Peri-pubertal Changes in Structure, Gene Expression, and Testosterone Production in Goat Testes

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Abstract

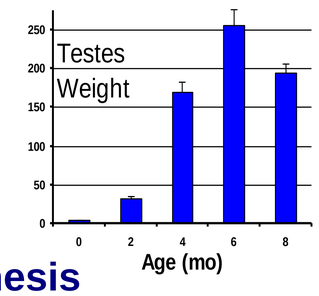
Goats are an important livestock species around the world, partly because they grow and mature quickly. The development of spermatogenesis and steroidogenesis was assessed in testes collected from bucks at 0, 2, 4, 6 and 8 months of age (n = 5 each age). Samples were split among five students. To determine when the seminiferous tubules of the testes grow and initiate spermatogenesis, histological sections were cut from formalin-fixed tissue and stained. Images were captured with a microscope and camera. The diameter of the seminiferous tubules, the lumen of the tubule, and the height of the seminiferous epithelium (determined in 30 tubules for each goat) increased from 0 to 4 months of age. RNA from the testes was reverse transcribed and used in PCR to amplify FSHR, CYP11A1, and RPLP0 cDNAs. Gel analysis revealed the desired amplicons in most reactions and eliminated others from quantitative PCR. The latter measured concentrations of FSHR, CYP11A1, LHR, and IGF1R mRNAs, normalized to GAPDH mRNA. IGF1R mRNA levels decreased from 2 to 4 months, while CYP11A1 decreased from 0 to 2 months. Testosterone was extracted from testes and measured with an enzyme-linked immunoassay. On a per testis basis, testosterone was greatest at 6 months. In conclusion, structural and functional maturity are evident at approximately 4 months of age in the Alpine goats used in this study. Presence of spermatids and spermatozoa was first evident at 4 months, correlating with the time of changes found in this study and initiation of puberty.

GENE NAME	ABBR.	PURPOSE
Follicle Stimulating Hormone Receptor	FSHR	Stimulates Sertoli cell function
Luteinizing Hormone Receptor	LHR	Stimulates testosterone synthesis
P450 side chain cleavage enzyme	CYP11A1	Steroidogenic enzyme
Insulin-like Growth Factor 1 Receptor	IGF1R	Stimulates testicular growth
Ribosomal Protein LP0	RPLP0	For normalization
GlycerAldehyde 3-Phosphate DeHydrogenase	GAPDH	For normalization

Introduction

The functions of mature testes, steroidogenesis and spermatogenesis, are linked by unknown mechanisms. They develop in mammals postnatally. This study was to assess changes in tissue growth, gene expression, and testosterone production during testis maturation.

Fig. 1. Means (±SEM) weight of the testes (g) of bucks at 0, 2, 4, 6, and 8 months of age (n = 5/age).



Hypothesis

Coordinated changes in seminiferous tubule morphology, testicular gene expression and testosterone production occur with attainment of reproductive maturity in bucks.

Experiment 1

Question: Does the diameter of the seminiferous tubules, lumen of the tubules and height of the seminiferous epithelium differ among age groups?

Experimental Approach:

Testis tissues were sectioned, mounted on glass slides & stained with hematoxylin and eosin Y. Images of 30 tubules per animal were captured using a Nikon microscope and analyzed.

Results: Tubule diameter, lumen diameter, and height of the seminiferous epithelium increased as goats matured (P<0.05).

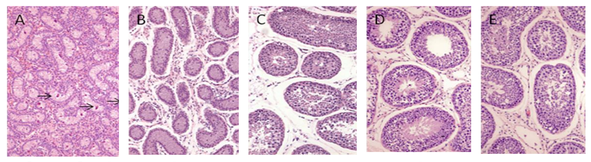


Fig. 2. Representative micrographs of testes stained with hematoxylin and eosin.

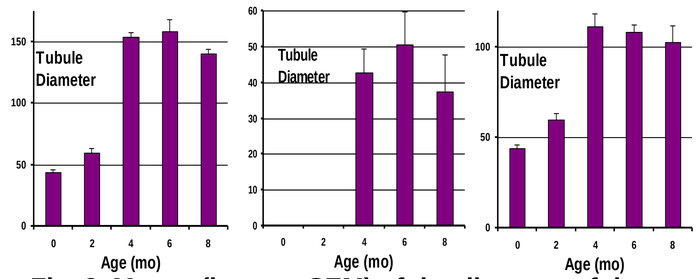


Fig. 3. Means (in um, ±SEM) of the diameter of the seminiferous tubules, diameter of lumina and height of the seminiferous epithelium in developing male goats.

Experiment 2

Question: During pubertal development, does expression of key genes in the testis differ?

Experimental Approach:

RNA extracted from testis tissue with TRIzol reagent was analyzed on a Nanodrop and a Bioanalyzer 2100. Reverse transcription of RNA used random hexamer and dT20 primers to synthesize cDNA. PCR amplification of FSHR, CYP11A1 and RPLP0 cDNAs were analyzed by electrophoresis on an acrylamide gel. Real-time RT-PCR data (threshold cycles) were used to calculate relative concentrations for LHR, FSHR, CYP11A1 and IGF1R mRNAs.

Results: Abundance of CYP11A1 and IGF1R mRNA decreased with age (P < 0.05).

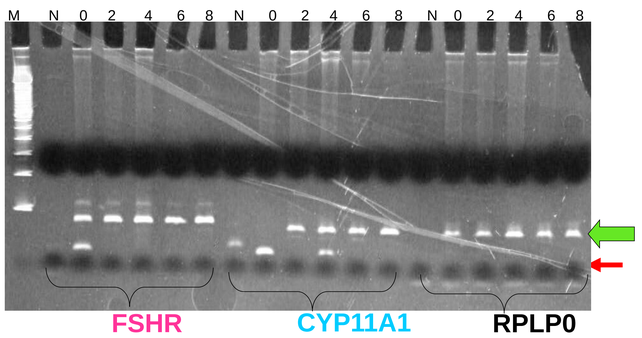


Fig. 4. A representative gel depicting RNA preparations from 0, 2, 4 6, or 8 month old goat testes that were reverse transcribed and used to amplify FSHR, CYP11A1, and RPLP0 cDNAs. The PCR products were electrophoresed on a 6% acrylamide gel stained with EtBr. Expected sized amplicons (green arrow) were apparent, but some lanes had smaller products (primer dimers, red arrow), artifacts that eliminated those from quantitative PCR reactions (below). Lanes: M = 100 bp markers; N = H20; 0,2,4,6,8 = age in months

Experiment 2 (cont'd)

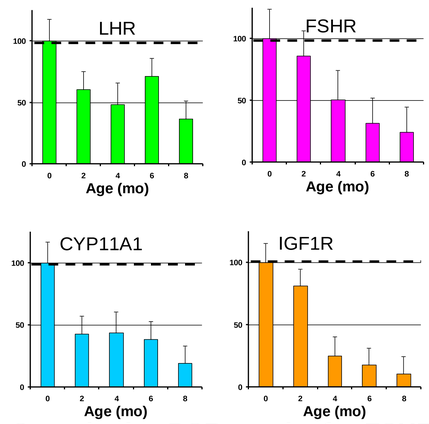


Fig. 5. Quantitative PCR results for FSHR, CYP11A1, LHR, and IGF1R. Data was normalized to GAPDH mRNA. Mean (±SEM) mRNA abundance for the age groups are depicted relative to 0 month of age group.

Experiment 3

Question: Does testosterone content in testis increase as bucks mature?

Experimental Approach:

Testis tissue was homogenized and extracted. Testosterone was analyzed with an enzyme immunoassay kit. A standard curve of testosterone was performed alongside to calculate testosterone concentration of samples.

Results: Testosterone concentrations per gram of testis were highly variable, but total testosterone per testis peaked at 6 months of age (P< 0.05).

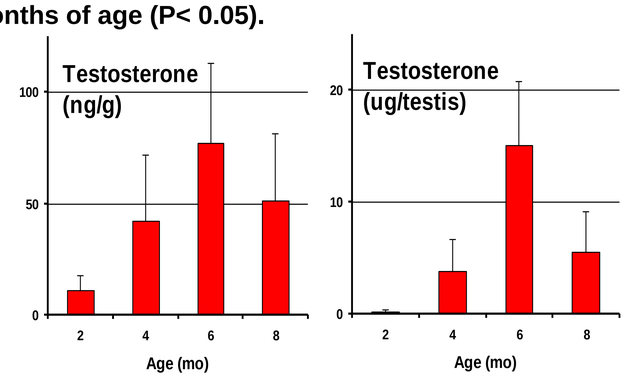


Fig. 6. Mean (±SEM) concentrations of testosterone in testis of developing goats shown on a per g and a per testis basis.

Conclusions

1. The structure of the testis changes dramatically between 0 and 4 months of age in the buck with increases in seminiferous tubule diameter, lumen and height of the seminiferous epithelium .
2. CYP11A and IGF1R mRNA concentrations decrease at 2 or 4 months of age.
3. Total testosterone content in the testis is peaks at approximately 6 months of age.

OVERALL, buck testes are active in steroidogenesis and expression of genes important to reproduction and growth at 0 and 2 months, while spermatogenesis begins between 2 and 4 months of age.

Acknowledgements

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