Gene Expression Changes in Goat Testes During Development and in Sperm During the Breeding and Non-breeding Seasons: A Novel Male Fertility Test?

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Objectives

1. To analyze alterations in gene expression in the goat testes during development.
2. To analyze alterations in gene products in goat sperm between breeding and non-breeding seasons

1. Activities

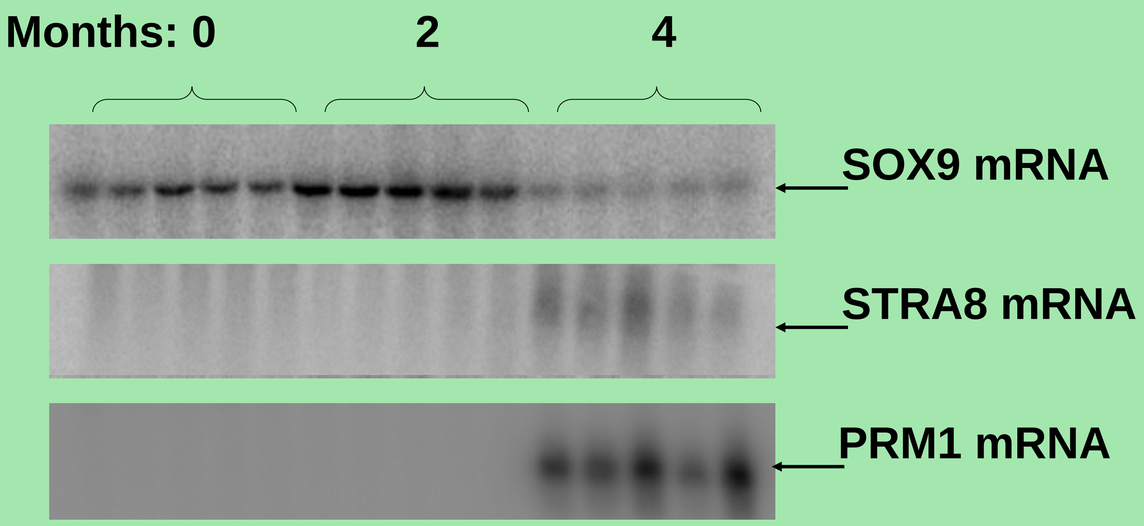
Goat testes were harvested from five Alpine bucks at 0, 2, 4, 6, and 8 months of age. Tissues were fixed in paraformaldehyde and used for in situ hybridization . In addition, tissues were frozen and RNA isolated for mRNA analyses by Northern blotting. Gene products chosen as markers for testis cells:

SOX 9 – Sertoli cells

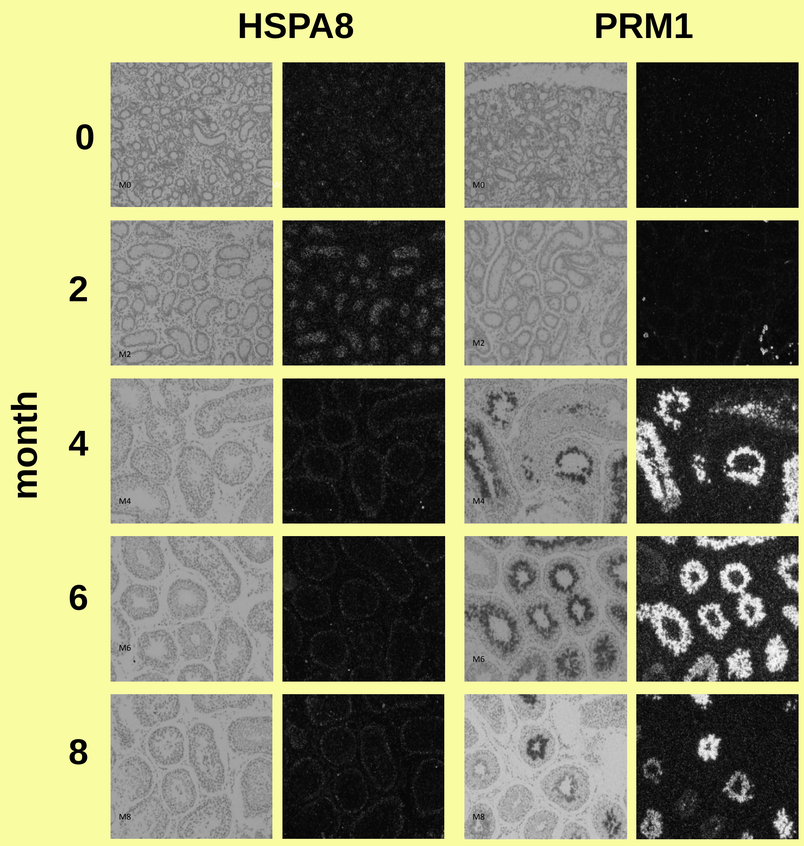
STRA8 and Heat shock protein A8 (HSPA-8) – early germ cells

Protamine 1 (PRM1) – maturing germ cells

Northern Blots of Testis RNA



In Situ Hybridization of Testis



[3] Utilization, Characterization and Preservation of Goat Genetic Resources II. Accession number 0204895; $194,953.
Animal Health and Production: Animal Bioinformatics and Development of Tools for Livestock.

2. Activities

Ejaculates were collected from mature Alpine and Boer bucks (n = 4 bucks each) in October (peak breeding) and April (not peak breeding). RNA was isolated from sperm, fluorescently labeled and hybridized to Agilent bovine microarrays. Gene products that were differentially expressed between months ($p < 0.05$) are reported in the Table. Selected genes are being analyzed with real time PCR in ongoing studies.

Microarrays detect altered mRNAs in sperm

Gene Name	Abbr.	Fold change*
Zinc finger CCHC domain containing 6	ZCCHC6	4.1
Microcephalin 1	MCPH1	3.6
Heat shock protein B9	HSPB9	3.4
Transmembrane protein 106A	TMEM106A	3.3
Limb region 1 homolog-like	LMBR1L	2.8
Calcium binding Y phosphorylation regulated	CABYR	2.8
HEAT repeat containing 7A	HEATR7A	2.7
Bovine chromosome 12 ORF 57	C5H12ORF57	2.6
Spermatogenesis associated 21	SPATA21	2.5
Eukaryotic translation initiation factor 6	EIF6	2.5
1-Acylglycerol-3-phosphate O-acyltransferase 2	AGPAT2	2.3
β-galactosamide α-2,6-sialyltransferase	ST6GALNAC5	-2.2 (DOWN)

*Breeding compared to non-breeding season, $P < 0.01$.

Conclusions

1. The largest changes in gene expression during testes development happen in the first 4 months in the goat. Sertoli cell marker Sox9 decreases at 4 months (because of dilution with germ cells) while germ cell markers increase.
2. Microarrays detected 12 gene products that are differentially expressed in sperm between peak breeding season and non-peak season. 11 of the 12 were up-regulated during the breeding season. Of those, 5 genes (in red) were more highly expressed (> 5.8 fold) in the sperm from Alpine bucks compared to sperm from Boer bucks. Two (in green) are already linked to spermatogenesis.

Beneficiaries, Evaluation and Expected Impact

Male sub-fertility is a costly problem in many farm species. Current clinical tests to screen for fertility include analyses of sperm number, morphology, mortality, chromatin quality, and acrosomal integrity. These endpoints may fluctuate due to factors that are unrelated to overall fertility.

Our working hypothesis is caprine seminal plasma proteins, developmentally regulated genes and/or sperm mRNAs may be useful predictors of male fertility. Collectively, this project has identified novel proteins and gene products that may provide the foundation for new male fertility test. Analysis of sperm mRNA may provide new ways to assess sperm health and testis function.

This award provided partial support for two African American students pursuing their doctoral (A. Faucette, Texas A&M) and masters (D. Rucker, Prairie View A&M) degrees and undergraduate research experiences for two additional African American students. Three refereed journal publications are anticipated. The foundation for a pathway to doctoral degrees in the animal sciences has been strengthened.

Access to genetic resources and tools will enable producers to increase their profitability. These experiments provide new research-based knowledge and increase our understanding of biological events that can be used to develop technologies to improve reproductive efficiencies in animal production systems.