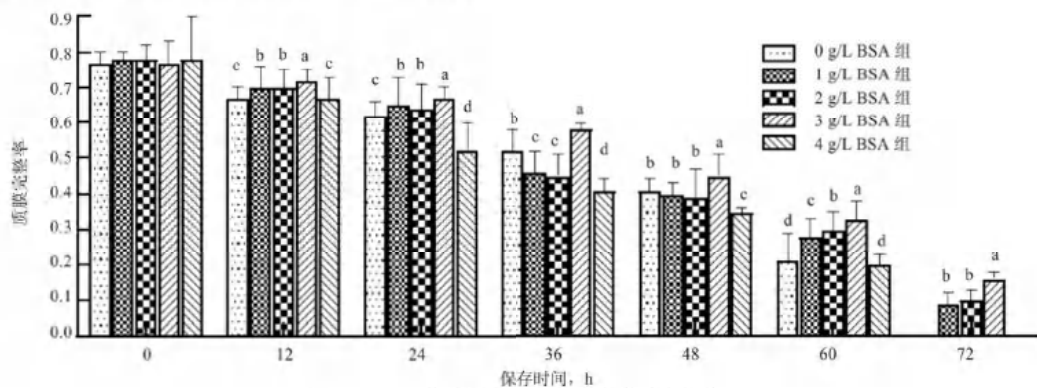


图 5 BSA 对山羊精子质膜完整率的影响

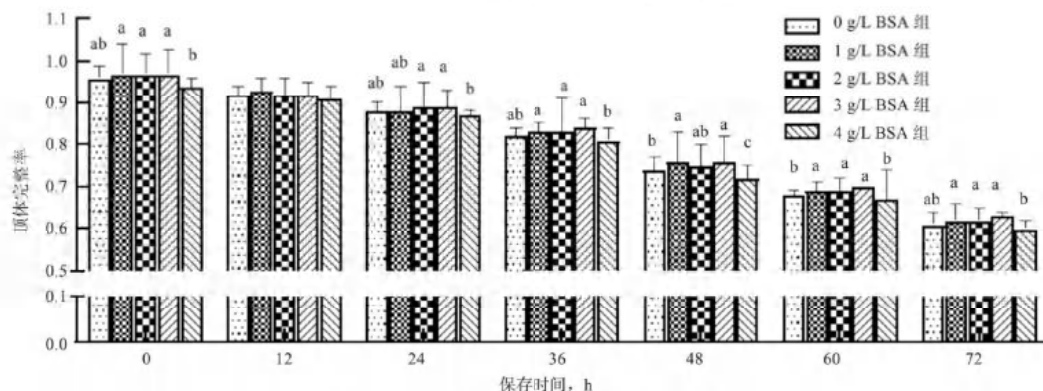


图 6 BSA 对山羊精子顶体完整率的影响

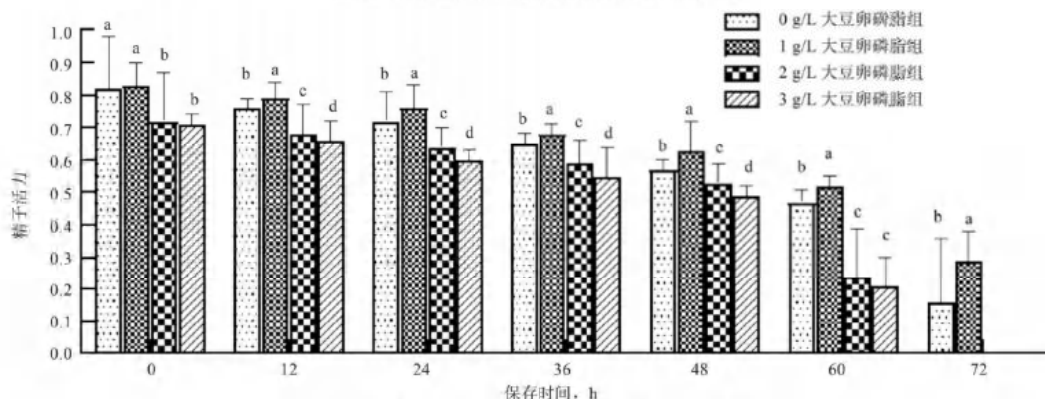


图 7 大豆卵磷脂对山羊精子活力的影响

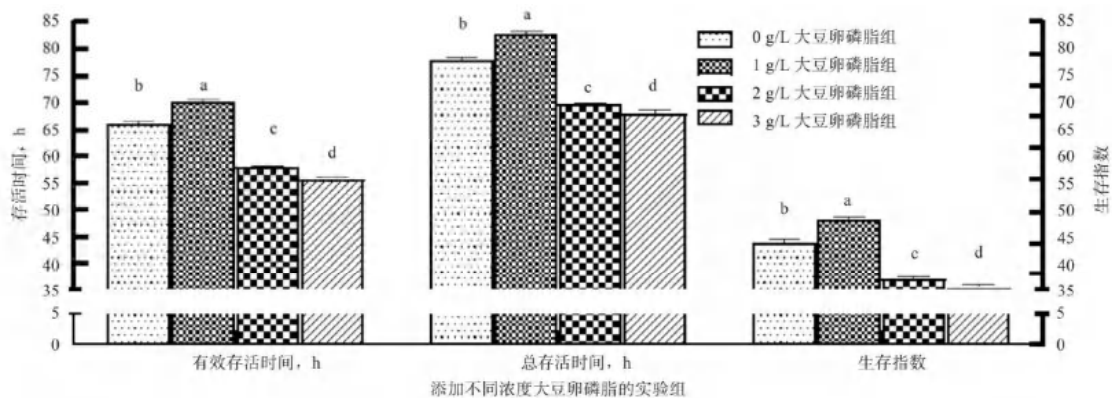


图 8 大豆卵磷脂对山羊精子保存时间的影响

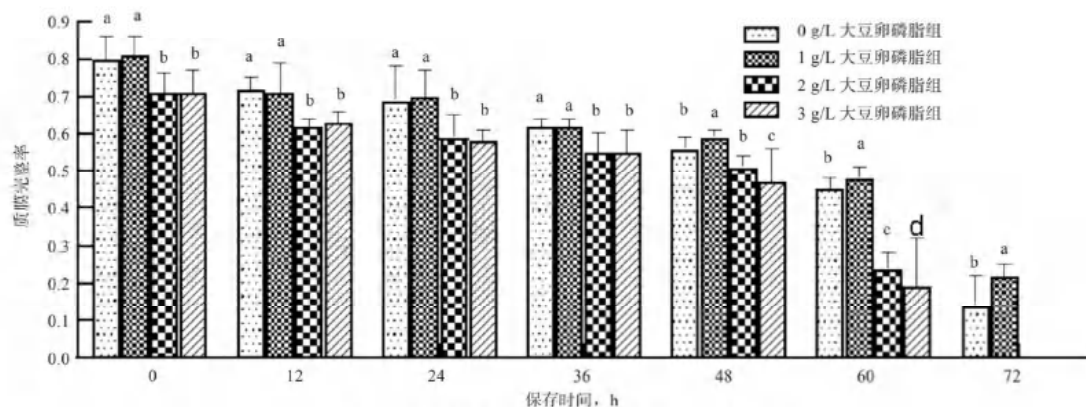


图 9 大豆卵磷脂对山羊精子质膜完整率的影响

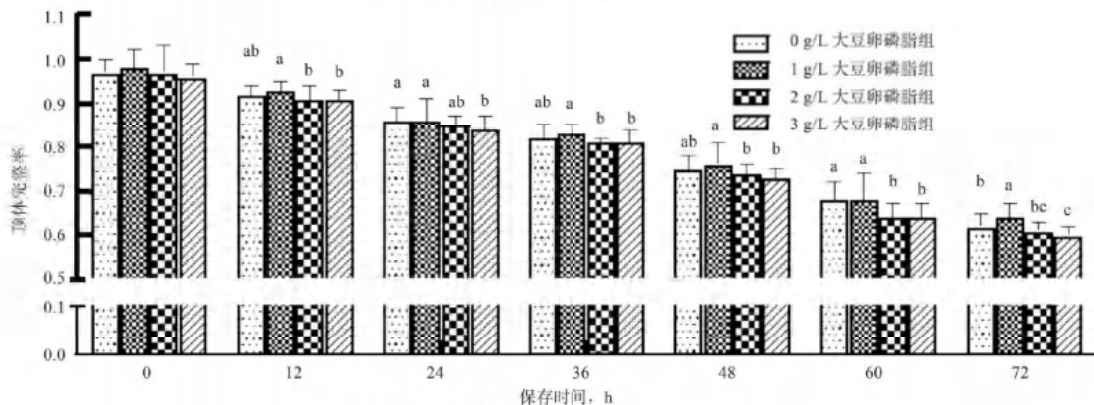


图 10 大豆卵磷脂对山羊精子顶体完整率的影响

糖, 这有待更进一步研究。

常温条件下, 在 3 g/L BSA 与配方 E 配制成的稀释液中, 山羊精子活力的保持状况最好。Naijian 等^[13]研究发现, 在冷冻 Tris 蛋黄稀释剂中加入 10 g/L 或 15 g/L BSA 对于 Mahabadi 山羊精子都有较好的保存效果。Uysal 等^[14]研究发现, 添加 20 g/L BSA 显著提高了冻融 Akkaraman 公羊精子的活力以及顶体与质膜的完整性。Fu 等^[15]研究发现, 在公猪精液中添加 4 g/L BSA 的精子活力比添加 2 g/L BSA 的要高, 但相同浓度的 BSA 和脱脂奶对公猪精液的保护作用没有显著差异。

本研究同样证明, 在精液稀释液中添加适量的 BSA 可延长山羊精子的保存时间, 然而本研究得出的最优添加浓度与文献报导的结果相异, 这可能由基础稀释液配方和实验动物品种之间的差异导致。此外, 添加 4 g/L BSA 组的精子活力和保存时间低于其他组, 说明过高浓度的 BSA 对山羊精子有害。并且结果还显示, 山羊精液稀释液中的适量 BSA 能降低山羊精子质膜氧化损伤并提高其质膜的完整率, 而对山羊精子顶体却没有保护作用。可能是作为抗氧化剂的 BSA 能够降低精液中丙二醛 (MDA) 浓度, 从而减少质膜的过氧化^[16], 并

保持DNA的高度完整^[17]。

本研究结果显示,在羊精液稀释液中添加大豆卵磷脂达到1 g/L时,精子的活力与细胞膜的完整性显著提高,精液的保存时间亦显著增长,但精子顶体完整率相对于其他浓度组并没有显著变化。当稀释液中大豆卵磷脂浓度达到2 g/L或3 g/L时,精子的活力下降增快、质膜完整率下降。栗瑞兰^[18]研究发现,大豆卵磷脂浓度为1.5%的精液稀释液中的内蒙古绒山羊精子DNA完整度最高,2.5%组最低,之间的差异不明显。孟娜娜^[19]研究显示,在4~5℃的保存温度下,稀释液中添加0.375%大豆卵磷脂绵羊精子保存的时效增强效果最好,但是大豆卵磷脂超过一定浓度后,精子的保存时间将随其浓度增加而逐渐变短。这可能是大豆卵磷脂难溶于水,当大豆卵磷脂浓度达到临界值后,其溶解能力变小,使得稀释精液变得黏稠,导致阻力增大,从而表现为精子能量消耗增高增快^[20]。Akhter等^[21]和Singh等^[22]研究认为,过高浓度的卵磷脂将影响精子正常运动,并且对精子的生存性能产生了相当程度的不利影响。世界各地的大量研究都已经证明,在含有大豆卵磷脂的精液稀释液能提高精子的保存效果,但是各研究的具体结论都不尽相同。应基于产品纯度、生产批次、试验动物和精液处理方式等因素的不同进行针对性的调整才能得出具体的最佳添加浓度。

4 结 论

本研究在前人研究基础上比较了不同浓度梯度BSA及大豆卵磷脂对山羊精子的保护作用,并根据其作用优化了山羊稀释液的配方。结果显示,在上述E配方基础稀释液中添加3 g/L BSA、1 g/L大豆卵磷脂的优化稀释液保存山羊精子的效果最佳。因此,每1 L蒸馏水含有葡萄糖19 g、果糖19 g、柠檬酸二钠16 g、EDTA-Na₂ 1.7 g、BSA 3 g或大豆卵磷脂1 g、青霉素钠和硫酸链霉素各100万单位的常温精液稀释液,具有较好的山羊精子保存效果,最适合山羊人工授精使用。

参考文献:

- [1] 赵兴绪. 羊的繁殖调控 [M]. 北京: 中国农业出版社, 2008: 526.
- [2] As Aku N S P S. The effect of phyto-lecithin on preservation and cryopreservation of semen: A review [J]. Anim Prod Sci, 2007, 9(1): 49-52.
- [3] 马青, 李颖康, 贾小梅, 等. 绵羊精液常温保存稀释液筛选 [J]. 宁夏农林科技, 2004(6): 26-27.
- [4] 刘丽清, 周佳勃, 刘娣. BSA在精子获能中的作用及其替代物研究进展 [J]. 动物医学进展, 2005(6): 40-43.
- [5] Osman K, Nang C F, Ibrahim K S F. Albumin Improved spermatozoa quality and DNA integrity for freezing-free preservation [J]. Int Jour Biol Medi Res, 2012, 3(2): 3.
- [6] Layek S S, Mohanty T K, Kumaresan A, et al. Cryopreservation of bull semen: Evolution from egg yolk based to soybean based extenders [J]. Anim Reprod Sci, 2016, 172: 1-9.
- [7] 中华人民共和国国家质量监督检验检疫总局. 山羊冷冻精液 [S]. 北京: 中国国家标准化管理委员会, 2006.
- [8] 薛慧良. 稀释液营养剂和抗菌素对常温保存布尔山羊精液品质和受胎效果的研究 [D]. 太谷: 山西农业大学, 2001.
- [9] 王占赫, 郭玉琴, 周双海, 等. 不同精液稀释液常温保存绵羊精液的效果 [J]. 当代畜牧, 2006(2): 34-36.
- [10] 赵金红, 彭海梅, 周鹏, 等. 酉州乌羊精液品质评定及稀释液配方研究 [J]. 中国农学通报, 2014(20): 6-10.
- [11] 曲宏伟, 铁成, 何云梅, 等. 阿拉善型绒山羊精液稀释液配方的筛选与优化 [J]. 家畜生态学报, 2018(2): 45-49.
- [12] 宣小龙, 李福林. 夏洛莱绵羊精液稀释液冷冻试验 [J]. 草业与畜牧, 2012(8): 36-38.
- [13] Naijian H R, Kohram H, Shahneh A Z, et al. Effects of various concentrations of BSA on microscopic and oxidative parameters of Mahabadi goat semen following the freeze-thaw process [J]. Small Ruminant Res, 2013, 113(2-3): 371-375.
- [14] Uysal O, Bucak M N. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen [J]. Acta Vet Brno, 2007, 76(3): 383-390.
- [15] Fu J, Li Y, Wang L, et al. Bovine serum albumin and skim-milk improve boar sperm motility by enhancing energy metabolism and protein modifications during liquid storage at 17℃ [J]. Theriogenology, 2017, 102: 87-97.
- [16] Banday M N, Lone F A, Rasool F, et al. Use of antioxidants reduce lipid peroxidation and improve quality of crossbred ram sperm during its cryopreservation [J]. Cryobiology, 2017, 74: 25-30.
- [17] Van Thuan N, Wakayama S, Kishigami S, et al. New preservation method for mouse spermatozoa without freezing [J]. Biol Reprod, 2005, 72(2): 444-450.
- [18] 栗瑞兰. 内蒙古绒山羊精液冷冻保存方法优化及精液稀释液主要成份的作用机制研究 [D]. 呼和浩特: 内蒙古农业大学, 2018.
- [19] 孟娜娜. 脱脂奶粉和大豆卵磷脂对绵羊精液低温保存效果的影响 [D]. 保定: 河北农业大学, 2015.
- [20] 金海. 无动物源性水牛精液冷冻保存稀释液的研究 [D]. 武汉: 华中农业大学, 2014.
- [21] Akhter S, Ansari M S, Andrabi S, et al. Soya-lecithin in extender improves the freezability and fertility of buffalo (bubalus bubalis) bull spermatozoa [J]. Reprod Domest Anim, 2012, 47(5): 815-819.
- [22] Singh A K, Singh V K, Narwade B M, et al. Comparative quality assessment of buffalo (bubalus bubalis) semen chilled (5℃) in egg yolk- and soya milk-based extenders [J]. Reprod Domest Anim, 2012, 47(4): 596-600.

(责任编辑: 周会会)

67

1953-2020



《中国畜牧杂志》创刊于1953年，是中国科学技术协会主管、中国畜牧兽医学会主办、中国农业大学为学术支撑的畜牧行业综合指导类期刊，已被全国中文核心期刊数据库、中国科技核心期刊数据库、中国学术期刊综合评价数据库、中国核心期刊（遴选）数据库、维普网等多家数据库收录。

作为中国畜牧人的老朋友，《中国畜牧杂志》一贯秉承严谨、求实的办刊理念，坚持内容为王，始终把学术导向、传播讯息、交流成果作为己任，记录并服务着畜牧产业的发展。我们真诚邀请国内外科研学者、行业精英、优秀企业以《中国畜牧杂志》作为平台，分享您的研究成果和智慧，共同促进行业健康、持续发展。

ISSN 0258-7033
CN11-2083/S

国内邮发代号：82-147

国外发行代号：SM452

定价：35.00元



微信订阅



• 全国中文核心期刊 • 国家首批A类学术期刊
• RCCSE中国学术期刊 (A-) • 国际著名检索系统《化学文摘》(美国) 刊源

ISSN 1004-7034
CN 23-1205/S

黑龙江畜牧兽医

HEILONGJIANG ANIMAL SCIENCE
AND VETERINARY MEDICINE

主管：黑龙江省畜牧兽医局
主办：黑龙江畜牧兽医杂志社

15 **2018**
半月刊



黑龙江畜牧兽医

(2018年第15期 总第555期)

目次

试验研究

- 热灭活大肠杆菌及其活菌对仔猪腹泻率、消化酶活性和脏器指数的影响 李媛媛,郭乾鹏,梁世忠,向明,黄怡(1)
- uPA 对外成熟牛卵母细胞生发泡破裂的影响 段云娇,刘新宇,塔娜,赵玉芬,于泊洋,钱英红,李海军(6)
- HSP27 在热应激致岭南黄鸡雏鸡免疫器官损伤中的作用
..... 阮炜珊,许丹宁,杨舒展,付晶,郭斯璇,曾依翎,黄运茂,田允波,曹楠(11)
- 降解赤霉烯酮芽孢杆菌的筛选、鉴定及性质研究 薛晓华,郭晓军,赵蕊蕊,郭威,张宸毓,朱宝成(16)
- 芥子碱降解菌 1 对昆明小白鼠 LD₅₀ 的研究 余丹,邹成义,李斌,屈东,汪林书(20)
- GC_SBE 元件突变对绵羊 Myomaker 基因 5' 调控区活性的影响 朱自欣,路盟,张枫惠,秦健,李颖靓,杜荣(23)
- 唾液乳杆菌发酵条件的研究 修立颖,韩雪冰,孙远芳,陆金荣,单春乔,江国托,余丽芸(28)

专论与综述

- 卵黄抗体的应用及纯化研究进展 李明亮,冷超粮,宋安东,秦鑫鑫,阎云超(34)
- 原虫感染对宿主免疫应答的影响 赵丽娟,祝学珍,唐磊,秦亚,孙浩杰,蔡亚南(39)
- microRNA 与生殖 单鑫,朱根宝,刘亚(43)
- 白腐真菌处理稻草秸秆饲料的研究进展 张仲卿,张爱忠,姜宁(47)
- 禽类 Toll 样受体抗感染作用研究进展 李孟聪,牛鑫鑫,方红,李靖,曾祥伟(51)
- ALDH1A1 基因研究进展 胡忠昌,曹阳,秦立红,赵玉民(54)
- 细菌生物被膜耐药机制研究进展 刘燕霏,杨建德(57)
- 果园生态养鸡模式的研究进展 王育伟,岳华,周爱民,张晓晖,李廷见,肖龙(60)
- 小鼠腹腔巨噬细胞的分离与鉴定方法研究进展 吕娟容,冯军,陈志胜,池仕红(63)
- 马垂体中间部机能障碍研究进展 吴殿君,周晓菲,刘佳丽,曹永国,张文龙,付云贺,张乃生(65)
- 三氮唑药理作用及其机制研究进展 张超,毕海林,肖裕章,袁启峰,唐建华(69)

畜牧科学

- 不同音乐对奶牛神经递质水平和泌乳性能影响的研究 马小艳,吴雨霏,王东海,余雄(73)
- 胎教音乐、早教音乐和儿童音乐对奶牛部分产奶性能指标的影响
..... 吴雨霏,王东海,马小艳,张宝刚,王卓东,王成成,余雄(77)
- 围生期蒙古牛外周血中性粒细胞 CD11b 表达研究 张新颖,杨晓燕,杜立银(80)
- 不同断奶时间对藏香仔猪生长性能和部分血液生化指标的影响 王玉玲,孙雪,王桂英,李路胜(84)
- 荷斯坦奶牛乳腺干细胞的成脂诱导及鉴定 韩哲先,陈钰萌,刘迎春,高峰(87)
- 嘉积鸭生长性能的测定及相关分析 顾丽红,徐铁山,刘志勇,林大捷,杨少雄,叶保国,邢漫萍,林哲敏(89)
- 郧巴黄牛体重和体尺性状的相关及回归分析 索效军,张年,杨前平,陶虎,熊琪,李晓锋,陈明新(94)
- 中国荷斯坦奶牛 PRL 基因第 3 外显子多态性对牛乳腺上皮细胞增殖的影响 侯佳妮,牛淑玲,刘春杰,徐晶(97)
- 杂交肉牛背最长肌 PPAR γ 基因与脂肪代谢的研究 王小梅,敖日格乐,王纯洁(101)
- 英纯血马运动 2 000 m 前后生理生化指标的研究 张小宇,文强,范奎奎,任宏,赵宇飞,王勇,郭永清(103)

(期刊基本参数) CN 23-1205/S* 1958* s* A₄* 245* zh* P* ¥ 40.00* 2650* 67* 2018-15



黑龙江畜牧兽医

(2018年第15期 总第555期)

目次

湖羊和川中黑山羊 GDF9、BMPR-1B、CaRIIR 基因多态性及其与产羔数的关联分析

杨新月,周多恩,李斌,李耀坤,刘德武,孙宝丽,柳广斌(106)

负离子复合制剂对冷藏期猪肉微生物数量及 pH 值的影响 王震,乔鹏飞,王传龙,张敏(110)

瑶山鸡慢羽羽型与出壳体重相关性研究

林家栋,张福平,龚俞,李雪松,张游宇,任丽群,李洪林,祖盘玉,吴立鑫,李维(114)

能量限饲对肉种鸡肉品质的影响 李佳凝,刘洋,刘大伟,王超,曹淑鑫,陈盈霖,徐良梅(116)

兽医科学

马梨形虫病双重 PCR 检测方法的建立 瓦热斯·吐尔松,王振宝,阿兵,宋瑞琪,刘世芳,闻秀秀,巴音查汗(120)

仔猪粪便中大肠杆菌 O₁₅₇:H₇ 的分离鉴定及敏感药物筛选 薛力刚,孙亮,王丹(124)

鲫鱼金黄杆菌的分离、生理生化鉴定及分子生物学鉴定 李晨阳,王利,吴开年(127)

2015—2016 年黑龙江三个地区猫冠状病毒 N 基因分子流行病学调查 刘秋瑾,孙东波,王欣宇,李春秋,武瑞,郭东华(130)

传染性法氏囊病病毒 RT-LAMP 检测方法的建立 陆冰洋,刘华栋,李婷婷,唐娟,丁树荣,王彩先,詹丽娥(135)

小鹅瘟病毒 PCR 检测条件的优化 童艳梅,李娴妍,陈义旺,马晓强,顾江凤,杨梦琪,刘雨,杨晓伟,赵光伟(139)

三种检测多杀性巴氏杆菌毒素灭活方法的研究 郝成武,何海,韩瑞,贺笋(141)

一株鸡传染性支气管炎病毒的分子流行病学研究 谢景伟,刘金,覃建萍,陈峰(144)

饲草、饲料与添加剂

扎龙湿地野大麦对 NaCl 胁迫的生理响应 焦德志,宋士伟(147)

不同处理对新疆大叶紫花苜蓿种子萌发的影响

钟雯,唐明祥,张晓君,沈景林,王鹏,伊鹏霏,包国章,张晶,丁雪梅(151)

黑麦、青稞的生产性能及营养价值综合评价 何鸿源,李清,毛华明,陈艳美,金贞,孙志海(156)

2-甲基丁酸和纤维素酶对泌乳牛生产性能的影响 周胜花,宋献艺(160)

动物保健品研究

兽用生物制品中支原体污染 PCR 检测方法的建立与应用 高广仁,万玉林,崔治亮,葛玉凤,刘学荣(163)

Bacillus subtilis S21 固态发酵黄荆子微生态制剂工艺及成分变化研究 胡美忠,莫正昌,郁建生(167)

浅析银黄可溶性粉和板蓝根颗粒对 PRRS 的防治效果 李慧,颜久武,张子荣,张莉,齐亚银(171)

磺胺二甲嘧啶的毒性预测 武英豪,程林丽,刘莹莹,胡焯(175)

24 味中药水提物对宠物犬源致病性大肠杆菌 NJ-1 株体外抑菌试验研究 白建,曹靖,王汝都,薛建娥(177)

特种动物研究

黄河三角洲国家级自然保护区越冬鹤类调查及受胁因素分析 刘昌景,周用武(180)

DNA 分类和形态分类共同界定一只草海死亡鸟类残体 王野影,孙喜娇,熊勇,张明明,栗海军(184)

四川白兔种质性能测定研究 李思聪,李金良,杨锐,李旭廷,张翔宇,郭志强,肖嵩杨,王吴燕(188)

大宁河云南盘幽的胚胎发育初步研究 周燕,蒲德成,邓星星,王建,周朝伟,郑宗林,朱成科(192)

黑化雄鸡体尺性状和屠宰性能测定及相关性分析 吴琼,宁浩然,宋超,邢秀梅(195)

不同饵料投喂对南美白对虾亲虾性腺成熟及 E75 基因表达的影响 吕峰,巩杰,倪建忠,张跃群,张敏,陆德祥(198)

凡杂志有印刷不清、破损、倒装、错装、脱页等问题,请寄回本社,以便与印刷厂联系调换

黑龙江畜牧兽医



(2018年第15期 总第555期)

目次

- Cd^{2+} 对黄河鲤鱼肝胰脏抗氧化酶活性的影响 王 凡,孙欢欢,黄琼波,刘海芳(200)
- 硫酸铜和硫酸亚铁(5:2)合剂对黄河鲤鱼的急性毒性试验 林 伟,杨治国,杨东辉(203)

实验室检测

- 畜禽肠道组织 H.E.染色石蜡切片制作要点及关键技术 刘慧军,杨海明,胥 蕾,张李荣,王志跃(206)
- 三种免疫学检测方法对呋喃唑酮代谢物的检测 何方洋,彭庆军,吴小胜,郑迎春,江雪梅,冯月君,赵 颖,张 颖(210)
- 苯酚/氯仿抽提法提取牛血与鸡血 DNA 部分条件的比较研究
..... 母 童,陈佳萍,赵 平,顾亚玲,冯小芳,陈亚飞,刘丽元,蒋秋斐,张 娟(214)
- 高效液相色谱法测定兽药樟脑磺酸钠注射液的含量 张人俊,陈锡龙,孙真峥,王庆红(217)

教育与教学

- 野生动物与自然保护专业“3S 技术与应用”课程的教学改革 刘 畅,段玉宝(220)
- 多导师制“创业基础”课程教学模式改革探究 刘少宝,田起香(222)
- 高校动物医学专业课堂教学有效性提升的探索 于蕾妍,吕 浩,邹本革,王海娟,李 爱,李华涛(226)
- 高等农业院校青年教师素质提升途径研究 赵洪亮,刘 江,谢立勇,李建东,衣 莹(228)
- 省级在线开放课程“兽医寄生虫病学”的建设 殷光文,杜金苓,马玉芳,廖吕燕,江和基,黄志坚(230)
- 我国高校“双一流”建设研究 杨应红(233)

- 彩图 (238)

信息

- 华诞景慕——贺《黑龙江畜牧兽医》创刊六十周年 (50)
- S8(除 S812)畜牧、动物医学、狩猎、蚕、蜂(除草地学、草原学)类核心期刊表 (134)

广告发布单位

河北威远动物药业有限公司

版权声明

《黑龙江畜牧兽医》(以下简称“本刊”)已许可《中国学术期刊(光盘版)》电子杂志社有限公司以数字化方式复制、汇编、发行、信息网络传播本刊全文。本刊稿酬中含有中国知网的使用费,不再另行支付。作者向本刊提交文章发表的行为即视为同意黑龙江畜牧兽医杂志社的上述声明。如作者不同意文章被中国知网的电子方式收录,请在来稿时向本刊杂志社声明,本刊杂志社将做适当处理。

本刊部分文章已经在中国知网以“网络首发”形式出版,网络首发出版文章的页码可能与纸质样刊不一致;文章中的彩色图片在纸质样刊正文后统一彩色排版,与数字化排版时页码也不一致,特此告之。



湖羊和川中黑山羊 GDF9、BMPR-IB、GnRHR 基因多态性及其与产羔数的关联分析

杨新月¹, 周多恩², 李 斌¹, 李耀坤¹, 刘德武¹, 孙宝丽¹, 柳广斌^{1,2*}

(1. 华南农业大学 动物科学学院, 广州 510642; 2. 新兴县温氏新旺羊业有限公司 广东 云浮 527300)

中图分类号: S826.2

文献标识码: B

文章编号: 1004-7034(2018)15-0106-05

摘 要: 为了研究湖羊和川中黑山羊生长分化因子 9 (GDF9) 基因、骨形态蛋白受体 (BMPR-IB) 基因和促性腺激素释放激素受体 (GnRHR) 基因多态性及其与产羔数之间的关系, 试验以 594 只湖羊和 333 只川中黑山羊为研究对象, 用 PCR-RFLP 技术分析了湖羊和川中黑山羊 BMPR-IB、GnRHR、GDF9 基因序列的多态性, 并与产羔数进行关联分析。结果表明: 在湖羊和川中黑山羊中均未检测到 GDF9 基因的 G8 突变, 说明该突变在这两种羊中比较保守, 不存在多态性; 湖羊 BMPR-IB 基因存在 FecB 突变, 表现出三种基因型 (GG、GA、AA), 等位基因 G 比等位基因 A 频率高, G 为优势等位基因, 基因型分布偏离了 Hardy-Weinberg 平衡, GG 型平均产羔数分别比 GA 型和 AA 型多 0.19、0.33 只 ($P>0.05$); 川中黑山羊 GnRHR 基因存在 G698A 突变, 表现出三种基因型 (GG、GA、AA), 达到了 Hardy-Weinberg 平衡, 不同基因型个体间的产羔数差异不显著 ($P>0.05$)。

关键词: 湖羊; 川中黑山羊; GDF9; BMPR-IB; GnRHR

产羔数是山羊和绵羊最重要的繁殖性状, 受基因和环境等因素的影响, 是影响肉羊产业发展的主要因素。分子标记辅助选择 (MAS) 可弥补常规表型选择的不足, 找到与这些数量性状基因座相关联的分子遗传标记是实现 MAS 的先决条件。

生长分化因子 9 (growth differentiation factor 9, GDF9) 是由卵母细胞分泌, 对卵泡各阶段的生长发育起着重要调节作用。J. P. Hanrahan 等^[1]发现, G8 (又称 FecG^{II}) 突变是编码区第 1 184 bp 处发生了 C→T 碱基突变, 致使编码的第 395 位丝氨酸突变为苯丙氨酸, 该突变杂合子在 Belclare 绵羊和 Cambridge 绵羊上均表现出排卵数显著增加, 分别增加了 (1.79±0.55) 枚、(2.35±0.39) 枚, 所以认为 GDF9 基因可能是影响绵羊高繁殖力的主效基因。目前, 对 GDF9 基因的研究比较多, 而在山羊上的研究相对较少。骨形态蛋白受体 (Bone morphogenetic protein receptor IB, BMPR-IB) 对雌性动物维持正常繁殖具有重要的调节作用。绵羊

BMPR-IB 基因方面的研究较多, 其中 FecB 突变对繁殖的影响比较大, 该突变是 BMPR-IB 基因 746 bp 处 A→G 突变, 导致氨基酸序列的第 249 位谷氨酸 (Glu) 残基转变为精氨酸 (Arg) 残基。孙红霞^[2]研究发现, 滩羊和小尾寒羊 BMPR-IB 基因编码序列第 746 位相邻两处碱基发生了与 Booroola 绵羊相同的突变 (A746G), 该位点分别处于中度多态和高度多态。促性腺激素释放激素受体 (GnRHR) 在生殖功能的调节中起着重要作用。阿布来提·苏来曼^[3]采用 PCR-SSCP 方法对多浪羊 GnRHR 外显子 1 进行基因多态性分析, 结果只在多浪羊 (多胎群体) 中检测到三种基因型 (AA、AB 和 BB), BB 基因型的平均产羔数分别比 AA 和 AB 基因型增加 0.65 只和 0.76 只, 说明 GnRHR 基因对多浪羊的高繁殖性状有显著影响。

本试验以湖羊和川中黑山羊为研究对象, 分析了湖羊和川中黑山羊 GDF9、BMPR-IB、GnRHR 基因的多态性, 并与产羔数进行关联分析, 以期从分子水平筛选出具有高繁殖力的湖羊和川中黑山羊群体, 为进一步筛选出湖羊和川中黑山羊的高繁分子标记基因提供理论依据, 现报道如下。

1 材料与方法

1.1 试验动物

湖羊和川中黑山羊, 均来自广东云浮某羊场, 全年实行高床舍饲。选择湖羊母羊 594 只、川中黑山羊

收稿日期: 2017-08-26; 修回日期: 2017-09-25

基金项目: 广东省公益研究与能力建设项目 (2015A020209122)

作者简介: 杨新月 (1993—), 女, 硕士研究生, 研究方向为动物遗传育种与繁殖, 798975224@qq.com。

* 通信作者: 柳广斌 (1982—), 男, 讲师, 博士, 研究方向为动物遗传育种与繁殖, gblu@suau.edu.cn。

母羊 333 只,颈静脉采血 2 mL,进行抗凝处理,于-20℃保存,备用。

1.2 血液基因组 DNA 的提取

采用血液基因组 DNA 提取试剂盒(购自天根生化科技有限公司)提取血液基因组 DNA,以 1% 琼脂糖凝胶电泳检测,并用紫外分光光度计检测 DNA 浓

度与纯度, OD_{260}/OD_{280} 1.8~2.0,合格,可用于后续试验,所得 DNA 样品于-20℃保存。

1.3 引物的设计

参照参考文献[4-6]设计 3 对引物,引物由苏州金唯智生物科技有限公司合成。引物为-20℃保存的干粉剂,使用前稀释,备用。引物序列见表 1。

表 1 扩增引物及相关信息

基因	引物序列	SNP 位点	突变碱基	大小/bp	退火温度/℃	内切酶
BMPR-IB	F 5'-CTCGCTATCGGGAAGTTTGGATG-3'	A746G	G>A	140	62	Ava II
	R 5'-CAAGATGTTTTTCATGCCTCATCAACAGGTC-3'					
GDF9	F 5'-CTTTAGTCAGCTGAAGTGGGACAAC-3'	C1184T	T>C	139	62	Dde I
	R 5'-ATGGATGATGTTCTGCACCATGGTGTGAACCTGA-3'					
GnRHR	F 5'-TCTTGAAGCTGTATCAGCCATA-3'	G698A	A>G	187	56	Msp I
	R 5'-GTGTTGAAAATTGTGGAGAGTAGA-3'					

1.4 PCR 扩增

反应体系(10 μL):基因组 DNA 模板 1 μL,上、下游引物各 0.2 μL,5 μL Taq DNA 聚合酶,以去离子双蒸水补充至终体积。

反应程序:94℃预变性 2 min;94℃变性 30 s,不同温度(因不同引物而异,见表 1)退火 30 s,72℃延伸 30 s,共 35 个循环;72℃延伸 5 min,4℃保存。

1.5 酶切反应

PCR 反应结束后即进行候选基因扩增产物的酶切。酶切体系:10×Buffer 1 μL,双蒸水 8.5 μL,10 U/μL 限制性内切酶 0.5 μL,PCR 反应产物 5 μL。振荡混匀,37℃水浴 30 min。酶切产物用 3% 琼脂糖凝胶电泳分型。

1.6 多态性分析及与产羔性的关联分析

用 PCR-RFLP 技术分析湖羊和川中黑山羊 BMPR-IB、GnRHR、GDF9 基因序列的多态性,并与产羔数进行关联分析。

1.7 数据的统计分析

试验数据采用 Excel 2003 软件进行初步处理,用 SPSS 20.0 软件的 GLM(General Linear Model)程序配合构建的单位点效应模型对各 SNP 位点进行统计分析,并采用 LSD 和 Dunnett's T3 法进行多重比较。

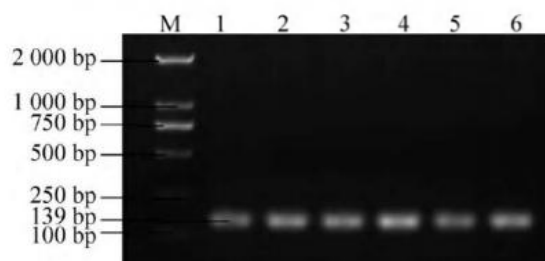
配合下列模型进行最小二乘方差分析: $y_{jkl} = \mu + P_j + G_k + e_{jkl}$ 。式中: y_{jkl} 为产羔数记录值, μ 为群体平均值, P_j 为第 j 个胎次的固定效应, G_k 为第 k 种基因型的固定效应, e_{jkl} 为随机残差效应。

2 结果与分析

2.1 PCR 扩增结果

样品的 OD_{260}/OD_{280} 在 1.8~2.0 之间,可用于后续试验。用所设计的引物对获得的 DNA 样本进行 PCR 扩增,所得产物经 3% 琼脂糖凝胶电泳检测。结果显示,各对引物扩增片段均与目的片段长度一致且特异性好,可直接进行后续试验。

2.2 湖羊和川中黑山羊 GDF9 基因多态性分析结果见图 1。

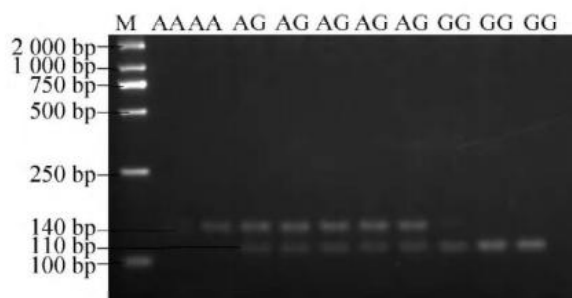


M, DL-2 000 Marker; 1~3, 湖羊; 4~6, 川中黑山羊。

图 1 湖羊和川中黑山羊 GDF9 基因分型结果

由图 1 可见:湖羊和川中黑山羊 GDF9 基因 PCR 扩增片段经 Dde I 内切酶酶切后仅有 1 种条带(139 bp),说明湖羊和川中黑山羊群体中可能均不存在 G8 突变。

2.3 湖羊和川中黑山羊 BMPR-IB 基因多态性分析结果见图 2、图 3。



M, DL-2 000 Marker。

图 2 湖羊 BMPR-IB 基因分型结果

湖羊和川中黑山羊 BMPR-IB 基因的 PCR 扩增片段经 Ava II 内切酶酶切后,湖羊群体中出现了 140, 110, 30 bp 3 种条带(因 30 bp 条带太弱,凝胶图片上没有显示),表现出三种基因型,即 AA(140 bp)、AG(140, 110 bp)、GG(110 bp);川中黑山羊群体中仅出

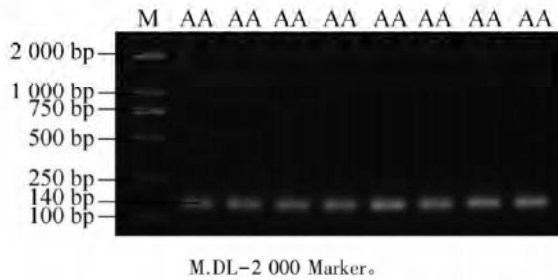


图3 川中黑山羊 BMPR-1B 基因分型结果

现 1 条 140 bp 条带,说明川中黑山羊群体中可能不存在 FecB 突变。

2.4 湖羊和川中黑山羊 GnRHR 基因多态性分析 结果见图 4、图 5。

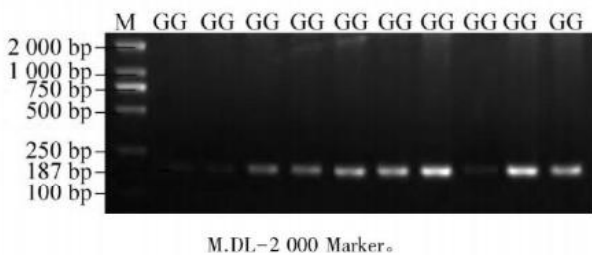


图4 湖羊 GnRHR 基因分型结果



图5 川中黑山羊 GnRHR 基因分型结果

由图 4、图 5 可见: GnRHR 基因的 PCR 扩增片段经 Msp I 内切酶酶切后,湖羊样本只出现 1 种条带,说明湖羊 GnRHR 基因不存在 A698G 突变;川中黑山羊样本出现 3 种条带,表现出 GG、AG 和 AA 3 种基因型。

2.5 高繁殖力候选基因的遗传多态性分析

BMPR-1B 和 GnRHR 基因的遗传多态参数见表 2。

由表 2 可见:湖羊 BMPR-1B 基因在 A746G 位点存在等位基因 G 和 A,G 基因频率较高,为优势等位基因;GG 型个体数远多于 AA 型和 GA 型个体数,GG 型为优势基因型。卡方检验结果显示,湖羊的基因型

表2 BMPR-1B 和 GnRHR 基因的遗传多态参数

基因	品种	基因频率		基因型频率			χ^2	多态信息含量 (PIC)	纯合度 (Ho)	杂合度 (He)	有效等位基因数 (Ne)
		A	G	AA	AG	GG					
BMPR-1B	湖羊(594)	0.053 0	0.947 0	0.002 8(15)	0.100 4(33)	0.896 8(546)	118.74	0.095 3	0.899 6	0.100 4	1.111 6
GnRHR	川中黑山羊(333)	0.504 5	0.495 5	0.254 5(93)	0.500 0(150)	0.245 5(90)	3.50	0.375 0	0.500 0	0.500 0	2.000 0

注:括号内数字表示该基因型的个体数 $df=2$ $\chi^2_{0.05(2)}=5.99$ $\chi^2_{0.01(2)}=9.21$ 。PIC>0.5 为高度多态;0.25<PIC≤0.5 为中度多态;PIC≤0.25 为低度多态。

分布偏离 Hardy-Weinberg 平衡,差异极显著 ($P<0.01$),说明湖羊中 BMPR-1B 基因处于非自然平衡状态,这或许是人们长期对 BMPR-1B 基因相关联的某一性状进行人为选育的结果。此位点的多态信息含量为 0.095 3,属于低度多态;纯合度较高,为 0.899 6;杂合度为 0.100 4,与纯合度相差较大;有效等位基因数为 1.111 6,与检测到的等位基因数差异较大,说明这两个等位基因在该群体中分布不均匀。

川中黑山羊 GnRHR 基因在 G698A 位点存在等位基因 G 和 A,且 AG 型个体数远多于 AA 型和 GG 型个体数。卡方检验结果显示,川中黑山羊的基因型分布符合 Hardy-Weinberg 平衡定律,差异不显著 ($P>0.05$)。此位点的多态信息含量为 0.375 0,属于中度多态;纯合度和杂合度均为 0.500 0;有效等位基因数为 2.000 0,与检测到的等位基因数一致,说明这两个等位基因在该群体中分布均匀。

2.6 高繁殖力候选基因与产羔数的关联分析

2.6.1 BMPR-1B 基因与湖羊产羔数关联分析 见表 3。

表3 BMPR-1B 不同基因型与湖羊

基因型	产羔数关联分析	
	样本数	产羔数
AA	15	1.50 ^a ±0.51
GA	33	1.64 ^a ±0.63
GG	546	1.83 ^a ±0.71

注:同列数据肩标小写字母相同表示差异不显著 ($P>0.05$)。

由表 3 可见,不同基因型个体间的产羔数差异不显著 ($P>0.05$),GG 型平均产羔数分别比 GA 型和 AA 型多 0.19 和 0.33 只。说明 FecB 突变并不是影响湖羊品种内产羔率差异的主要因素,也可能与 AA 型和 GA 型湖羊个体数较少有关。

2.6.2 GnRHR 基因与川中黑山羊产羔数关联分析 对川中黑山羊 GnRHR 基因 G698A 位点的多态性与产羔数进行最小二乘分析,结果见表 4。

由表 4 可见,基因型效应对产羔数的影响不显著 ($P>0.05$),因此推断, GnRHR 基因 G698A 突变不是影响川中黑山羊高繁殖力的多态位点。

表4 GnRHR 不同基因型与川中黑山羊

产羔数关联分析

只

基因型	样本数	产羔数
GG	90	1.81 ^a ±0.66
AG	150	1.85 ^a ±0.67
AA	93	1.84 ^a ±0.70

注:同列数据肩标小写字母相同表示差异不显著($P>0.05$)。

3 讨论

3.1 候选基因的遗传多态性分析

基因频率和基因型频率是群体的遗传结构,可以表现群体某一性状的基本遗传特征。所有随机交配的群体均应适用于 Hardy-Weinberg 平衡,但实际生产中会受到一些因素的干扰,例如选择性交配、人工选择、自然选择、近亲交配、群体再分和自然选择等,因此某一特定群体内的基因型频率并不一定遵循 Hardy-Weinberg 平衡。本试验中,湖羊在 *FecB* 位点出现了 Hardy-Weinberg 平衡偏离,分析可能是湖羊生活的环境发生了改变或其在培育过程中受到了人为选择造成的。

3.2 候选基因与产羔数的相关性分析

Belclare 绵羊和 Cambridge 绵羊的 *G8* 突变,导致其排卵数量明显增加,说明 *GDF9* 基因可以作为绵羊的高繁殖力候选基因^[1]。管峰等^[5]在 310 只湖羊中检测到了 *G8* 突变,但发生率极低,仅为 0.645%。孟丽娜^[6]在美姑黑山羊等 4 个山羊品种中也对该突变进行了研究,但均未检测到 *G8* 突变。姬云涛^[7]和汤继顺等^[8]分别对济宁青山羊和安徽白山羊 *GDF9* 基因的编码区进行遗传多态性检测,结果均存在 SNPs,且与产羔数密切相关,说明该基因可能是影响济宁青山羊和安徽白山羊产羔数的一个主效基因。本试验中,在湖羊和川中黑山羊均未检测到 *G8* 突变,说明 *GDF9* 基因比较保守,但不能说明 *GDF9* 基因与湖羊和川中黑山羊的高繁殖力无关,可能在该基因的其他部位存在影响湖羊和川中黑山羊高繁殖力的多态位点;也可能是试验所选群体样本量偏小或样本群分布不够广泛,致使没有检测到 *G8* 突变。因此,后期可对湖羊和川中黑山羊 *GDF9* 基因序列的其他位点进行多态性分析,也可适当扩大样本群和样本分布范围做进一步筛查。

FecB 是在 Booroola 羊中最早被发现的高繁殖力主效基因^[9],随后的研究表明,*FecB* 基因实际为 *BMPR-1B* 基因^[10-11],在所有的湖羊群体中均能检出该基因的 BB(突变纯合)基因型,且证实湖羊经选育后产羔率显著提高。G. H. Davis 等^[9]报道,在 Booroola 羊中,B+母羊的平均排卵数比 B++多 1.24 枚,平均产羔数则多 0.69 只。在本试验中,将湖羊 *FecB* 基因的不同基因型与产羔数进行关联分

析,结果表明,基因型效应对产羔数影响显著,但不同基因型个体间的产羔数差异不显著,说明 *FecB* 基因并不是影响湖羊品种内产羔率差异的主效基因,这与管峰等^[5]的研究结果一致。当然这也说明湖羊产羔率的差异还可能受其他基因或因素的影响;也可能与 GA、AA 型个体数较少有关,后期可加大样本群,做进一步的验证。此外,本试验中川中黑山羊在此位点不存在多态性,说明 *FecB* 基因对川中黑山羊的高繁殖力没有影响,这与方鸿滨等^[12]的研究结果一致。

GnRHR 是一种高亲和 G 蛋白耦联受体,它和 GnRH 结合后,可调控哺乳动物的繁殖性能^[13],因而在寻找影响动物高繁殖力候选基因的研究中,GnRHR 基因也备受研究者的关注。张育军等^[14]的研究表明,黄淮山羊和波尔山羊均在 GnRHR 基因外显子 1 的 58 bp 处发生了 A→C 突变,导致编码的氨基酸由赖氨酸改为谷氨酰胺,且在黄淮山羊中突变纯合型(BB)个体比野生型(AA)个体平均产羔数多 0.66 只。本试验结果表明,川中黑山羊也存在 G698A 突变,表现出三种基因型(GG、AG、AA),但不同基因型个体间的产羔数差异不显著,这可能是所选择的样本群太少或样本群分布不够广泛所致,推测 GnRHR 基因 G698A 突变可能不是影响川中黑山羊多胎性的一个多态位点。此外,本试验中湖羊在 GnRHR 基因 G698A 位点处不存在多态性,与王琼^[15]的研究结果一致,说明 GnRHR 基因不能作为湖羊多胎性状的主效基因,在湖羊的选育工作中可以不予考虑。

4 结论

在湖羊和川中黑山羊中均不存在 *GDF9* 基因的 *G8* 突变,说明该突变在这两种羊中比较保守,均不存在多态性。川中黑山羊 *BMPR-1B* 基因不存在 *FecB* 突变;而在湖羊中存在此突变,并表现出三种基因型(GG、GA、AA),其中等位基因 G 比等位基因 A 频率高,G 为优势等位基因,基因型分布偏离了 Hardy-Weinberg 平衡,GG 型平均产羔数分别比 GA 型和 AA 型多 0.19 只和 0.33 只,但差异不显著($P>0.05$),说明 *FecB* 基因并不是影响湖羊品种内产羔率差异的主基因。在湖羊 GnRHR 基因中未检测到 G698A 突变;而川中黑山羊在此位点存在多态性,表现出三种基因型(GG、GA、AA),达到了 Hardy-Weinberg 平衡,但不同基因型个体间的产羔数差异不显著($P>0.05$),说明 G698A 突变可能不是影响川中黑山羊多胎性的一个多态位点。

参考文献:

- [1] HANRAHAN J P, GREGAN S M, MULSAN T P et al. Mutations in the genes for oocyte-derived growth factors *GDF9* and *BMP15* are associated with both increased ovulation rate and sterility in cambridge and belclare sheep (*Ovis aries*) [J]. Biol Reprod, 2004, 70 (4): 900-909.



负离子复合制剂对冷藏期猪肉微生物数量及 pH 值的影响

王 震, 乔鹏飞, 王传龙, 张 敏*

(延边大学 农学院动物科学系, 吉林 延吉 133000)

中图分类号: S879.2

文献标识码: B

文章编号: 1004-7034(2018) 15-0110-04

摘 要: 为了研究负离子复合制剂对冷藏期猪肉微生物、pH 值和硒含量、锗含量的影响, 试验将 60 头 45 日龄左右的健康杜长大三元杂交仔猪(公母各半, 公猪去势) 随机分为 2 组, 对照组饲喂基础日粮, 试验组饲喂基础日粮+0.25% 负离子复合制剂, 生长到 (115.0±4.5) kg 进行屠宰, 屠宰后采集前腿、里脊和后腿肉, 测定微生物数量、pH 值、硒含量、锗含量。结果表明: 试验组有害菌数少于对照组; 在贮藏期间不同部位猪肉 pH 值均呈上升趋势, 但试验组上升速度低于对照组; 试验组猪肉硒、锗含量显著高于对照组 ($P<0.05$)。说明日粮中添加负离子复合制剂可抑制冷藏期间猪肉 pH 值的增长和猪肉微生物数量, 减缓猪肉腐败速度; 还能够提高猪肉中硒、锗含量, 改善猪肉品质。

关键词: 负离子复合制剂; 冷藏期; 硒锗; 猪肉 pH 值; 微生物数量

硒、锗是人体必需的微量元素, 具有独特的生物活性和功能^[1]。适当补充硒、锗可提高人体免疫力、

预防肿瘤和心血管疾病的发生^[2]。猪肉营养丰富, 含有丰富的蛋白质、人体所需必需氨基酸及钙、铁、磷及多种维生素等, 是一种性价比非常高的肉类食品^[3]。冷藏肉在卫生、营养及口感方面均优于热鲜肉, 因而许多肉类加工企业将其列为主打产品^[4]。从肉类消费结构可知, 冷藏肉在肉类消费中占有非常大的比重。猪肉在冷藏过程中会受到微生物的污染, 冷藏过程中微生物数量变化也受到人们的关注。试验将负离子复合制剂添加到育肥猪日粮中, 研究其对猪肉营养物质和冷藏猪肉微生物数量的影响, 现报道如下。

收稿日期: 2017-10-31; 修回日期: 2018-01-20

基金项目: 吉林省重点专项科技研发项目“肉猪无抗系列饲料的研发与应用”(201802074NY)

作者简介: 王 震(1993—), 男, 硕士研究生, 研究方向为动物营养与饲料科学, 1056352492@qq.com。

* 通信作者: 张 敏(1963—), 女, 教授, 硕士, 硕士生导师, 研究方向为饲料添加剂与畜产品质量调控, 276129497@qq.com。

- [2] 孙红霞. 3 个绵羊品种 BMPR-IB、BMP15、GDF9 和 INHA 基因多态性及与产羔数的相关性 [D]. 杨凌: 西北农林科技大学, 2009.
- [3] 阿布来提·苏来曼. 多浪羊繁殖性状相关的几个候选基因的多态性研究 [D]. 乌鲁木齐: 新疆农业大学, 2012.
- [4] 王启贵, 钟发刚, 李辉, 等. 绵羊 BMPR-IB 基因多态性与其产羔数的相关研究 [J]. 草食家畜, 2003, 119(2): 20-23.
- [5] 管峰, 艾君涛, 庞训胜, 等. 绵羊 GDF9 和 BMP15 基因多态性检测 [J]. 生命科学研究, 2005, 9(2): 184-188.
- [6] 孟丽娜. 5 个候选基因在 4 个山羊品种的多态性分析 [D]. 保定: 河北农业大学, 2014.
- [7] 姬云涛. 山羊多胎性状主要候选基因及其表达量研究 [D]. 杨凌: 西北农林科技大学, 2007.
- [8] 汤继顺, 章薇, 朱德建, 等. 安徽白山羊 GDF9 基因群体遗传效应分析 [J]. 中国草食动物科学, 2016, 36(6): 1-5, 74.
- [9] DAVIS G H, MONTGOMERY G W, ALLISON A J, et al. Segregation of a major gene influencing fecundity in progeny of booroola sheep [J]. New Zeal J Agr Res, 1982, 25(4): 525-529.
- [10] WILSON T, WU X Y, JUENGEL J L, et al. Highly prolific booroola

sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein 1B receptor (ALK-6) that is expressed in both oocytes and granulosa cells [J]. Biol Reprod, 2001, 64(4): 1225-1235.

- [11] SOUZA C J, MACDOUGALL C, MACDOUGALL C, et al. The booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene [J]. J Endocrinol, 2001, 169(2): 1-6.
- [12] 方鸿滨, 王永, 序向东, 等. BMP15 基因和 BMPR-IB 基因与乐至黑山羊繁殖性状之间关系的初步研究 [J]. 西南民族大学学报 (自然科学版), 2010, 36(2): 206-209.
- [13] 胡敏兰. 山羊 GnRHR 和 ESR 基因多态性及其与繁殖力的关联分析 [D]. 合肥: 安徽农业大学, 2009.
- [14] 张育军, 胡敏兰, 张子军, 等. 山羊 GnRHR 基因部分序列多态性及其与产羔数的关联性分析 [J]. 中国草食动物科学, 2010, 30(4): 13-16.
- [15] 王琼. 新疆三个绵羊品种高繁殖力候选基因的遗传多态性及其表达量研究 [D]. 乌鲁木齐: 新疆农业大学, 2013. (010)



微信平台



QQ群

ISSN 1004-7034



9 771004 703181

1 5>



• 全国中文核心期刊 • 国家首批A类学术期刊
• RCCSE中国学术期刊 (A-) • 国际著名检索系统《化学文摘》(美国)刊源

ISSN 1004-7034
CN 23-1205/S

黑龙江畜牧兽医

HEILONGJIANG ANIMAL SCIENCE
AND VETERINARY MEDICINE

主管：黑龙江省畜牧兽医局
主办：黑龙江畜牧兽医杂志社

03 2018
半月刊



黑龙江畜牧兽医

(2018年第03期 总第543期)

目次

试验研究

步态评分对泌乳性能、体况评分和体细胞的影响

杨影,吴帆,师睿,张海亮,宋朗,王晔,罗汉鹏,徐伟,郭刚,王雅春(1)

玉米赤霉烯酮致大鼠睾丸支持细胞氧化损伤及NAC的保护效应

郭宏元,薛画眉,王光光,邹辉,袁燕,顾建红,刘学忠,刘宗平,卞建春(5)

MYL2基因在肌肉生长过程中的研究

刘瑞莉,吴磊,袁玮,董雅娟(10)

山羊痘病毒毒力因子KLPI的表达及多克隆抗体的制备

陈宏伟,刘涛,宋书婷,李金娜,焦海宏,胡建军,李有文(15)

不同条件处理的氧化玉米秸秆对体外瘤胃发酵的影响

张勤,姜雨轩,于满满,张美美,赵新德,谢小来(20)

鲤鱼IgM的分离纯化及其抗血清的制备

姜娜,邢薇,罗琳,李铁梁,李文通,刘彩霞,袁丁,徐冠玲,马志宏(24)

河流型与沼泽型水牛FSHR基因密码子偏好性分析

周芳廷,张永云,钱林东,李卫真,袁峰,滕晓红,苗永旺(28)

驯鹿Chrelin基因组DNA克隆及序列分析

赵琪,王哲,张曼,刘骄,蒲星宇,王晨映,杨银凤(35)

奶牛乳房炎血清学相关指标的变化

葛影影,王根林,张震,薛永康(39)

专论与综述

细菌生物被膜形成的影响因素及检测方法的研究进展

孔静雅,郭强强,邬琴,孔科委,李劫,齐亚银,周霞(44)

猪胸膜肺炎放线杆菌生物被膜形成与抑制机制的研究

刘燕霞,杨建德(49)

大环内酯类抗生素对细菌生物被膜作用的研究进展

丁双元,官丽莉,马红霞,刘树明(52)

肉鸡腹水综合征病因及防治的研究进展

王玲,王天成,吴聪,王立琦,郭小权,胡国良,刘平(55)

速激肽及其受体基因在哺乳动物生殖领域的研究

王玉琴,吴至博,张小辉,杨芳,田志龙,施会彬,王建平,李元晓(59)

奶牛隐性乳房炎诊断技术研究新进展

郝景锋,李静姬,张宇航,尹柏双,付连军,曾荣荣,李东贺,要斌,黄岩,李心慰,李小兵,赵晨旭,刘国文(63)

富血小板血浆在马属动物的临床应用进展

杨珊珊,李云章,罗园渠,王煜(66)

猪SLA免疫学相关研究进展

奚衍洋,赵云蛟,钱爱东(70)

表面麻醉在犬眼科中的应用

向邦全(73)

营养素、生长因子与激素对脂肪细胞生长发育影响的研究

雒诚龙,邵伟,任万平,余雄(76)

刺参肠道菌群的研究进展

刘美如,王力,叶仕根,黎睿君,李强(81)

生物活性蛋白饲料的研究进展

赵甜,赵晓雨,黄正端,刘建涛(85)

壳聚糖-海藻酸钠胶囊性能及释药的影响因素

史同瑞,王丽坤,张莹,王岩,骆宏伟,鹿凌岩,于万才(87)

畜牧科学

哈萨克马遗传资源特征研究

丁丽媛,杜拉提·卡衣马尔旦,Исхан Кайрат,艾地尔汗·沙合多拉,格明古丽·木哈台(91)

五种糖对马精子低温保存及冷冻效果的影响

徐文慧,郑新宝,李海,罗永明,孟军,于维浩,赵海丽,张亚昂,毋状元,姚新奎(95)

马精准人工授精技术的研究

张志鹏,简洪禹,麻兆晖,潘庆杰,周欢敏(101)

高湿热条件下蜀宣花牛与西门塔尔牛、荷斯坦牛血液生化指标比较研究

王巍,付茂忠,唐慧,甘佳,冯华,方东辉,王淮,易军(104)

(期刊基本参数) CN 23-1205/S* 1958* s* A₄* 262* zh* P* ¥40.00* 2650* 68* 2018-03



黑龙江畜牧兽医

(2018年第03期 总第543期)

目次

华南地区不同耐热性荷斯坦奶牛血液生化指标比较 廖迎新,刘德武,李耀坤,王 坤,廖高峰,柳广斌(108)

黑龙江省放牧与舍饲肉牛肝功能状态的评价

..... 何 平,黄宝银,于洪江,沈泰钰,高三思,刘润琪,董志昊,李蕊蕊,杨 威,陈媛媛,徐 闯(112)

南方冬季钟楼式肉牛舍通风系统应用效果研究 刘玉欢,曹子薇,陈昭辉,刘继军,杨食堂(116)

HNPI 对牛支持细胞中细胞周期因子的影响 王 晨,王 皓,于 娜,王 雪,张 晗,许钟峯,张贵学(121)

京海黄鸡二系、三系杂交效果研究 储冬生,俞亚波,顾云飞,施会强,王宏胜,王金玉,张跟喜,邱 聪(123)

前列腺素 F_{2α} 对奶牛产后子宫机能的影响 王国卿,许文利,金佳钰,龙 博,吴雪晴(126)

黄体酮在防治德州驴妊娠早期流产症状中应用效果的观察 马运峰,孙玉江,潘庆杰,嵇传良,姜桂苗,吕承林,刘海兵(129)

从江香猪 ApoA1、ApoC3、ApoE 基因在组织器官中表达差异分析 赵佳福,杨远青,段志强,阮 涌,倪萌萌,嵇辛勤(132)

集约化猪场垂直通风模式分析 付典林,杨卫平,刘仁鑫,杨圣文,张利群(137)

坝上长尾鸡线粒体基因组特征与系统进化分析 陈 烨,王 涵,岳巧娟,赵彩娟,周荣艳,李兰会,张振红,李祥龙(140)

鸡性别伴性遗传规律的研究 张蓝艺,李 维,李洪林,祖盘玉,林家栋,张福平,陈 颖(144)

兽医科学

西藏小型猪对猪瘟病毒的敏感性评价 廖洁丹,黄允真,范双旗,丁红星,赵明秋,陈金顶(148)

牛肠道病毒 3D 抗体与感染相关性研究 周萍萍,张海丽,安 东,廉乐乐,李 嘉,高明春,王君伟(151)

有丝分裂原刺激多浪羊淋巴细胞体外培养研究 高学丽,李莲瑞(155)

猪流行性腹泻病毒 ORF3 基因云南株的克隆及序列分析

..... 刘丽娟,马光强,杨丽彦,张双翔,胡兴义,周碧君,王开功,文 明,程振涛(158)

猪流行性腹泻病毒的分离鉴定及 S1 基因序列分析 樊 平,颜邦斌,王怀禹(162)

鸡网状内皮细胞增生症病毒分离株的鉴定及 pol 基因序列分析 王 璇,潘淑惠,文正常(166)

邯郸地区鸡白痢沙门氏菌的分离鉴定与耐药性分析 史瑞雅,马邯生,刘彦威,刘 娜,李 博(169)

沙门氏菌现场快速检测方法的建立 姚贵哲,王伟利,夏 明,孟庆峰(172)

副猪嗜血杆菌的分离及 ERIC-PCR 指纹图谱分析 刘 杰,孙 哲,刘维康,赵坤坤,刘守川(175)

仔猪初免时间及猪口蹄疫、猪瘟、猪三联疫苗免疫效果研究 张成图,陈永忠,孟 茹,王淑琴(179)

饲草、饲料与添加剂

日粮中添加梧桐子油和罗格列酮对绵羊背最长肌中脂代谢相关酶活性的影响

..... 史晓雪,牛占宇,李 超,张三润,闫婉姝,张润厚(182)

3 种食药用真菌发酵物对 SPF 鸡生产和免疫性能的影响 李海燕,张 琳,于 森,徐敏丽,白俊平,吴家强,林冬梅,丁志勇(185)

发酵棉粕对安格斯肉牛生长性能和日粮养分消化率的影响 梁 敏,张文举,杨 亮,张晓羊,陈 宁,尹君亮(189)

不同水平复合酶制剂在断奶仔猪生产中的应用 张 洁,梁兴龙,党文庆,何 敏,杨晋青,梁信宇(192)

大蒜多糖的提取纯化及对鸡体外周血淋巴细胞增殖的影响 王 慧,胡元亮,邱树磊,陈晓兰,刘海霞,郭长明,武彩虹(195)

动物保健品研究

高效液相色谱法测定泰拉霉素注射液中泰拉霉素的含量 黎乃添,张桂君,吴志玲,梁惠莲,梁劲康,方炳虎(199)

兽用烟酸阿莫西林可溶性粉的制备及稳定性研究

..... 孟冬霞,刘晓东,武果桃,武晋孝,闫益波,任 杰,卫顺生,赵 娟,马政禹,夏艳婷(203)

凡杂志有印刷不清、破损、倒装、错装、脱页等问题,请寄回本杂志社,以便与印刷厂联系调换

黑龙江畜牧兽医



(2018 年第 03 期 总第 543 期)

目次

大鲵皮肤分泌物抗菌肽 Andricin 01 生物信息学分析	金文刚, 裴金金, 贺屹潮, 陈德经 (206)
氟苯尼考纳米乳的制备及性状研究	朱术会, 王成森, 王加才, 孙新堂, 宿志民, 孙 燕, 郭洪梅 (209)
五加芪粉对鸡的田间试验研究	桑卡娜, 张晓东, 李召斌 (212)
中药复方对雏鸡大肠杆菌病受损脏器治疗效果	于苗苗, 姜晓文, 于文会, 李子佳, 黄 慧, 王海彬 (215)

特种动物研究

拉布拉多犬低剂量性控冻精的人工授精试验研究	高一龙, 温 海, 秦海斌, 强京宁, 贺星亮, 张汇东, 马大君, 朱 粤 (219)
小鼠精子冷冻及冻融精子体外受精研究	罗 林, 吴 梦, 兰国成, 梁 浩, 葛良鹏 (223)
青海省生长期牦牛体重与体尺指标关系模型的构建	孙国强, 李九花, 郝力壮, 刘书杰, 牛建章 (225)
雁鹅消化系统 EST 和 SOD 同工酶电泳分析	杨 爽, 金建丽, 曹明星, 刘艳爽 (229)

实验室检测

一种基于 ITR 序列的羊痘病毒 TaqMan 探针荧光 PCR 检测方法的建立	王建华, 李 宁, 赵祥平, 贺 艳, 郑文杰, 刘 伟 (233)
几种蜂花粉多糖含量的比较	胡景华, 王添奕, 颜伟玉 (237)
水杨酸酶法检测牛奶中尿素氮含量	马建民, 孙 健, 段素云 (239)

教育与教学

虚拟仿真技术在动物医学实验(实践)教学中的应用	贺建忠, 井 波, 陶大勇, 焦海宏, 吐尔逊江·库尔班, 周东海, 郭定宗 (242)
动物医学专业实验教学体系改革	张 旭, 韦春波, 郭景茹, 王建华, 郭东华, 孙东波 (245)
以创新创业为导向的“专题讲座式”遗传学教学改革探索与实践	黄雅琴, 叶兆伟, 李尽哲, 王德芝 (247)

信息

重要启示	(19)
S8(除 S812)畜牧、动物医学、狩猎、蚕、蜂(除草地学、草原学)类核心期刊表	(107)
《黑龙江畜牧兽医》被国际著名检索系统《化学文摘》(美国)收录	(181)

彩图

.....	(251)
-------	--------

广告发布单位

河北威远动物药业有限公司
长城国际展览有限公司

版权声明

《黑龙江畜牧兽医》(以下简称“本刊”)已许可《中国学术期刊(光盘版)》电子杂志社有限公司以数字化方式复制、汇编、发行、信息网络传播本刊全文。本刊稿酬中含有中国知网的使用费,不再另行支付。作者向本刊提交文章发表的行为即视为同意黑龙江畜牧兽医杂志社的上述声明。如作者不同意文章被中国知网的电子方式收录,请在来稿时向本刊杂志社声明,本刊杂志社将做适当处理。

本刊部分文章已经在中国知网以“网络首发”形式出版,网络首发表文章页码可能与纸质样刊不一致;文章中的彩色图片在纸质样刊正文后统一彩色排版,与数字化排版时页码也不一致,特此告之。



华南地区不同耐热性荷斯坦奶牛血液生化指标比较

廖迎新, 刘德武, 李耀坤, 王 坤, 廖高峰, 柳广斌*

(华南农业大学 动物科学学院, 广州 510642)

中图分类号: S823.4; S854.4⁺3

文献标识码: B

文章编号: 1004-7034(2018)03-0108-05

应激是机体受到各种内外环境因素及社会、心理因素刺激时所出现的全身性非特异性适应反应, 又称为应激反应。引发应激的因素——应激源有多种, 主要有寒冷、酷热、潮湿、强光、雷电、气压、噪音、营养缺乏、感觉剥夺、刺激过量、心理社会因素等。热应激的应激源为高热高湿。热应激是指在高环境温度中的机体对热环境机体提出的任何要求所做的非特异性的生理反应的总和, 表现为热喘息、心率加快、体内氧化代谢增加、过氧化物增加、膜系统损伤、甲状腺和肾上腺功能的加强、水和电解质平衡紊乱。H. Selye^[1]在1976年曾定义了奶牛热应激, 他证明奶牛热应激指的是一种非特异性防御反应和包括特异性障碍在内的全身性适应综合征, 刺激源为超过机体本身体温调节能力过高的温度刺激, 在垂体-肾上腺皮质系统上起作用。奶牛热应激的下限温度范围为25~26℃, 超过这个温度范围, 奶牛将处于热应激状态, 直肠温度和产奶量都会受到影响^[2]。R. H. Ingraham等^[3]利用温湿度指数(THI)判断热应激程度时认为: 当THI<72时, 奶牛无热应激反应; 当THI为≥72~<78时, 奶牛会产生轻微热应激; THI为≥78~<90时, 奶牛会产生中度热应激; 当THI≥90时, 奶牛会产生严重热应激反应。

在热应激反应的作用下, 动物的生理和生化指标都会发生变化。T. L. Mader等^[4]研究结果表明, 在热应激环境下, 牛易出现体温升高、呼吸频率加快、食欲下降等现象, 严重影响养牛的经济效益。目前, 关于奶牛耐热性指标的研究非常少, 而且都不尽相同, 但选育耐热性的荷斯坦奶牛势在必行。为了降低热应激反应给奶牛带来的不良影响, 选育耐热奶牛品种, 本研究根据夏季(热应激期)和秋季(非热应激期)产

奶量下降率选择出高耐热组和低耐热组奶牛, 研究两组奶牛的直肠温度和相关血液生化指标的变化, 为进一步探究荷斯坦奶牛的热应激作用机理和筛选耐热指标提供理论依据。

1 材料与方法

1.1 试验牛的选择及分组

根据某奶牛场的生产性能数据, 在健康、胎次相同, 且处于泌乳中期的100头荷斯坦奶牛中筛选, 选择了夏季和秋季产奶量下降率处于极端的各10头共20头作为试验牛, 其中产奶量下降率(0.09%)低的极端个体为高耐热组, 产奶量下降率(0.56%)高的极端个体为低耐热组。分别在9月中旬和12月中旬测量直肠温度并采集血液。

1.2 温湿度指数的记录

在牛舍中部南墙距地面1.5 m处挂置干湿电子温度计, 每天08:00、14:00测定相对湿度和温度, 并换算成THI。THI=0.81×温度+(0.99×温度-14.3)×相对湿度+46.3。

1.3 直肠温度的测定

每天08:00、14:00测定两组奶牛的直肠温度, 并记录, 连续测量5 d, 求平均值。具体的测定是将兽用玻璃体温计的水银柱先甩至35℃以下。用乙醇消毒棉球擦净, 感应部分插入奶牛直肠10 cm, 3 min后取出读数。

1.4 血液样本的采集及预处理

用促凝管(购自广州康龙生物技术有限公司)收集血液样本。采集尾静脉血13 mL左右, 分离血清, 采集到的血液样本均上下颠倒混匀, 加入促凝管5 000×g离心10 min, 取所得血清6 mL左右分装4管, 每管1.5 mL。所得全部样本立即放入-20℃冰箱, 加干冰后运回实验室。

1.5 血液生化指标的测定

将采集到血液和离心得到血清进行血液生化指标的测定: 碱性磷酸酶(ALP)、肌酸激酶(CK)、总蛋白(TP)、肌酐(CREA)、乳酸脱氢酶(LDH)、钙离子(Ca²⁺)、钾离子(K⁺)、钠离子(Na⁺)、超氧化物歧化

收稿日期: 2016-09-08; 修回日期: 2017-12-10

作者简介: 廖迎新(1992—), 女, 硕士研究生, 研究方向为动物分子遗传育种, 838780264@qq.com。

* 通信作者: 柳广斌(1982—), 男, 讲师, 博士, 研究方向为动物分子遗传育种, 129818@qq.com。

酶(SOD)、丙二醛(MDA)、谷胱甘肽过氧化物酶(GSH-Px)、三碘甲腺原氨酸(T₃)、甲状腺激素(T₄)、皮质醇(cortisol)全自动生化分析仪、全波长酶标仪、全自动电解质分析仪检测;热休克蛋白70(HSP70)用酶联免疫吸附试验(ELISA)检测试剂盒检测。试剂盒购自南京建成生物有限公司。

1.6 数据的统计分析

本试验数据由Excel软件统计,利用SPSS 20.0软件进行独立样本 t 检验和显著性分析。

2 结果与分析

2.1 夏季和秋季荷斯坦奶牛直肠温度的变化

结果见表1。

表1 夏季和秋季荷斯坦奶牛的直肠温度变化

项目	夏季	秋季
直肠温度/℃	38.75 ^a ±0.08	38.19 ^b ±0.05
温湿度指数	87.30	52.26
热应激强度	中度	非热应激

注:同行数据肩标字母不同表示差异显著($P<0.05$),无肩标表示差异不显著($P>0.05$)。

由表1可知,夏季的荷斯坦奶牛处于中度热应激状态,秋季处于非热应激状态,夏季荷斯坦奶牛的直肠温度显著高于秋季($P<0.05$)。

2.2 夏季和秋季荷斯坦奶牛血液生化指标的变化

结果见表2。

表2 非热应激期和热应激期荷斯坦奶牛的血液生化指标

血液生化指标	非热应激期	热应激期	P 值
T ₃ /(ng·mL ⁻¹)	2.29±0.14	1.33±0.08	0.00
T ₄ /(ng·mL ⁻¹)	115.27±6.87	73.80±10.61	0.00
cortisol/(ng·mL ⁻¹)	5.03±0.75	12.46±0.80	0.00
ALP/(U·L ⁻¹)	48.75±3.69	35.63±2.46	0.01
CK/(U·L ⁻¹)	169.38±14.89	203.69±40.95	0.44
TP/(g·L ⁻¹)	76.25±1.63	81.04±2.09	0.08
CREA/(μmol·L ⁻¹)	77.26±3.17	62.41±3.28	0.00
LDH/(U·L ⁻¹)	702.44±29.85	799.88±25.55	0.02
Ca ²⁺ /(mmol·L ⁻¹)	2.37±0.03	2.34±0.03	0.52
K ⁺ /(mmol·L ⁻¹)	4.45±0.05	4.79±0.14	0.04
Na ⁺ /(mmol·L ⁻¹)	139.41±0.52	144.71±0.34	0.00
SOD/(U·mL ⁻¹)	107.43±0.92	107.19±0.97	0.87
MDA/(nmol·mL ⁻¹)	9.50±0.20	9.70±0.14	0.41
GSH-Px/(U·mL ⁻¹)	876.71±16.18	929.24±13.47	0.02
HSP70/(ng·mL ⁻¹)	1.97±0.03	1.51±0.07	0.00

由表2可知,荷斯坦奶牛非热应激期的T₃、T₄、CREA、HSP70极显著高于热应激期($P<0.01$)。热应激期的cortisol、Na⁺极显著高于非热应激期($P<0.01$)。热应激期的ALP、LDH、K⁺、GSH-Px与非热应激期相比差异显著($P<0.05$)。其他指标差异不显著($P>0.05$)。

2.3 高耐热组荷斯坦奶牛夏季和秋季血液生化指标的变化

结果见表3。

由表3可知,高耐热组荷斯坦奶牛热应激期T₃、HSP70极显著低于非热应激期($P<0.01$)。ALP显著低于非热应激期($P<0.05$)。cortisol、Na⁺极显著高于非热应激期($P<0.01$)。GSH-Px显著高于非热应激期($P<0.05$)。其他指标差异不显著($P>0.05$)。

2.4 低耐热组荷斯坦奶牛夏季和秋季血液生化指标的变化

结果见表4。

由表4可知,低耐热组荷斯坦奶牛热应激期T₄、HSP70极显著低于非热应激期($P<0.01$)。T₃、CREA显著低于非热应激期($P<0.05$)。cortisol、Na⁺极显著高于非热应激期($P<0.01$)。其他指标差异不显著($P>0.05$)。

2.5 高耐热组和低耐热组荷斯坦奶牛夏季和秋季显著变化的血液生化指标的比较

结果见图1。

由图1可知,高耐热组荷斯坦奶牛在热应激期和非热应激期T₃、cortisol、ALP、Na⁺、HSP70、GSH-Px相比差异显著或极显著,而低耐热组荷斯坦奶牛在热应激期和非热应激期T₃、T₄、cortisol、CREA、Na⁺、HSP70相比差异显著或极显著。不同的是高耐热组的T₄变化不显著,而低耐热组变化极显著;高耐热组的GSH-Px变化显著,而低耐热组变化不显著。推断T₄、GSH-Px可以作为评定奶牛耐热性的指标。



表 3 高耐热组荷斯坦奶牛热应激期和非热应激期血液生化指标

血液生化指标	非热应激期	热应激期	P 值
T3/(ng·mL ⁻¹)	2. 27 ± 0. 16	1. 21 ± 0. 10	0. 00
T4/(ng·mL ⁻¹)	113. 95 ± 8. 79	86. 72 ± 16. 86	0. 17
cortisol/(ng·mL ⁻¹)	5. 01 ± 0. 81	12. 01 ± 0. 63	0. 00
ALP/(U·L ⁻¹)	48. 89 ± 4. 45	34. 89 ± 3. 82	0. 03
CK/(U·L ⁻¹)	189. 22 ± 16. 38	181. 22 ± 50. 89	0. 88
TP/(g·L ⁻¹)	75. 42 ± 2. 55	80. 61 ± 3. 38	0. 24
CREA/(μmol·L ⁻¹)	73. 52 ± 4. 08	61. 74 ± 4. 59	0. 07
LDH/(U·L ⁻¹)	694. 33 ± 26. 01	788. 22 ± 36. 72	0. 05
Ca ²⁺ /(mmol·L ⁻¹)	2. 36 ± 0. 04	2. 35 ± 0. 04	0. 85
K ⁺ /(mmol·L ⁻¹)	4. 49 ± 0. 06	4. 90 ± 0. 24	0. 13
Na ⁺ /(mmol·L ⁻¹)	139. 99 ± 0. 78	144. 40 ± 0. 32	0. 00
SOD/(U·mL ⁻¹)	107. 26 ± 1. 46	106. 65 ± 1. 13	0. 74
MDA/(nmol·mL ⁻¹)	9. 58 ± 0. 28	9. 81 ± 0. 21	0. 52
GSH-Px/(U·mL ⁻¹)	869. 47 ± 20. 98	942. 92 ± 16. 86	0. 02
HSP70/(ng·mL ⁻¹)	1. 98 ± 0. 04	1. 41 ± 0. 08	0. 00

表 4 低耐热组荷斯坦奶牛热应激期和非热应激期血液生化指标

血液生化指标	非热应激期	热应激期	P 值
T3/(ng·mL ⁻¹)	2. 31 ± 0. 27	1. 48 ± 0. 10	0. 02
T4/(ng·mL ⁻¹)	116. 96 ± 11. 67	57. 20 ± 8. 65	0. 00
cortisol/(ng·mL ⁻¹)	5. 05 ± 1. 46	13. 04 ± 1. 69	0. 00
ALP/(U·L ⁻¹)	48. 57 ± 6. 63	36. 57 ± 3. 07	0. 13
CK/(U·L ⁻¹)	143. 86 ± 24. 76	232. 57 ± 69. 95	0. 23
TP/(g·L ⁻¹)	77. 31 ± 1. 93	81. 60 ± 2. 31	0. 18
CREA/(μmol·L ⁻¹)	82. 06 ± 4. 71	63. 26 ± 4. 99	0. 02
LDH/(U·L ⁻¹)	712. 86 ± 62. 45	814. 86 ± 36. 77	0. 19
Ca ²⁺ /(mmol·L ⁻¹)	2. 37 ± 0. 04	2. 32 ± 0. 06	0. 50
K ⁺ /(mmol·L ⁻¹)	4. 40 ± 0. 08	4. 65 ± 0. 14	0. 13
Na ⁺ /(mmol·L ⁻¹)	138. 66 ± 0. 59	145. 11 ± 0. 68	0. 00
SOD/(U·mL ⁻¹)	107. 65 ± 1. 08	107. 89 ± 1. 75	0. 91
MDA/(nmol·mL ⁻¹)	9. 40 ± 0. 30	9. 56 ± 0. 17	0. 64
GSH-Px/(U·mL ⁻¹)	886. 03 ± 26. 75	911. 65 ± 21. 40	0. 47
HSP70/(ng·mL ⁻¹)	1. 97 ± 0. 04	1. 63 ± 0. 09	0. 00



图 1 高耐热组和低耐热组荷斯坦奶牛夏季和秋季变化显著的血液生化指标韦恩图

3 讨论

3.1 热应激对生产性能的影响

众多研究结果表明,热应激会影响奶牛的生产性能,体现在采食量、奶产量、乳品质、繁殖性能方面。宋代军等^[5]选用胎次相近的荷斯坦奶牛 18 头,证明了热应激条件下,奶牛的产奶量和干物质采食量显著降低。张凡建等^[6]选取了 36 头泌乳中期奶牛,研究不同程度热应激对产奶量和乳成分的影响,结果表明,与无热应激相比,中度热应激使奶牛产奶量降低 16. 33%,这与本研究结果一致。热应激使得奶牛的产奶量下降,原因可能是热应激引起奶牛代谢机能改变,导致饲料采食量减少和饲料利用率降低。

3.2 热应激对生理指标的影响

在热应激条件下,动物交感神经兴奋,体温明显升高,呼吸频率加快。多年来的研究表明,热应激能够显著影响奶牛温度和呼吸频率。奶牛的直肠温度变化反映的是奶牛对湿热环境的一种调节适应,正常的奶牛直肠温度为 38.5°C 。许多研究表明,排除其他非热应激因素影响,当奶牛的直肠温度 $\geq 39^{\circ}\text{C}$ 时,奶牛便进入热应激状态。安代志^[7]的试验结果表明,当THI升高时,直肠温度也升高,这与本研究结果一致,可能是奶牛拥有体表短而密的被毛和不发达的汗腺,当环境温度和湿度升高时,奶牛自身不能及时调节,环境热与奶牛内部消化产热及奶牛维持产热,三方面的热量作用于奶牛机体,奶牛发生各种特异性或者非特异性反应产生热应激反应,导致直肠温度升高。

3.3 热应激对血液生化指标的影响

当奶牛处于热应激状态时,其内分泌会发生紊乱,影响机体新陈代谢,相关血液生化指标也会发生变化。在热应激状态下,动物血糖含量下降,原因是糖原分解能量,增强机体对热应激的抵抗力。热应激初期,敏感神经兴奋,肾上腺髓质功能增强,分泌肾上腺素,分解肝糖原,血糖浓度升高。随着热应激时间的持续,垂体-肾上腺皮质激素(ACTH)释放糖皮质激素,分解蛋白,糖异生增强,为确保血糖稳定,抑制促甲状腺激素的分泌,从而使得T₃、T₄水平下降,减少糖异生程度。

奶牛处于热应激状态下,机体的生理和生化方面发生很大变化,这也对微量矿物元素影响显著。Na⁺是一种维持体液渗透压、酸碱平衡、神经肌肉应激性及细胞外液容量的重要电解质。K⁺参与多种新陈代谢的过程,激活和提高酶的活性,是酶促反应的主要离子,是调节代谢的第二信使。热应激引起血清Ca²⁺含量显著降低。刘学剑^[8]研究表明,夏季热应激期奶牛血清Ca²⁺含量与非热应激时期相比显著下降。李建国等^[9]研究表明,夏季热应激期奶牛血清中K⁺、Na⁺、Ca²⁺的含量显著低于春、秋、冬季。这与本研究结果不一致,热应激改变血液离子浓度的原因可能是热应激加剧新陈代谢,矿物质活动增强。

热应激也对很多其他血液生化指标有显著影响。在正常状态下,血清酶能保持相对稳定,但当动物处于热应激环境时,血清酶活力和含量也会随之发生改变。ALP是一种反映成骨细胞活性、骨生成状况和钙磷代谢的重要指标^[10],ALP活性降低意味着ATP的分解减少,伴随着血钙和血磷含量减少。宋小珍等^[11]研究表明,在热应激环境下ALP降低,这与本研究结果一致。LDH在糖分解代谢和糖异生中发挥重要作用,CK是能量代谢的关键酶,一般把CK

的升高作为发生应激时的一个评价指标^[12]。近年来,关于热应激有一种自由基理论:在正常状态下,体内自由基的产生和清除处于动态平衡,SOD和GSH-Px作用都是清除和破坏体内产生的氧自由基,当动物受到应激时,自由基的产生急剧增加,清除不足,组织氧化会受到损伤。在本研究中,GSH-Px在夏季热应激期与非热应激期时相比增多,可能是加速清除自由基防止组织氧化^[13]。cortisol在热应激中具有动员能源,促进脂类、蛋白质分解和糖异生作用,防止发炎,从而提高机体总抵抗力^[14]。杨淑晶^[15]研究表明,夏季热应激期红细胞钾含量、MDA显著高于春季非热应激期,夏季血浆中SOD活性、GSH-Px活性极显著低于春季。李朝明^[16]研究表明,从热应激期到非热应激期奶牛血清中总胆固醇(TC)、TP、白蛋白(Alb)与HSP70的含量是下降的,而SOD的含量却是上升的。

热应激也与甲状腺的功能密切相关。甲状腺激素的合成和分泌受垂体分泌的促甲状腺激素(TSH)的控制,其分泌过程与大脑皮层接受外界冷热刺激有关。大量研究表明,体温升高时,甲状腺分泌机能降低,且甲状腺体积变小、萎缩。这主要是为了降低体内的基础代谢,减少体热的产生。李朝明^[16]研究表明,从热应激到非热应激奶牛血清中T₃、T₄的含量是上升的。何钦^[17]研究表明,热应激时,各泌乳阶段奶牛的血清T₃、T₄水平均有所下降,且随着热应激程度的加深,影响越严重。热应激时,各泌乳阶段奶牛的cortisol、T₃、T₄和催乳素(PRL)等激素水平降低,HSP70表达量减少,CK等酶活性发生改变。李朝明等^[18]研究表明,热应激降低了奶牛血清中T₃和T₄的含量,其中娟杂组降低幅度最大。以上结论都与本研究结果一致。热应激条件下T₃和T₄的含量显著下降,可能是热应激时为了降低基础代谢,减少体热的产生。

4 结论

本研究结果表明,热应激能显著或极显著影响奶牛的T₃、T₄、ALP、CREA、HSP70、cortisol、Na⁺、LDH、K⁺、GSH-Px水平,高耐热组荷斯坦奶牛在夏季和冬季T₃、cortisol、ALP、Na⁺、HSP70、GSH-Px差异显著或极显著,而低耐热组荷斯坦奶牛在夏季和冬季T₃、T₄、cortisol、CREA、Na⁺、HSP70差异显著或极显著。不同的是高耐热组的T₄变化不显著,而低耐热组变化极显著,由此推断T₄在高耐热组奶牛中的变化受热应激影响较小,可以作为评价奶牛耐热性的指标。高耐热组GSH-Px变化显著,而低耐热组差异不显著,也可以作为参考指标。期望本研究能为以后耐热性荷斯坦奶牛的选育提供一定理论依据,为后续筛选耐热荷斯坦奶牛奠定基础。(下转第120页)



- 境的研究[J]. 黑龙江畜牧兽医, 2016(01 上): 94-96, 100.
- [4] 栾冬梅, 赵靖, 冯春燕, 等. 黑龙江省不同类型肉牛舍冬季环境的研究[J]. 东北农业大学学报, 2011, 42(6): 66-70.
- [5] 高玉红, 赵伍祥, 赵凤存, 等. 河北省不同地区冬季肉牛舍内外温湿环境研究[J]. 家畜生态学报, 2013, 34(12): 68-72.
- [6] 魏天盛, 贾斌, 洪小华, 等. 钟楼式单层彩钢瓦屋面肉牛棚舍的环境评价[J]. 畜牧与兽医, 2012, 44(6): 26-31.
- [7] 高玉红, 李宏双, 郭建军, 等. 坝上寒区不同建筑类型奶牛舍冬季温湿环境评价[J]. 东北农业大学学报, 2015, 46(12): 52-57, 93.
- [8] 张言, 程琼仪, 陈昭辉, 等. 环境自动控制系统在冬季南方肉牛舍上的研究与应用[J]. 黑龙江畜牧兽医, 2015(07 上): 6-10.
- [9] 蔡景义, 冯堂超, 师筑俊, 等. 敞篷牛舍接力送风及饲料添加铬改善肉牛生产性能[J]. 农业工程学报, 2015, 31(15): 191-195.
- [10] 汪银锋, 李素平, 高腾云, 等. 肉牛舍温湿环境对肉牛生理指标的影响[J]. 家畜生态学报, 2009, 30(3): 56-59.
- [11] 仲庆振, 周海柱, 纪术远, 等. 夏季不同类型牛舍内环境的比较研究[J]. 黑龙江畜牧兽医, 2010(11 上): 73-74.
- [12] 姜玉杰, 纪术远, 周海柱. 不同类型肉牛舍冬季环境质量测定与分析[A]//2010 年全国家畜环境与生态学术研讨会. 论文集[C]. 通辽: 出版社, 2010.

Study on the effect of ventilation system of campanile beef barn in southern China during winter

LIU Yuhuan^{1,2}, CAO Ziwei^{1,2}, CHEN Zhaohui^{1,2}, LIU Jijun^{1,2*}, YANG Shitang³

(1. College of Animal Science and Technology, China Agricultural University, Beijing 100193, China; 2. State Key Laboratory of Animal Nutrition, Beijing 100193, China; 3. Gao'an Yufeng Agriculture Co., Ltd, Gao'an 330800, China)

Abstract: The aim of the present study was to evaluate the effect of the campanile beef barn ventilation system in the southern of China during winter. In this study, the campanile beef barn (A) and the double-slope curtain house (B), which were similar to the construction size of Gao'an beef cattle experimental station in Jiangxi province, were selected as the experimental beef barn. The three sections of beef barn were selected to detect the environmental parameters such as temperature, relative humidity, wind speed, carbon dioxide concentration, ammonia concentration and so on. The results showed that the average daily temperature of A was (10.2 ± 2.3) °C, which was significantly lower than that of B (10.5 ± 2.3) °C ($P < 0.05$). The average daily relative humidity of A and B were (90.2 ± 14.2) % and (91.5 ± 13.3) %, respectively. There was no significant difference between A and B ($P > 0.05$). The average wind speed of A was (0.18 ± 0.64) m/s, which was significantly higher than that of B (0.14 ± 0.52) m/s ($P < 0.05$). The contents of carbon dioxide and ammonia in A were significantly lower than those in B ($P < 0.05$), and the daily carbon dioxide concentration values were (185.50 ± 76.82) (1260.41 ± 64.58) mg/m³, respectively. The average daily ammonia concentration was (1.37 ± 1.34) (2.12 ± 1.48) mg/m³. The results indicated that the campanile beef barn had better ventilation, better air quality and more suitable temperature compared with the double-slope curtain house.

Keywords: Gao'an area; campanile beef barn; thermal environment; ventilation; air quality

(010)

(上接第111页)

参考文献:

- [1] SELYE H. Forty years of stress research: principal remaining problems and misconceptions[J]. Can Med Assoc J, 1976, 115(1): 53-56.
- [2] BERMAN A, FOLMAN Y, KAIM M, et al. Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical climate[J]. J Dairy Sci, 1985, 68(6): 1488-1495.
- [3] INGRAHAM R H, GILLETTE D D, WAGNER W D. Relationship of temperature and humidity to conception rate of Holstein cows in subtropical climate[J]. J Dairy Sci, 1974, 57(4): 476-481.
- [4] MADER T L, DAVIS M S, BROWN-BRANDL T. Environmental factors influencing heat stress in feedlot cattle[J]. J Anim Sci, 2006, 84(3): 712-719.
- [5] 宋代军, 何钦, 姚焰础. 热应激对不同泌乳阶段奶牛生产性能和血清激素浓度的影响[J]. 动物营养学报, 2013, 25(10): 2294-2302.
- [6] 张凡建, 徐聪, 翁晓刚, 等. 不同程度热应激对泌乳中期奶牛产奶量和乳成分的影响[J]. 中国兽医学报, 2014, 34(10): 1686-1688.
- [7] 安代志. 高温环境的评定及其高产奶牛体温调节特性[D]. 南京: 南京农业大学, 2005.
- [8] 刘学剑. 气象因子对奶牛生产性能的影响[J]. 中国奶牛, 1995(1): 47-48.
- [9] 李建国, 桑润滋, 张正珊, 等. 热应激对中国荷斯坦牛血液生化指标及产奶性能的影响[J]. 中国畜牧杂志, 1999, 35(2): 25-26.
- [10] 王秋颖. 碱性磷酸酶特性及其应用的研究进展[J]. 中国畜牧兽医, 2011, 38(1): 157-161.
- [11] 宋小珍, 付戴波, 瞿明仁, 等. 热应激对肉牛血清内分泌激素含量、抗氧化酶活性及生理生化指标的影响[J]. 动物营养学报, 2012, 24(12): 2485-2490.
- [12] 李俊杰, 桑润滋, 田树军, 等. 热应激与牛血液成分的变化[J]. 家畜生态, 2001, 22(4): 56-59.
- [13] 董淑丽, 王占彬, 雷雪芹, 等. 热应激对动物血液生化指标的影响[J]. 家畜生态, 2004, 25(2): 54-56.
- [14] LEE C, SARNA S K. Central regulation of gastric emptying of solid nutrient meals by corticotropin releasing factor[J]. Neurogastroenterol Motil, 1997, 9(4): 221-229.
- [15] 杨淑晶. 荷斯坦奶牛血液部分生化指标的季节性变化及其与奶牛耐热性相关性的研究[D]. 乌鲁木齐: 新疆农业大学, 2005.
- [16] 李朝明. 荷斯坦奶牛、娟姗牛和娟荷杂交牛热适应性的研究[D]. 重庆: 西南大学, 2013.
- [17] 何钦. 热应激对不同泌乳阶段奶牛生产性能及其营养代谢的影响[D]. 重庆: 西南大学, 2012.
- [18] 李朝明, 魏学良, 徐玉薇, 等. 热应激对荷斯坦和娟姗及娟荷杂交奶牛生理生化指标的影响[J]. 中国畜牧杂志, 2015, 51(21): 72-76.

(015)



微信平台



QQ群

ISSN 1004-7034



9 771004 703181

0 3>



广东省农业技术推广奖

获奖证书

为表彰在农业技术推广工作中做出贡献
的单位和个人，特颁发此证书，以资鼓励。

获奖项目：华南地区奶牛健康养殖关键技术与示范推广

奖励等级：二等奖

奖励个人：柳广斌

奖励日期：2024年12月25日

证书号：2023-2-X12-R06





广东省农业技术推广奖

获奖证书

为表彰在农业技术推广工作中做出贡献
的单位和个人，特颁发此证书，以资鼓励。

获奖项目：畜禽种质资源保护与利用技术示范推广

奖励等级：一等奖

奖励个人：柳广斌

奖励日期：2022年12月15日

证书号：2021-1-X04-R04





广东省农业技术推广奖

获奖证书

为表彰在农业技术推广工作中做出贡献
的单位和个人，特颁发此证书，以资鼓励。

获奖项目：华南地区羊的繁育及配套技术研发与示范推广

奖励等级：一等奖

奖励者：柳广斌

奖励日期：2021年12月8日

证书号：2020-1-X03-R02

广东省农业技术推广奖

评审委员会
评审委员会



广东省农业技术推广奖

获奖证书

为表彰在农业技术推广工作中做出贡献的单位和个人，特颁发此证书，以资鼓励。

获奖项目：优质肉牛生态高效养殖技术与示范

奖励等级：二等奖

获奖者：柳广斌

奖励日期：2020年12月17日

证书号：2019-2-X05-R11



证书号第 4387287 号



发明专利证书

发明名称：一种提高湖羊产羔数的多位点聚合效应分析的育种方法

发明人：柳广斌;杨新月;刘德武;李耀坤;孙宝丽

专利号：ZL 2018 1 0680809.1

专利申请日：2018 年 06 月 27 日

专利权人：华南农业大学

地址：510642 广东省广州市天河区五山路 483 号

授权公告日：2021 年 04 月 27 日

授权公告号：CN 108950008 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

其他事项参见续页

证书号第 3865519 号



发明专利证书

发明名称: BMP6 基因作为黑山羊产羔数性状的分子标记

发明人: 柳广斌;杨新月;孔令旋;何玉强;刘德武;孙宝丽;李耀坤

专利号: ZL 2018 1 0066944.7

专利申请日: 2018 年 01 月 24 日

专利权人: 华南农业大学

地址: 510642 广东省广州市天河区五山路 483 号

授权公告日: 2020 年 06 月 30 日

授权公告号: CN 108531608 B

国家知识产权局依照中华人民共和国专利法进行审查, 决定授予专利权, 颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年, 自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

其他事项参见续页

证书号第 5603834 号



发明专利证书

发明名称: chi-miR-128-3p 作为山羊卵泡成熟 miRNA 标志物的应用

发明人: 刘德武; 邹娴; 柳广斌; 李耀坤; 邓铭; 冯光杭

专利号: ZL 2020 1 1461363.7

专利申请日: 2020 年 12 月 11 日

专利权人: 华南农业大学

地址: 510640 广东省广州市天河区五山路 483 号

授权公告日: 2022 年 11 月 25 日

授权公告号: CN 112410439 B

国家知识产权局依照中华人民共和国专利法进行审查, 决定授予专利权, 颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年, 自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

其他事项参见续页

证书号第 4419238 号



发明专利证书

发明名称：一种影响湖羊产羔性状的 SNP 标记及其应用

发明人：李耀坤；杨新月；柳广斌；刘德武；孙宝丽；郭勇庆；邓铭

专利号：ZL 2018 1 0072300.9

专利申请日：2018 年 01 月 25 日

专利权人：华南农业大学

地址：510642 广东省广州市天河区五山路 483 号

授权公告日：2021 年 05 月 11 日

授权公告号：CN 108410994 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

证书号第5616381号



发明专利证书

发明名称: chi-miR-450-5p 作为山羊卵泡成熟 miRNA 标志物的应用

发明人: 刘德武;赵智锋;邹娴;柳广斌;李耀坤

专利号: ZL 2019 1 1388884.1

专利申请日: 2019年12月30日

专利权人: 华南农业大学

地址: 510642 广东省广州市天河区五山路 483 号

授权公告日: 2022年11月29日

授权公告号: CN 111118170 B

国家知识产权局依照中华人民共和国专利法进行审查,决定授予专利权,颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年,自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第1页(共2页)

其他事项参见续页

证书号第 4387288 号



发明专利证书

发明名称：一种提高山羊产羔数的多基因聚合效应分析的育种方法

发明人：李耀坤；杨新月；刘德武；柳广斌；孙宝丽

专利号：ZL 2018 1 0681847.9

专利申请日：2018 年 06 月 27 日

专利权人：华南农业大学

地址：510642 广东省广州市天河区五山路 483 号

授权公告日：2021 年 04 月 27 日

授权公告号：CN 108950009 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

其他事项参见续页

证书号第 4293534 号



发明专利证书

发明名称：一种影响湖羊产羔性状的 SNP 标记及其应用

发明人：刘德武;杨新月;李斌;柳广斌;李耀坤;郭勇庆;邓铭

专利号：ZL 2018 1 0009018.6

专利申请日：2018 年 01 月 04 日

专利权人：华南农业大学

地址：510642 广东省广州市天河区五山路 483 号

授权公告日：2021 年 03 月 12 日

授权公告号：CN 108103208 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

证书号第 5542536 号



发明专利证书

发明名称：一个与中国南方荷斯坦奶牛 305 天产奶量显著相关的 SNP 分子标记及应用和选育方法

发明人：李耀坤；林小觉；刘德武；王志英；柳广斌；郭勇庆；孙宝丽
邓铭

专利号：ZL 2021 1 1299513.3

专利申请日：2021 年 11 月 04 日

专利权人：华南农业大学

地址：510640 广东省广州市天河区五山路 483 号

授权公告日：2022 年 10 月 28 日

授权公告号：CN 113832240 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



第 1 页 (共 2 页)

其他事项参见续页

证书号第6059180号



发明专利证书

发 明 名 称：一个与南方荷斯坦奶牛乳脂率相关的SNP分子标记、试剂盒及应用和选育方法

发 明 人：李耀坤;林小觉;刘德武;王志英;柳广斌;郭勇庆;孙宝丽
邓铭

专 利 号：ZL 2021 1 1298942.9

专利申请日：2021年11月04日

专 利 权 人：华南农业大学

地 址：510640 广东省广州市天河区五山路483号

授权公告日：2023年06月16日

授权公告号：CN 113897443 B

国家知识产权局依照中华人民共和国专利法进行审查，决定授予专利权，颁发发明专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为二十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



证书号第18565865号



实用新型专利证书

实用新型名称：一种保定、称重和体尺测量一体式装置

发 明 人：邓铭;刘谭;李驰;罗丽霞;刘进芳;李耀坤;柳广斌;罗顺辉
黄祺

专 利 号：ZL 2022 2 1653005.0

专 利 申 请 日：2022年06月28日

专 利 权 人：华南农业大学
珠海市现代农业发展中心（珠海市金湾区台湾农民创业园
地 址：510642 广东省广州市天河区五山路483号

授 权 公 告 日：2023年03月07日

授 权 公 告 号：CN 218572356 U

国家知识产权局依照中华人民共和国专利法经过初步审查，决定授予专利权，颁发实用新型专利证书并在专利登记簿上予以登记。专利权自授权公告之日起生效。专利权期限为十年，自申请日起算。

专利证书记载专利权登记时的法律状况。专利权的转移、质押、无效、终止、恢复和专利权人的姓名或名称、国籍、地址变更等事项记载在专利登记簿上。



局长
申长雨

申长雨



“建行杯”“互联网+”
 第九届中国国际
 大学生创新创业大赛 广东分赛
 The 9th China International College Students' "Internet+" Innovation and Entrepreneurship Competition



荣誉证书

吕建达、冯嘉斌、刘婷婷、蔡泽曦、湛紫祺、蒙晓莹、陈科霖、朱易、赖宣尔、陈家宝、李浪浪、黄启轩、安春羽、邓远豪、宁冬冬：

你们的项目《五羊开泰-引领南方黑山羊高质量发展新模式》，在第九届中国国际“互联网+”大学生创新创业大赛广东省分赛中荣获

银 奖

指导老师：柳广斌、刘德武、陈永晴、张素敏、黄国胜
 特发此证，以资鼓励。





获奖证书

证书编号: 202102T010135

华南农业大学 柳广斌:

担任指导教师的队伍在中国研究生乡村振兴科技强农+创新大赛“光明杯”第四

届牛精英挑战赛中荣获肉牛组 **特等奖**

特发此证，以资鼓励。

指导单位:

教育部学位管理与研究生教育司

教育部学位与研究生教育发展中心

主办单位:

中国学位与研究生教育学会

中国科协青少年科技中心





荣誉证书

刘金洋、罗丽梅、范希阳、冉红丽、陈家宝、刘思莹、黄桢桢、岳希蒙、方思成、鲍国梁、
宋洋洋、汪晶：

你们的项目《领头羊——南方黑山羊产业高质量发展引领者》在中国国际大学生创新大赛（2024）广东省分赛中荣获

银 奖

指导老师：陈永晴、柳广斌、贾坤、刘德武、张慧慧
特颁此证，以资鼓励。

广东省教育厅
二〇二四年八月
编号：20240037H

获奖证书

华南农业大学

吕建达、湛紫祺、蔡泽曦、陈家宝、范希阳、冉红丽、黄桢荣、刘思莹、

张淇菲、罗丽梅 同学：

你们的作品《五羊开泰——引领南方黑山羊高质量发展新模式》在第十四届

“挑战杯”广东大学生创业计划竞赛中荣获

铜 奖

指导老师：陈永晴、柳广斌、刘德武



二〇二四年七月



牛精英计划

第三届全国农林高校
牛精英挑战赛

THE 3RD DAIRY-BEEF OPPORTUNITY & CHALLENGE

荣誉证书
CERTIFICATION

华南农业大学 谢青梅 郭勇庆 柳广斌 陈飞洋

担任指导教师的参赛队伍在

第三届全国农林高校“牛精英挑战赛”中荣获

二 等 奖

特发此证，以兹奖励。

DBOC team supervisors Xie Qingmei Guo Yongqing Liu Guangbin Chen Feiyang from
South China Agricultural University

together with the team has earned

Second Place Honor

in the 3rd Dairy-Beef Opportunity & Challenge.

This certificate of commendation is hereby awarded as an encouragement.



全国农业专业学位研究生教育指导委员会
China National Steering Committee of Agricultural Graduate Education

中国学位与研究生教育学会农林科工作委员会
A&F Committee, Chinese Society of Academic Degrees and Graduate Education

农林学科工作委员会

2018年7月

荣誉证书

柳广斌同志：

在华南农业大学2022年“三育人”评选中，
被评为“教书育人”先进个人。

特发此证，以资鼓励。



荣誉证书

柳广斌 同志：

被评为华南农业大学动物科学学院 2022 年度
“教学十佳”。

特发此证，以资鼓励。

中国共产党华南农业大学动物科学学院委员会 华南农业大学动物科学学院

2023 年 4 月 19 日

荣誉证书

柳广斌同志:

被评为华南农业大学动物科学学院 2021 年度

“教学十佳”。

特发此证，以资鼓励。

中国共产党华南农业大学动物科学学院委员会

华南农业大学动物科学学院

2022 年 1 月 10 日



荣誉证书

柳广斌：

在华南农业大学动物科学学院 2019 年度青年教师
教学观摩比赛中表现突出，荣获一等奖。

特发此证，以资鼓励。

华南农业大学动物科学学院

2020 年 1 月 6 日

证 明

柳广斌老师（身份证：210103198204261211），于 2014 年 10 月 21 日至 2017 年 12 月 4 日期间，由华南农业大学动物科学学院派驻到我公司做技术支持服务，主要负责我公司山羊育种及繁殖技术指导工作。工作期间认真负责，促进公司羊业生产成绩提高。特此证明。

新兴县温氏新旺羊业有限公司

负责人：

2017-12-04



广东省科学技术厅文件

粤科农字〔2024〕200号

广东省科学技术厅关于下达“百千万工程” 农村科技特派员第二轮重点派驻人员 名单和任务清单的通知

各有关地市科技局，各农村科技特派员派出单位：

为深入贯彻落实省委关于实施“百县千镇万村高质量发展工程”有关决策部署，进一步推动农村科技特派员下乡助力城乡区域协调发展，按照《广东省农村科技特派员科技助力百县千镇万村高质量发展行动计划（2023-2026）》《广东省科学技术厅关于落实“百千万工程”开展新一轮农村科技特派员重点派驻任务（2024-2026年）选派管理工作的通知》要求，在首轮农村科技特派员3年帮扶重点派驻任务即将到期的情况下，按照“镇村

发榜+地市组织选派+省级认定支持”的新型组织管理方式，第二轮共选派 2960 名农村科技特派员组建 984 个团队，“一对一”结对科技帮扶全省涉农县（市、区）辖内 984 个乡镇。现将“百千万工程”农村科技特派员第二轮重点派驻人员名单和任务清单印发给你们：

一、各地市科技管理部门、派出单位应按照《广东省科学技术厅关于落实“百千万工程”开展新一轮农村科技特派员重点派驻任务（2024-2026 年）选派管理工作的通知》（粤科函农字〔2023〕1649 号）、《广东省科学技术厅关于印发〈农村科技特派员助力“百千万工程”管理工作指引（2024-2026 年）〉的通知》（粤科函农字〔2024〕192 号）有关要求，做好本辖区、本单位农村科技特派员组织、管理、考核和验收相关工作。

二、各农村科技特派员团队应聚焦落实省委实施“百千万工程”决策部署，在省、市科技管理部门指导下，扎根基层一线，配合驻镇帮扶工作队开展科技帮扶工作，农村科技特派员团队纳入驻镇帮扶工作队（乡镇人民政府）统一管理，本轮帮扶任务周期为 3 年。

三、农村科技特派员承担重点派驻任务经费保障，按年度以科技支撑“百千万工程”专项资金下达到各地市，各地市科技管理部门要加强管理，压实责任，专款专用，提高资金使用效率，应将专项资金拨付到相关派出单位，用于开展科技帮扶工作。

附件：“百千万工程”农村科技特派员第二轮重点派驻人员名单和任务清单



（联系人及电话：赵清泉，020-83163909）

800	KTP20240800	连山壮族瑶族自治县吉田镇	广东科贸职业学院	李淑红、黄敏、梁多	吉田镇现代稻渔综合种养项目
801	KTP20240801	连山壮族瑶族自治县禾洞镇	广东科贸职业学院	杨承鸿、李欣、童湘颖	禾洞镇山区特色经济作物科技提升及推广
802	KTP20240802	连山壮族瑶族自治县小三江镇	华南农业大学	柳广斌、刘德武、陈永晴	黑山羊养殖技术示范推广及科技小院平台建设
803	KTP20240803	连山壮族瑶族自治县上帅镇	广东省农业科学院	于琳、桑文、何自福	作物高产种植与病虫害绿色防控技术推广应用
804	KTP20240804	连南瑶族自治县三江镇	仲恺农业工程学院（负责人、成员2）、华南农业大学（成员1）	刘文俊、范双旗、黄婷	黑山羊健康养殖技术推广应用
805	KTP20240805	连南瑶族自治县寨岗镇	广东省农业科学院	黄华林、陈海强、赵崇真	寨岗镇茶叶品质提升与示范推广
806	KTP20240806	连南瑶族自治县三排镇	仲恺农业工程学院	支庆庆、贺竹梅、何磊	油茶优质高产栽培技术推广与推广
807	KTP20240807	连南瑶族自治县大坪镇	广东科贸职业学院	周欢欢、刘旭华、米博瀚	农文旅融合模式升级及稻鱼茶精深加工推广
808	KTP20240808	连南瑶族自治县香坪镇	广东财贸职业学院	程玖、王国霞、邓莉平	科技助力七星西瓜、丝苗米和稻鱼鸭稳质增产增效
809	KTP20240809	连南瑶族自治县大麦山镇	华南农业大学	晏嫦好、黄亚辉、周玲	黄莲茶栽培管理技术指导及品牌营销技术服务
810	KTP20240810	连南瑶族自治县涡水镇	广东省林业科学研究院	郭文冰、龙永彬、曾明	山苍子特色产业关键技术开发与推广应用
811	KTP20240811	阳山县秤架瑶族乡	华南农业大学	张晓萍、肖南、张曼	发掘地方文旅资源，提升地区农产品加工技术和产业升级
812	KTP20240812	阳山县大良镇	仲恺农业工程学院	梁关生、梁佳勇、谢勇	蔬菜、茶叶产业高效提质关键生产技术的集成应用
813	KTP20240813	阳山县杜步镇	广东省农业科学院（负责人、成员1）、华南理工大学（成员2）	胡晓丹、胡建广、李理	水稻玉米蔬菜轮作种植及新品种引进示范与推广

河源市农村科技特派员 证书

编号: HYKTP2022063

实施乡村振兴战
略, 不忘初心、牢记使
命, 心系群众、扎实肯
干, 努力做出无愧于时
代的业绩。

姓名: 柳广斌

单位: 华南农业大学动物科学学院

服务地区: 河源市

期限: 3年



聘书

尊敬的 柳广斌 老师：

特聘请您担任连山壮族瑶族自治县乡村振兴人才驿站黑山羊产业发展专家，聘期自2023年4月21日至2026年4月20日。

连山壮族瑶族自治县乡村振兴人才驿站
2023年4月21日

聘书

LETTER OF APPOINTMENT

兹聘请柳广斌为

广东省草食动物产业联盟
专家委员会特聘专家

广东省草食动物产业联盟
2019年3月16日

华南农技通社会服务工作量统计

个人绩效统计

查询月份 2024-01 - 2024-12 确定

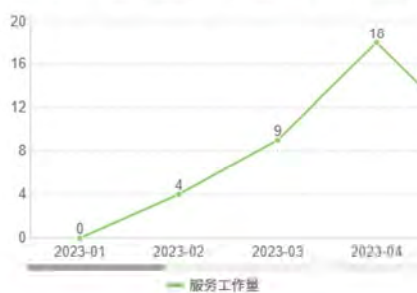
82 6 0 39
总工作量 开展培训/讲座 发放技术资料数 培训人数



个人绩效统计

查询月份 2023-01 - 2023-12 确定

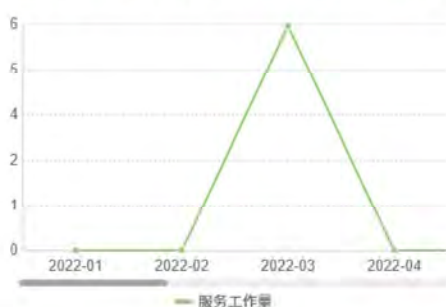
73 5 0 102
总工作量 开展培训/讲座 发放技术资料数 培训人数



个人绩效统计

查询月份 2022-01 - 2022-12 确定

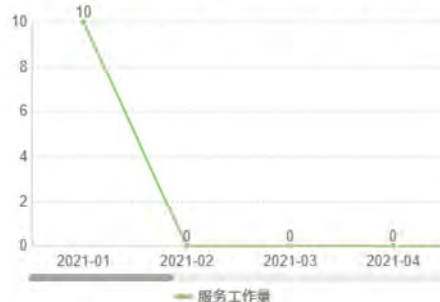
16 0 0 0
总工作量 开展培训/讲座 发放技术资料数 培训人数



个人绩效统计

查询月份 2021-01 - 2021-12 确定

15 0 0 0
总工作量 开展培训/讲座 发放技术资料数 培训人数



个人绩效统计

查询月份 2020-01 - 2020-12 确定

36 0 0 0
总工作量 开展培训/讲座 发放技术资料数 培训人数

