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Prospective ultrasonographic and endocrine predictors of spermatogenic onset in ram lambs

Pawel M. Bartlewski*, Jennifer L. Giffin, Olutobi A. Oluwole, Ann C. Hahnel

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada

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ABSTRACT

The objective of this study was to examine testicular ultrasonographic characteristics and endocrine profiles in prepubescent ram lambs for correlations with the age at first detection of elongated spermatids (ESt age). Bi-weekly ultrasound examinations and weekly testicular biopsies began at 10 weeks of age or at the time that testicular volume reached 15 cm^3, and continued until 1–2 weeks after Est's were first detected by histological examination of testicular biopsies in twenty-two spring-born Rideau Arcott × Polled Dorset lambs. Computer-assisted analysis of testicular ultrasonograms was performed using commercially available image analytical software. Blood samples were drawn before each ultrasonographic examination and were used for measurements of free triiodothyronine (fT_3) and thyroxine (fT_4), and follicle-stimulating hormone (FSH) concentrations. The mean (±SEM) age at first detection of ESts was 15.9 ± 0.5 weeks. Testicular volumes recorded between 10 and 12 weeks of age correlated inversely with the ESt age ($r = -0.44$ to -0.50, $P \leq 0.05$). Statistically significant correlations were recorded between the ESt age and numerical pixel values of testicular parenchyma at 10 ($r = -0.48$, $P = 0.05$) and 15 ($r = 0.52$, $P = 0.05$) weeks of age, and between the ESt age and testicular pixel heterogeneity in ram lambs aged 14.5 weeks ($r = 0.60$, $P = 0.007$). Lastly, circulating FSH concentrations at 10 weeks ($r = -0.43$, $P = 0.05$), serum fT_3 concentrations at 13 weeks ($r = 0.44$, $P = 0.04$) and fT_4 concentrations at 11.5 weeks of age ($r = 0.48$, $P = 0.03$) were all correlated with the ESt age. The present results show that testicular volume has the most stable relationship with pubertal onset; however, testicular echotexture as well as circulating concentrations of FSH and free fractions of thyroid hormones at specific ages may be indicative of more intricate developmental events heralding puberty.

1. Introduction

The age at which animals reach puberty dictates their future reproductive potential and lifetime productivity (Chandolia et al., 1997a,b; Aravindakshan et al., 2000; Bagu et al., 2004; Rawlings et al., 2005). Recent studies have also found that the attainment of puberty is a significant predictor of general and reproductive health in later life; relatively early or delayed onset of puberty in humans is associated with increased prevalence of nearly 50 different diseases (Day et al., 2015; Ruth et al., 2016). Since pubertal timing can act as a biological marker of fertility and certain health conditions in mammalian species, it would be an invaluable asset to be able to accurately predict the onset of sexual maturity in individual animals and human beings.

Prepubertal testicular development is associated with dynamic changes in testicular macro- and microstructure (Oluwole et al., 2013; Giffin et al., 2014). Due to the

* Corresponding author at: University of Guelph, Ontario Veterinary College, Department of Biomedical Sciences, 50 Stone Road, Guelph, ON N1G 2W1, Canada.
E-mail address: pmbart@uoguelph.ca (P.M. Bartlewski).

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onset of spermatogenesis, there is a tremendous increase in cellular number and diversity within the seminiferous tubules (STs). Thyroid hormones and follicle-stimulating hormone (FSH) exert stimulatory effects on ST maturation, facilitating the differentiation of Sertoli cells in prepubertal ram lambs (Kilgour et al., 1998; Sharpe et al., 2003; Oluwole et al., 2013). Ultrasonography is the primary imaging modality for evaluating the structure and development of the scrotal content owing to its non-invasiveness and versatility (Ragheb and Higgins, 2002; Ahmadi et al., 2012). Ultrasonography in conjunction with computer-assisted image analysis has also ousted other imaging and diagnostic techniques in the studies of histomorphological changes in the testes (Ragheb and Higgins, 2002; Omer et al., 2012). Scrotal ultrasonography can aid in the evaluation of ST lumination (Giffin et al., 2014), cell density (Omer et al., 2012; Giffin et al., 2014), shifts in the proportions of various germ cell types in prepubescent males (Ahmadi et al., 2013; Giffin et al., 2014), chemical composition of the testis (Ahmadi et al., 2013), and future semen morphology (Ahmadi et al., 2012). Recent advances in the application of ultrasonographic technology have led to new observations on cellular and subcellular changes in the testis that are only now being studied and have yet to be completely understood (Giffin et al., 2014).

Based on previously documented developmental changes in the gonads of prepubescent ram lambs and existing quantitative correlations among echotextural and histophysiological attributes of the testis, we hypothesized that ultrasonographic and hormonal variables recorded during the first wave of spermatogenesis in prepubescent ram lambs would correlate with the age at spermatogenic onset. Hence, the primary objective of this study was to examine ultrasonographic characteristics of the testes and variations in serum concentrations of FSH and free fractions of thyroid hormones for correlations with the age at first detection of elongated spermatids in growing ram lambs.

2. Material and methods

All experimental procedures described in this section were approved by the Animal Care Committee at the University of Guelph. Twenty-two spring-born Rideau Arcott × Polled Dorset ram lambs were housed in a field research station near Guelph, ON, Canada (latitude: 43°33′ N), and kept constantly under ambient light and temperature conditions (Fig. 1). All lambs were born within one week from one another. From 7 to 100 days after birth, they received a 16% crude protein lamb grower mix (Shur-Gain Feedmills, St. Marys, ON, Canada) *ad libitum*, and it was estimated that from weaning at 50 days of age until 100 days of age, when all ewe and ram lambs were penned together, daily consumption of the grower averaged 1.1 kg per lamb. Hay was also offered after weaning. After 100 days of age, ram lambs were penned separately and fed a diet of 80% barley and 20% corn with the addition of a 36% crude protein sheep supplement (Shur-Gain Feedmills, as above) and hay; daily food intake averaged 0.5 kg of grain and 0.2 kg of the supplement per ram. Water and mineralized salt licks were constantly available to the rams.

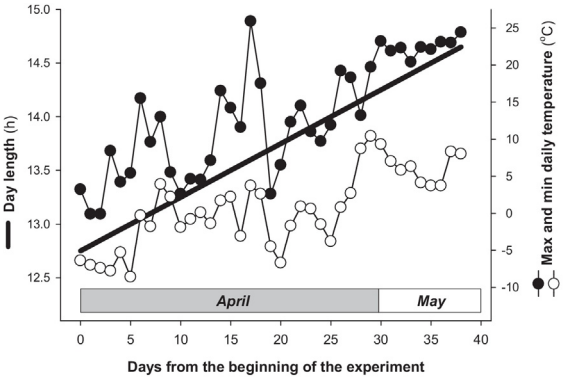

Fig. 1. Duration of day lengths (light hours) and maximum and minimum temperatures recorded throughout the present experiment. All meteorological data were obtained from https://www.timeanddate.com/sun/canada/guelph and http://climate.weather.gc.ca.

Bi-weekly ultrasound evaluations and weekly testicular biopsies began at 10 weeks of age or at the time when testicular volume reached 15 cm^3 (*i.e.*, approximately 3 weeks before the onset of the post-mitotic phase of testicular development characterized by the presence of spermatocytes and round spermatids as the most mature germ cell types; Giffin et al., 2017), and continued until 1–2 weeks after elongated spermatids (ESt's) were first detected by histological examination of testicular biopsies. Scrotal ultrasonography utilized an Aloka SSD-900 portable ultrasound scanner connected to a 7.5-MHz linear-array transducer (Aloka Inc., Tokyo, Japan) with the settings for main (80% of maximum), near and far gains (50% of maximum), and focal points (0.5 and 3.5 cm) kept constant throughout the study. Both testes were scanned in longitudinal and transverse planes, and all images were recorded (Pioneer DVD Recorder DVR-510H; Pioneer Electronics of Canada Inc., Markham, ON, Canada) for later image analysis. Testicular dimensions were measured using internal electronic calipers and testicular volume (TV) was calculated with the formula $TV = 1/6\pi \times length \times width^2 \times 0.945$ developed by Wrobel (1990) for domestic ruminants. Core needle biopsies (12- to 16-gauge E-Z Core Single Action Biopsy Device; Products Group International, Lyons, CO, USA) of the left testis only were collected from all animals; the right testis served as a control of an invasive procedure. Prior to taking biopsies, animals were sedated with xylazine (Rompun, Bayer, Toronto, ON, Canada; 0.2 mg/kg i.m.). Biopsies were left overnight in modified Davidson's fixative, washed in 70% ethanol and embedded in paraffin wax blocks before sectioning at a thickness of 5 μm, and then deparaffinized in xylene and rehydrated in a graded isopropanol-water series for staining with hematoxylin and eosin, using standard procedures. Subsequent histological analyses were performed using Image ProPlus® 7.0 analytical software (Media Cybernetics Inc., San Diego, CA, USA) on biopsy micrographs obtained with Q Capture (Quorum Technologies Inc., Guelph, ON, Canada) at 200x image magnification. Computer-assisted analysis of testicular ultrasonograms was also performed with Image ProPlus®, using the "spot meter" technique (six spots in a longitudinal view and four spots in a transverse view,