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## Understanding and evaluating bovine testes

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#### ABSTRACT

The objective is to briefly review bovine testes and how they are assessed, with an emphasis on articles from Theriogenology. Scrotal circumference (SC) is the most common method to assess testicular size; it varies among individual bulls and breeds and is highly heritable. In general, a large SC is associated with early puberty, more sperm, a higher percentage of morphologically normal sperm, and better reproductive performance in closely related females. Consequently, there are minimum requirements for SC for breeding soundness. In prepubertal bull calves, there is an early rise (10–20 weeks of age) in LH, which is critically related to onset of puberty and testicular development. Feeding bulls approximately 130% of maintenance requirements of energy and protein from approximately 8 to 30 weeks of age increased LH release during the early rise, hastened puberty (approximately 1 month), and increased mature testis size and sperm production (approximately 20%-30%). However, high-energy diets after weaning (>200 days) often reduced sperm production and semen quality. A bull's testes and scrotum have opposing (complementary) temperature gradients, which keep the testicular temperature 2 °C to 6 °C cooler than core body temperature for production of fertile sperm (increased testicular temperature reduces semen quality). Infrared thermography, a quick and noninvasive method of assessing scrotal surface temperature, may be beneficial for evaluations of breeding soundness. The primary clinical use of ultrasonography in assessment of reproductive function in the bull is characterization of grossly detectable lesions in the testes and scrotum. In conclusion, testis size and function are critical for bull fertility, affected by nutrition, and readily assessed clinically.

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#### 1. Introduction

To optimize reproductive function for either natural service or artificial insemination, bulls should produce large numbers of morphologically normal fertile sperm. Therefore, knowledge regarding bovine testes and how they are evaluated, is of great importance. The purpose of this article is to briefly review factors controlling gonadal development and puberty, the importance of testis size in breeding soundness, and mechanisms regarding testicular thermoregulation. In addition, methods for evaluating testicular size, temperature, and integrity are also

discussed. Because this article is being prepared as part of an anniversary issue of *Theriogenology*, emphasis is given to publications from this journal.

#### 2. Measurement of testicular size

A comprehensive review of bovine testicular measurements published in *Theriogenology* in 1979 [1], still has considerable relevance. In that review, it was noted that testicular weight provides an accurate estimate of sperm producing potential [1]. Furthermore, because testicular weight cannot be directly measured in a noninvasive way in breeding bulls, indirect measurements of the testes (and scrotum) have been developed, including testis length and diameter, paired testis width, and scrotal circumference

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(SC) [1]. Linear measurements are done with a caliper, whereas SC is done with a tape (various commercial tapes are available). Although it has been suggested that using a caliper to measure testis size is better than measurement of SC [2], in field conditions, measurement of SC remains the most common approach. In that regard, SC is regarded as an accurate predictor of paired testis weight (correlations ranged from 0.89-0.95 in dairy bulls and were 0.95 in beef bulls [1]). Furthermore, the correlation between SC and sperm output in young dairy bulls was 0.81 [3]. However, there are indications that shape of the testes could affect SC and sperm production. In one study [4], 27 Holstein bulls were categorized according to testicular shape. In that study, bulls with long and slender testes had the lowest SC but produced the greatest number of sperm, whereas bulls with short, spheroid testes had the largest SC, but produced the fewest sperm. This is an interesting observation that is certainly worthy of follow-up study.

There were profound changes in SC with age (distinct sigmoid curve): slow growth before 25 weeks of age, very rapid growth during the peripubertal phase, slow growth in mature bulls, and if bulls were retained for many years, a subsequent decline (because of senescence) after approximately 144 months of age [1]. In addition to the effects of age, there was considerable variation among bulls in SC; because the average (of several studies) heritability for SC was approximately 0.67 [1], it was concluded that rapid progress could be made by selection of bulls with large SC. In a subsequent study involving more than 3000 yearling beef bulls, paternal half-sib estimates of heritability were  $0.41 \pm 0.06$  for age-constant scrotal circumference,  $0.34 \pm$ 0.06 for testicular length, and 0.37  $\pm$  0.06 for calculated paired testicular volume [5]. Furthermore, it was noted that SC might be a reliable measure of puberty and bulls with a smaller SC were much more likely to have unsatisfactory semen quality [1]. Finally, SC was positively associated with growth characteristics and with conception rates in closely related female animals [1], although in a subsequent study [6], there was very little association between growth traits and SC in young beef bulls.

There are substantial differences among breeds in SC. In the 1979 review paper on assessing testicular size, it was noted that there was a need to collect additional breed-specific SC data [1]. In a subsequent study [7], SC was measured on 7918 beef bulls (2-year-old) of several breeds. The authors' recommendations of minimum acceptable SC for 2-year-old bulls were: Simmental, 36.0 cm; Angus and Charolais, 35.0 cm; horned and polled Herefords and Shorthorn, 34.0 cm; and Limousin, 33.0 cm.

#### 3. Puberty

Puberty in bulls is usually defined as collection of an ejaculate that contains at least  $50 \times 10^6$  sperm, of which at least 10% are progressively motile [8]. Alternatively, an SC of 28 cm has been used as a measure of puberty (reviewed in Rawlings et al. [9]).

In prepubertal bull calves, there is an early transient rise (between 10 and 20 weeks of age) in gonadotropin secretion (particularly LH); there is ample evidence that this is of critical importance with regard to puberty and testicular

development [9]. In a comparison of beef bulls reaching puberty early versus late (41.9  $\pm$  0.3 and 48.3  $\pm$  0.7 weeks of age, respectively), serum LH concentrations were greater (P < 0.05) in the early maturing bulls at 12, 13, 15, 17, and 48 weeks of age [10]. In a subsequent similar study, beef bull calves that reached puberty either early (36.6–44.2 weeks, n = 12) or late (46.4–48.9 weeks, n = 8), serum LH concentrations in the former group of bulls were significantly greater at 12, 14, and 16 weeks of age [11]. Furthermore, in that study, mean serum LH concentrations at 4, 10, and 40 weeks of age and LH pulse frequency at 10 and 20 weeks of age were negatively correlated with age at puberty.

There is considerable interest in early-life prediction of future reproductive events. Sexual development and reproductive function were studied in 22 Angus × Charolais and 17 Angus bulls from 6 to 16 months of age [12]. Age, weight, SC, and paired-testes volume were all good predictors of pubertal and mature status, with moderate to high sensitivity and specificity (71.6%-92.4%). In another study using response to exogenous GnRH as a means of predicting onset of puberty in bulls [13], peak serum LH concentrations and area under the LH response curve were lower (P < 0.05) in early- versus late-maturing bulls at 4 and 20 weeks of age. Therefore, the authors concluded that the response of LH to GnRH in calves could be useful to select bulls with early puberty. In another study involving 264 young beef bulls, 200-day-old calves with SC >23.0 cm had a 95% probability of achieving SC >34.0 cm by 1 year, whereas those with an SC <23 cm at 200 days had only a 54% probability of having an SC >34.0 cm by 1 year [14].

In a study characterizing sexual development in early-and late-maturing Nelore (Bos indicus) and Canchim (3/8 Bos indicus × 5/8 Bos taurus crossbred) bulls, early-maturing bulls were lighter and had a smaller SC at puberty than late-maturing bulls [15]. Therefore, it was concluded that sexual precocity was not related to lower thresholds for body weight and SC. Furthermore, yearling SC could be used to predict onset of puberty, whereas age, weight, and SC were equally good predictors of sexual maturity in B. indicus bulls.

In an attempt to hasten the onset of puberty, bull calves were given 3 mg of bovine LH (n = 6) or 4 mg of bovine FSH (bFSH; n = 6) once every 2 days, from 4 to 8 weeks of age [16]. In this study, the age at which the bulls first had an SC  $\geq$ 28 cm (as a measure of puberty), occurred earlier (P < 0.05) in bFSH-treated than control calves (39.3  $\pm$  1.3 vs. 44.8  $\pm$  1.3 weeks, respectively). Furthermore, at 56 weeks, bulls given bFSH had significantly more Sertoli cells and elongated spermatids and spermatocytes. Therefore, exogenous bFSH from 4 to 8 weeks of age enhanced testicular growth (SC), hastened puberty (based on SC  $\geq$ 28 cm) and promoted spermatogenesis. In contrast, although exogenous bovine LH increased serum LH concentrations and appeared to enhance spermatogenesis to some extent, the improvements were not significant.

#### 4. Effects of nutrition on testicular development

In a series of experiments to determine the effects of postweaning nutrition on reproductive development and

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