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Anti-Müllerian hormone concentration and scrotal circumference as predictors of fertility-linked characteristics in male Brahman and Simmental bulls

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ABSTRACT - This study investigates whether pre-pubertal scrotal circumference (SC) and anti-Müllerian hormone (AMH) concentrations indicate sexual precocity in Brahman and Simmental bulls. Fifty-three bulls (Brahman, n = 27; Simmental, n = 26) were used. Scrotal circumference was measured at three periods: pre-puberty (Brahman = 19.99±0.20 months; Simmental = 9.13±0.17 months), puberty (Brahman = 21.42±0.20 months; Simmental = 18.26±0.17 months), and post-puberty (Brahman = 33.34±0.55 months; Simmental = 24.07±0.17 months). A single sample taken at the pre-pubertal period was used for the AMH determination. Two classes were defined based on the frequency distribution of AMH concentrations: low AMH <2.28 ng/mL and high AMH ≥2.28 ng/mL. Scrotal circumference was negatively correlated with the percentage of total defects (r = −0.5957; P = 0.0246) and positively correlated with the percentage of normal sperm (r = 0.595; P = 0.0265). There was a high correlation between SC and assessment periods (pre-puberty, puberty, and sexual maturity) in both breeds (Brahman: r = 0.8776; P = 0.0005; Simmental: r = 0.7483; P = 0.0002). At puberty, SC was higher (P = 0.0002) in animals of the low AMH class (34.78±0.78 cm) than in the high AMH class (32.47±0.42 cm). Males classified as having low AMH concentration had better (P<0.0001) andrological assessments, (vigorous oscillatory movement or vigor: 3.92±0.20; sperm mass movement or turbulence: 2.63±0.19; progressive motility: 77.55±3.80) than those classified as having high AMH concentration (vigor = 3.33±0.19; turbulence = 2.06±0.19; progressive motility = 65.14±3.79). Pre-pubertal AMH concentrations are negatively correlated with SC and are a good predictor of sexual precocity in Brahman and Simmental bulls.

Keywords: AMH, bull selection, sexual precocity, sperm

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1. Introduction

Selection of bulls for breeding, whether from an andrological or a genetic improvement point of view, serves to increase the reproductive potential of the sire, ensure longer life in the herd,

and efficiently pass on desirable traits to the offspring. Therefore, looking for features that indicate early reproductive potential in bulls is paramount to the success and speed of genetic improvement. Scrotal circumference (SC) is characterized by being easy to measure and having high heritability and repeatability (Bergmann et al., 1997); moreover, it positively correlates with qualitative and quantitative semen traits, body weight, and age at puberty in both males and females (Kastelic, 2014) and may also indicate greater sexual precocity (Freneau et al., 2006).

After birth, bulls undergo three stages of development: pre-pubertal, pubertal, and post-pubertal. Bulls that reach puberty at 15 months of age can be used as sires from the second stage. However, bulls that take more than 17 months to reach puberty can only be used as sires during the post-pubertal stage, producing progeny from the age of 36 months on. Therefore, shortening the pubertal stage of bulls reduces the generation interval, and production costs (Siddiqui et al., 2008; Fortes et al., 2012; Costa e Silva et al., 2013).

One of the selection goals in *Bos indicus* breeding is to reduce age at puberty (Baldi et al., 2016; Fernandes et al., 2018). In Nellore bulls, age at puberty has a moderate heritability; however, breeders have been successful in reducing it to 12 (0.33 adjusted to 365 days) or 15 (0.37 adjusted to 450 days) months (Quirino et al., 1999; Dias et al., 2003).

Predicting the onset of puberty in bulls is important because they must be available for market and herd replacement, in addition to lowering production costs by selecting the most precocious and fertile individuals (Schwengber et al., 2001; Silveira et al., 2010; Mello, 2014). After six months of age, the calf's testicular parenchyma rapidly increases, leading to significant testicular growth, which is due to the increased diameter and length of the seminiferous tubules (Coulter, 1986; Curtis and Amann, 1981). Concurrently with testicular growth and body growth rate of the bull-calf, spermatogenesis capacity increases gradually towards puberty (Coulter, 1986; Curtis and Amann, 1981). However, once the male reaches puberty, the rate of testicular growth decreases, and, despite this slowdown in growth, it remains constant until the bull reaches sexual maturity (Evans et al., 1995; Coulter, 1986).

A pubertal marker that has been studied is the anti-Müllerian hormone (AMH), which is a glycoprotein secreted by the Sertoli cells in the testicles. It is known that the expression of AMH is negatively regulated during puberty by androgen hormones and decreases dramatically in the seminiferous tubules (Okay, 2003). Furthermore, the reduced expression of AMH is closely related to the onset of spermatogenesis in the seminiferous tubules (Almeida et al., 2012). Additionally, AMH blood concentrations in bulls increase during the testicular cellular growth and proliferation phases (Coen et al., 2021). This phase is characterized by increases in the number of Leydig cells, mitosis of pre-Sertoli cells, and in the diameter of seminiferous tubules, along with the development of the lumen of the seminiferous tubules (Wrobel, 2000). Simultaneously, the anterior pituitary gland secretes follicle-stimulating (FSH) and luteinizing (LH) hormones in response to the gonadotropin-releasing hormone (GnRH), which trigger morphological changes in the testicles of bulls (Evans et al., 1996).

In addition, immature Sertoli cells secrete more AMH during sexual differentiation from the fetal phase to puberty (Rey et al., 1993; Ford and Wise, 2009), as indicated by the relationship between the increase in AMH concentrations and the higher proportion of immature Sertoli cells (Rajpert-De Meyts et al., 1999; Al-Qahtani et al., 2005). In turn, the percentage of mature Sertoli cells assessed by testicular cytology has been positively correlated with bull semen quality (Rajak et al., 2016; Rajak et al., 2014), besides determining testicular size, daily spermatogenesis rate, and sperm quality (Johnson et al., 2008; Rajak et al., 2014). These findings suggest that AMH serves as a puberty biomarker.

The relationship of AMH in peripheral blood plasma with ovarian follicular reserve and function has already been demonstrated in cows (Maculan et al., 2018), but further studies are needed to assess AMH expression and peripheral concentrations in bulls.

Anti-Müllerian hormone may be an efficient marker of sexual precocity, allowing bulls to be selected before they reach reproductive age. It is hypothesized that low AMH concentrations and higher scrotal circumference in pre-puberty indicate sexual precocity in young Zebu (Brahman) and Taurine

(Simmental) bulls. The objectives of the present study, therefore, were to investigate whether the pre-pubertal SC and AMH concentrations indicate sexual precocity in Brahman and Simmental bulls. We hypothesize that the higher the pre-pubertal AMH concentrations, the lower the semen quality in zebu and taurine bulls.

2. Material and methods

Research on animals was conducted according to the institutional committee on animal use with protocol number (064/18).

2.1. Animals and facilities

Fifty-three young bulls were selected from a private farm in Silvianópolis, Minas Gerais, Brazil (22°1'47" South, 45°50'7" West, with an elevation of 887 meters). This group included 27 Brahmans (*Bos taurus indicus*) aged between 20 and 22 months and 26 Simmentals (*Bos taurus taurus*) aged between nine and 18 months. The selection criteria were adopted according to breed (Taurine and Zebu), age group, and weight. The animals grazed on *Brachiaria brizantha* pasture, and their diet was supplemented *ad libitum* with corn silage and concentrate containing 18% crude protein provided in the trough. All animals had access to mineral supplementation and water *ad libitum*. Weighing was conducted during data collection days, with the animals restrained in a chute and weighed using an electronic balance (Progresso WeighTech®).

2.2. Scrotal circumference

Scrotal circumference was measured at pre-puberty (obtained from Brahmans aged 20 months and Simmentals aged nine months), puberty (obtained from Brahmans aged 22 months and Simmentals aged 18 months), and post-puberty (obtained from Brahmans aged 30-35 months and Simmentals aged 22-26 months). It was measured using a flexible tape measure at the widest part of the scrotum, with slight ventral-caudal traction applied to the gonads towards the distal end of the scrotum.

2.3. Andrological assessment

Semen collections were performed quarterly to determine the onset time of puberty in both breeds. Because semen quantity and quality can be a difficult phenotype to define, due to the logistics of frequent semen collection and analysis, more easily measurable proxy traits, such as reaching a SC of ≥28 cm, have also been used (Lunstra et al., 1978). Samples were collected quarterly to avoid stress and injuries as they were performed using an electro ejaculator. Animals were considered pubertal when they had an ejaculate with at least 10% motility and sperm concentration of 50×10^6, according to the methodology proposed by Wolf et al. (1965). The results from two of these three sample collections were used, with the first obtained during puberty (from Brahmans aged 22 months and Simmentals aged 18 months) and the second at a randomly determined stage of sexual maturity or post-pubertal development (from Brahmans aged 30-35 months and Simmentals aged 22-26 months).

The seminal parameters were evaluated using a microscope at 10X magnification, and turbulence (sperm mass movement on a scale of 0-5) was assessed. Then, a drop of semen was placed between the slide and coverslip (previously heated to 37 °C), and straight-line sperm motility (%) and vigorous oscillatory movement (0-5) were assessed at 400X magnification (CBRA, 1998).

For semen pathology analysis, ejaculate aliquots enough to cloud the solution were added to a tube containing 1 mL of buffered saline-formaldehyde solution (Hancoch, 1956). The technician counted 400 sperm cells per ejaculate and assessed the percentage of normal spermatozoa as well as abnormalities in the acrosome, head, midpiece, and tail, following the guidelines recommended by the Colégio Brasileiro de Reprodução Animal (Brazilian College of Animal Reproduction; CBRA, 1998).

The abnormalities were classified into major, minor, and total sperm defects based on the criteria established by Blom (1973). Any type of abnormality correlated with fertility impairment or a pathological condition of the testicle or epididymis was deemed a "major defect". Other, seemingly less important shape deviations, such as a slender, normal but small, giant, short and flat, or normal but detached head, detached acrosome, abaxial insertion, distal drop, bent or curled tail, and curled tail at the end, were deemed "minor sperm defects". The sum of all major and minor sperm defects will be henceforth referred to as "total sperm defects".

2.4. Blood sampling and AMH determination

A blood sample was taken from each calf before they reached puberty; the average age of blood collection was 20 months for Brahman and nine months for Simmental bulls. The samples were obtained through venipuncture at the tailbone using 10-mL vacuum tubes (BD VACUTAINER®, São Paulo, BR). After centrifugation at 3000 *g* for 15 min, the serum was collected and stored at −20 °C for future analysis. Serum AMH concentrations were determined by enzyme-linked immunosorbent assay (ELISA Kit; Ansh Labs, Webster, Texas, USA), previously validated for cattle (Ireland et al., 2008), catalog number Bovine AMH ELISA AL-114. An assay was performed with a sensitivity of 0.011 ng/mL and an intra-assay variability of 1.8-2.8. The tests were performed in the IgAc laboratory (GENESE Institute of scientific collections, São Paulo, BR). Classes were formed based on the frequency distribution of AMH concentrations: low AMH <2.28 ng/mL and high AMH ≥2.28 ng/mL.

2.5. Statistics

All analyses were performed using JMP-Pro 12 (SAS®). Data were subjected to analysis of variance as repeated measures in a mixed model according to normality (Shapiro-Wilk) and homoscedasticity tests, comparing the fixed effects of AMH class (low <2.28 ng/mL and high ≥2.28 ng/mL), period, breed, and interactions against semen quality data and the SC. The error term was the random effect of the animal within the breed. The dependent variables were: SC, turbulence, vigorous oscillatory movement, volume, concentration, progressive motility, major, minor, total, and standard errors. Variables were subjected to a Pearson's correlation test using the PROC CORR procedure. Data are presented as means±standard errors of the mean. Tukey's test compared the means; differences were considered significant when $P<0.05$. Scrotal circumference was normalized using the Johnson Sb conversion and subjected to ANOVA, with lsmeans compared by contrast. Progressive motility was analyzed using a generalized linear model with a Poisson distribution, adjusted for overdispersion by Pearson Chisq/DF. Vigorous oscillatory movement was also analyzed using a generalized linear model with a Poisson distribution, and means were compared using contrasts. The best-fit lines were tested under the regression analysis for linear, quadratic, and cubic models and the highest coefficient of determination (r) was assumed for each pubertal status.

3. Results

3.1. Scrotal circumference

The mean SC in pre-pubertal Brahman bulls (age 19.99±0.20 months) was 28.90±3.48 cm (n = 27). In pubertal bulls (age 21.42±0.20 months), the average SC was 32.24±3.40 cm. For Simmental bulls, the average SC at pre-puberty (age 9.13±0.17 months) was 24.46±2.85 cm (n = 26), and at puberty (18.26±0.17 months) it was 36.57±3.45 cm. Scrotal circumference was negatively correlated with the percentage of sperm with total defects ($r = -0.5957$; $P = 0.0246$) and positively correlated with the percentage of normal sperm ($r = 0.595$; $P = 0.0265$). There was a high correlation between the SC measurements taken at the different periods (pre-puberty, puberty, and sexual maturity) in both breeds (Brahman: $r = 0.8776$; $P = 0.0005$; Simmental: 0.7483; $P = 0.0002$), demonstrating the high repeatability of this variable. The SC at puberty was higher ($P = 0.0002$) in animals of the low AMH class

(34.78±0.78 cm) compared with those of the high AMH class (32.47±0.42 cm). The AMH concentration was associated with SC in the pre-pubertal, pubertal, and post-pubertal stages (P = 0.0044, P = 0.0038, P = 0.0078, respectively) (Figures 1, 2, and 3, respectively). Therefore, bulls with lower pre-pubertal AMH concentrations had higher SC. The best fit lines and adjusted coefficients of determination (r) for pre-pubertal, pubertal, and post-pubertal were cubic (r = 0.18, P = 0.004), linear (r = 0.14, P = 0.004), and quadratic (r = 0.21, P = 0.008), respectively (Figures 1, 2, and 3, respectively).

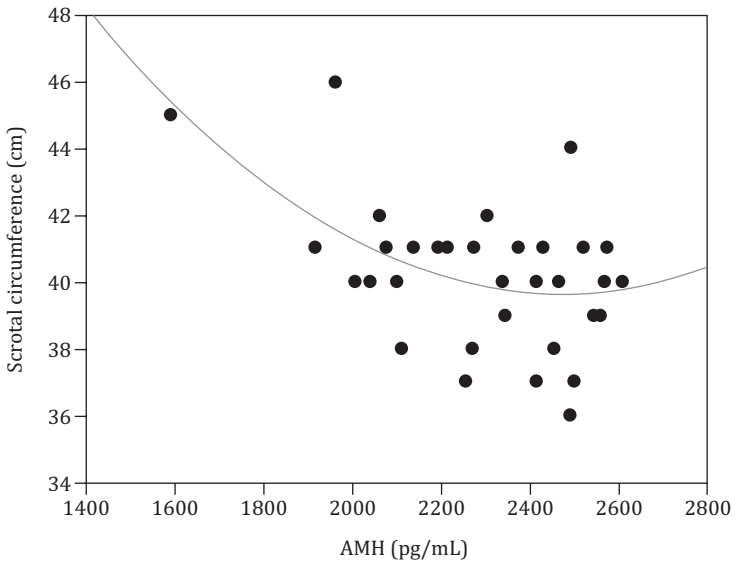

Figure 1 - Association between anti-Müllerian hormone (AMH) concentration and scrotal circumference in the pre-pubertal stage.

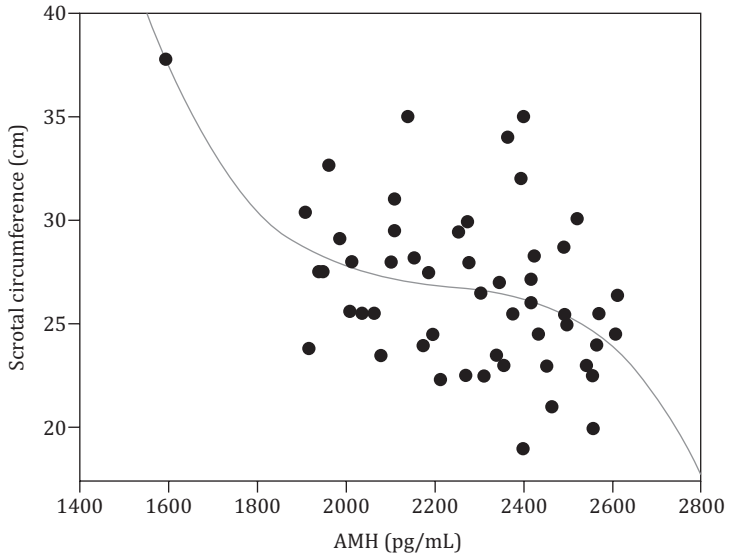

Figure 2 - Association between anti-Müllerian hormone (AMH) concentration and scrotal circumference in the pubertal stage.

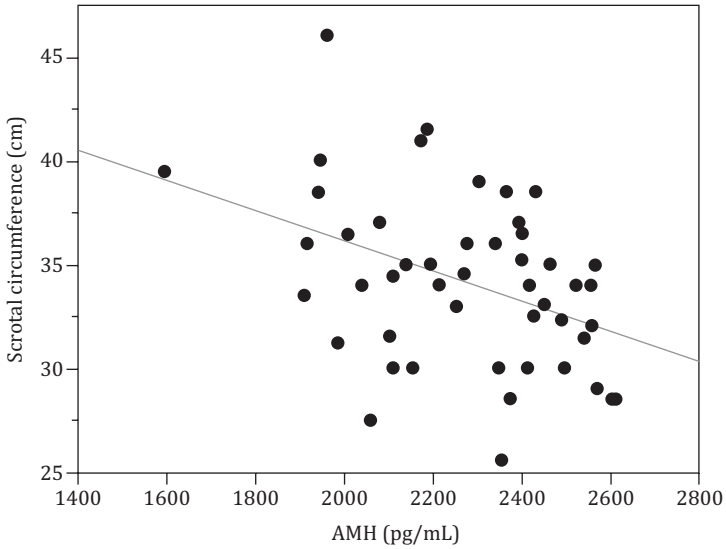

Figure 3 - Association between anti-Müllerian hormone (AMH) concentration and scrotal circumference in the post-pubertal stage.

3.2. Andrological assessment

Physical (volume, vigorous oscillatory movement, progressive motility, and turbulence) and morphological (percentage of large defects and normal spermatozoa) parameters differed between breeds, with better results observed in Simmental bulls (Table 1). It is important to consider environmental factors such as heat stress, which has been shown to influence semen production and quality by affecting ejaculate density and sperm motility (Popović et al., 2024). This highlights the necessity for environmental management in breeding programs to ensure optimal reproductive performance. The evaluation period (puberty and sexual maturity) influenced the semen parameters, and when the bulls reached sexual maturity, they showed better semen quality (Table 2). Progressive motility was higher (P<0.0001) in low AMH animals (Figure 4). The progressive motility in the Simental breed was higher (89.70±3.90) than in Brahman (52.99±3.69) (Table 1). Vigor was higher (P<0.05) in low AMH animals (Table 3). Vigor was higher (P<0.0001) in the Simmental breed (4.60±0.20) than in the Brahman breed (2.66±0.19). Turbulence was higher (P<0.01) in low

Table 1 - Influence of breed on physical and morphological semen parameters in Brahman and Simmental bulls at pubertal age

Parameter	Brahman (n = 27)	Simmental (n = 26)	P-value
Volume	4.91±0.52a	7.31±0.55b	0.0021
Vigorous oscillatory movement (1-5)	2.66±0.19a	4.60±0.20b	<0.0001
Concentration (10^6)	366.51±35.68a	646.03±39.46b	<0.0001
Progressive motility (%)	52.99±3.69a	89.70±3.90b	<0.0001
Turbulence (1-5)	1.19±0.18a	3.50±0.19b	<0.0001
Major defects (%)	7.84±1.47a	12.79±1.55b	0.0227
Normal sperm (%)	57.55±3.80a	74.55±4.02b	0.0028

Letters in the same row indicate differences (P<0.05).
Values are least-squares means±standard errors of the mean.

AMH animals and higher (P<0.0001) in Simmental breed (3.50±0.19) than in Brahman (1.19±0.18). Semen concentration was higher (P<0.0017) in Simmental breed (646.03±39.46) than in Brahman (366.51±35.68). There were no significant effects of breed, AMH, or their interaction in major and minor sperm defects. However, a breed effect was observed on total sperm defects.

Table 2 - Influence of reproductive period on seminal parameters in Brahman and Simmental bulls

Parameter	Puberty	Sexual maturity	P-value
Volume	4.47±0.50a	7.75±0.57b	0.0021
Vigorous oscillatory movement (1-5)	3.00±0.18a	4.24±0.21b	<0.0001
Concentration (10^6)	329.12±35.16a	683.42±39.72b	<0.0001
Progressive motility (%)	60.81±3.54a	81.88±4.04b	0.0002
Turbulence (1-5)	2.03±0.20a	2.67±0.17b	0.0185
Major defects (%)	13.80±1.41a	6.79±1.61b	0.0016
Total defects (%)	29.71±71	16.32±3.16	0.0020

Letters in the same row indicate differences (P<0.05).
Values are least-squares means±standard errors of the mean.

Table 3 - Influence of anti-Müllerian hormone (AMH) concentration in pre-puberty on the seminal quality of bulls after puberty

Parameter	Low AMH (n = 24)	High AMH (n = 29)	P-value
Vigorous oscillatory movement (1-5)	3.92±0.20a	3.33±0.19b	0.0351
Turbulence (%)	2.63±0.19a	2.06±0.19b	0.0344
Progressive motility (%)	77.55±3.80a	65.14±3.79b	0.0230

Letters in the same row indicate differences (P<0.05).
Values are least-squares means±standard errors of the mean.

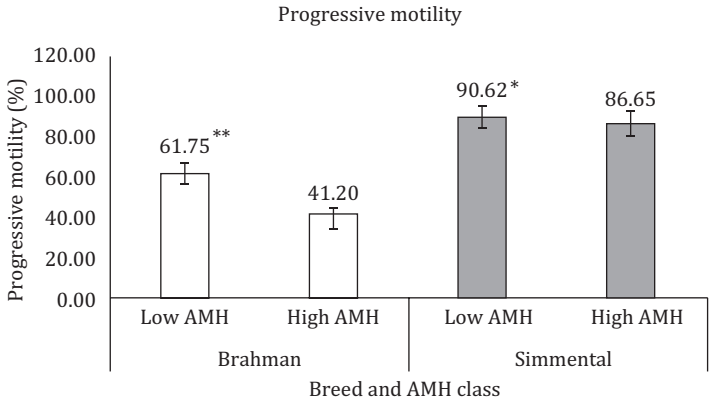

Figure 4 - Comparison between progressive motility and low and high anti-Müllerian hormone (AMH) concentrations in Simental and Brahman bulls.

3.3. Anti-Müllerian hormone concentration

Anti-Müllerian hormone concentrations averaged 2.275±0.22 ng/mL, with maximum and minimum values of 2.610 and 1.594 ng/mL, respectively. The frequency distribution is shown in Figure 5.

The mean AMH concentration was 2.275±0.22 ng/mL, with no difference between breeds (P>0.05). The AMH class influenced semen parameters, and semen quality was better in animals with lower AMH concentration at pre-puberty (Table 3). There was a tendency (P = 0.09) for AMH concentrations to be associated with body weight. Bulls with the lowest pre-pubertal and pubertal AMH concentrations had the highest body weight (Figures 6 and 7). The AMH concentration was not significantly associated with body weight in post-pubertal bulls (Figure 8).

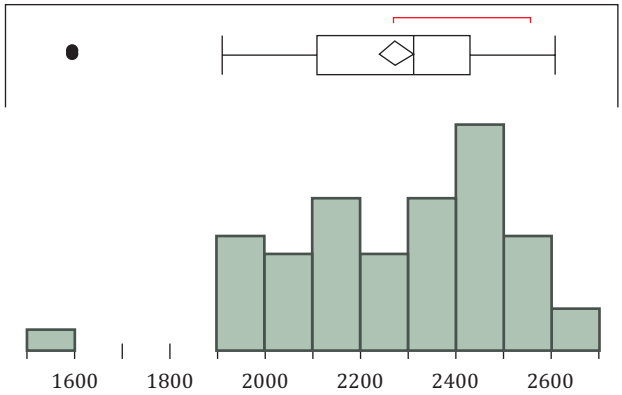

Figure 5 - Frequency distribution of anti-Müllerian hormone (AMH) concentrations: low AMH <2.28 ng/mL and high AMH ≥2.28 ng/mL.

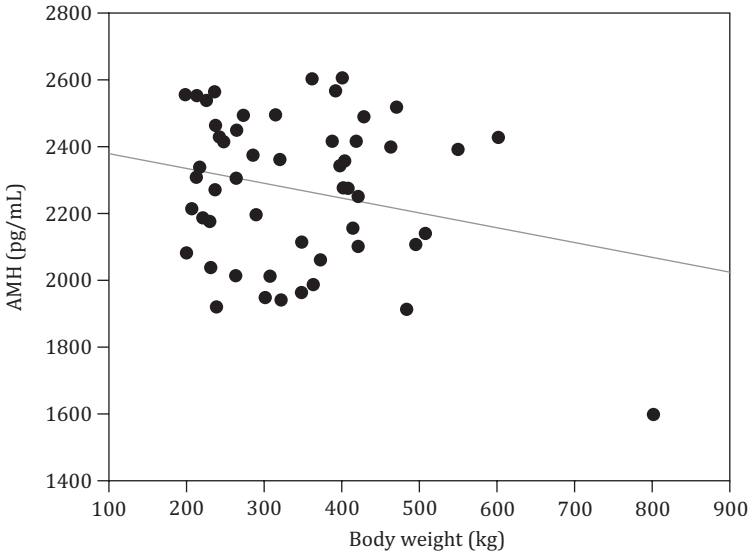

Figure 6 - Association between anti-Müllerian hormone (AMH) concentration and body weight in the pre-pubertal stage.

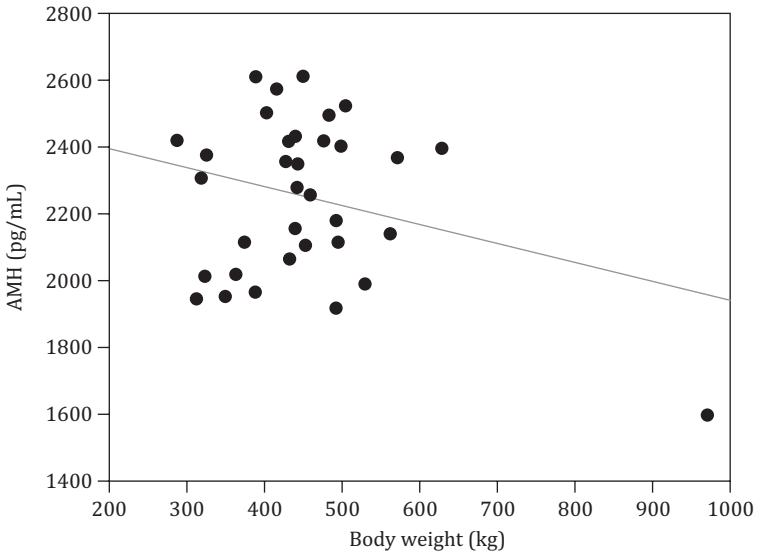

Figure 7 - Association between anti-Müllerian hormone (AMH) concentration and body weight in the pubertal stage.

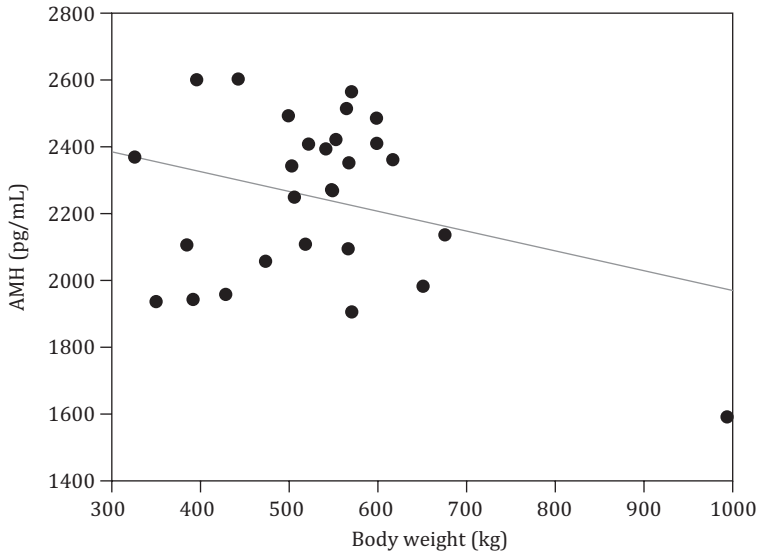

Figure 8 - Association between anti-Müllerian hormone (AMH) concentration and body weight in the post-pubertal stage.

4. Discussion

The SC of the Simmental breed assessed at sexual maturity was superior to that of the Brahman breed. According to Pinto et al. (1989), the SC of the Nellore breed ranged from 25 cm in animals aged 16-19 months to 27-28.5 cm in animals aged 20-22 months. In this study, SC was 36.57 cm for the Simmental breed, with an average age of 18 months. These data are similar to those of Miranda Neto et al. (2011), in which the SC was 34.88 cm for the animals at 21.43 months of age.

Similar data were observed in this study, in which the SC was 28.90 cm in Brahman animals with a mean age of 22 months. Oliveira et al. (2002), studying Limousin animals, and Kroetz et al. (2000), studying Charolais and Caracu animals, determined an average SC of 33.5, 34.4, and 32.2 cm, respectively, when they were assessed at 16 months of age.

There was a significant difference in the age of onset of puberty between the studied breeds, confirming previous findings (Costa e Silva et al., 2013) and suggesting genetic effects (Brito et al., 2004), as the animals on this farm are fed similarly. According to Costa e Silva et al. (2013), zebu animals enter puberty at approximately 25-28 months of age, while taurine animals typically enter puberty at an average age of 12-14 months. Additionally, taurine animals raised in tropical climates enter puberty later (at around 12-14 months) compared with those raised in temperate climates, where they reach puberty at approximately 8-10 months of age (Costa e Silva et al., 2013). In addition, it is observed that dairy cattle (*Bos taurus*) are more early-maturing than beef cattle (*Bos taurus*) (Lunstra et al., 1978; Guimarães et al., 2011; Costa e Silva et al., 2013). A pubertal age of 13.42±3.02 months has been observed in Simmental bulls reared under extensive conditions in tropical climates (Miranda Neto et al., 2011). Considering that the Simmental bulls used in this study have some dairy genetics and were kept under tropical climatic conditions, it could be argued that their age at puberty was slightly later (18 months) than expected.

It has been shown that testicular size is closely related to sexual development and can be used as a predictor of sexual precocity (Neves, 2007). There is a correlation between testicular measurements taken before and after puberty (Freneau, 1991). Almquist and Amann (1976) and Coulter and Keller (1982) observed high correlations between SC and age of 12 and 24 months (r = 0.76 and 0.80, respectively), which are in line with the results of the present study. This suggests that SC, measured in pre-puberty, can be used as a predictor of sexual precocity regardless of the genetic group.

The timing of male sexual maturity depends on the transient increase in LH pulsatility (Evans et al., 1995). This increase is accompanied by an increase in Leydig cell responsiveness to LH, which increases testosterone synthesis (Amann and Walker, 1983). In turn, Sertoli cell differentiation and onset of spermatogenesis depend on this pituitary-gonadal axis (Amann and Walker, 1983). In *Bos taurus* breeds such as Angus and Charolais, these events occur between weeks 10 and 18 (Rawlings and Evans, 1995), albeit varying to a large extent with the nutritional and metabolic status of the animal (Brito et al., 2007a; Brito et al., 2007b). Nevertheless, sexual precocity in *Bos indicus* occurs later than in *Bos taurus* (Lunstra and Cundiff, 2003; Lopez et al., 2006).

Scrotal circumference was an indicator of better semen quality, with high measurement repeatability in both breeds. The number of Sertoli cells determines the testicular size and daily sperm production (Johnson et al., 2008; Freneau, 1991). The percentage of morphologically normal sperm, better reproductive performance, higher sexual precocity, and greater sperm volume are parameters associated with the SC (Kastelic, 2014). Correlations between SC and sperm motility range from 0.13 to 1.00, while results between SC and sperm vigorous oscillatory movement range from 0.69 to 0.99 (Bergmann et al., 1997; Quirino et al., 1999; Sarreiro et al., 2002; Silveira, 2004; Dias et al., 2006, 2008). The heritability of the positive characteristics associated with bovine SC are moderate to high. Corbet et al. (2013) explained the importance of continuing recording these seminal features as part of the andrological assessment and including these measurements in pre-puberty assessments.

Notwithstanding the variation in SC and semen quality (Silva et al., 2002), animals with a younger age at puberty and larger testicular size may not necessarily have adequate sperm motility rates when they reach sexual maturity (Bailey et al., 1998). Therefore, the SC criterion, widely used in selection programs, is not particularly accurate (Siqueira et al., 2013; Buzanskas et al., 2017) despite the high correlation between changes in seminal parameters and SC.

Pre-pubertal AMH concentrations were similar in zebu and taurine animals. Rajak et al. (2017) performed five blood tests to determine the variation in AMH concentration in male zebu and crossbred animals from 1 to 24 months of age. There was no difference between the breeds, except

for sampling at 24 months of age, in which zebu males had higher AMH concentrations than crossbred males. This difference is likely due to the later onset of puberty of the zebu males compared with the crossbred males. Because dosing was performed in the pre-pubertal period, we did not detect a difference in AMH concentration with respect to the genetic group in this study.

The AMH concentration was a good indicator of sexual precocity. Animals classified as having low AMH concentrations performed better on andrological assessment at puberty. The AMH expression is higher in males than in females, and its concentration is higher at pre-puberty and decreases with testicular maturation (El-Sheikh Ali et al., 2017; Kitahara et al., 2016; Rota et al., 2002). Additionally, nutritional factors play a crucial role in modulating reproductive outcomes, as dietary composition can significantly affect endocrine function and reproductive development (Macedo et al., 2023). This highlights the importance of carefully considering dietary supplements in breeding programs to optimize reproductive performance. According to Ford and Wise (2009), immature Sertoli cells secrete large amounts of AMH. Immature Sertoli cells proliferate in cattle aged 4-20 weeks (Rawlings et al., 2008). Kitahara et al. (2016) observed that in Japanese black bulls, AMH levels increase from birth to two months of age and begin to decrease after the fifth month. According to Rajak et al. (2017), the transcriptional abundance of the AMH gene was higher at one month of age and then decreased significantly with age, reaching its baseline value at 24 months of age in crossbred males. The reduced expression of AMH by Sertoli cells is directly related to the onset of spermatogenesis in seminiferous tubules (Almeida et al., 2012). Low AMH concentrations lead to morphological and functional changes in the reproductive tract and support spermatogenic activity in pre-puberty. Thus, AMH concentration and the onset of spermatogenesis are opposing events. In a study conducted on horses by Almeida et al. (2012), a decrease in mRNA expression for AMH and its receptor was observed, while mRNA expression for androgen receptors increased in Sertoli cells.

The aforementioned drop in plasma AMH concentrations, which occurs when males reach puberty, may be explained by a change in tight junctions between Sertoli cells and germline cells and in AMH secretion from the basal to the adluminal compartment (Fénichel et al., 1999; Fujisawa et al., 2002). In addition, after puberty, AMH is primarily secreted by the apical pole of Sertoli cells, which is why AMH concentrations are higher in the seminal plasma than in serum, as previously reported. The AMH concentrations are higher in the semen than in the serum, potentially due to the role of this hormone in spermatogenesis and sperm motility. These findings taken together corroborate the observations of better seminal quality in the present study in lower AMH bulls.

Conversely, other studies have found that the *CLAUDIN11* and *AMH* genes, both of which are expressed in Sertoli cells, affect the maturation of these cells. The *CLAUDIN11* gene is involved in changes in tight junctions between adjacent Sertoli cells in rams (Guan et al., 2014), while the *AMH* gene is associated with an increased number of differentiating Sertoli cells (Vigier et al., 1984). These findings may explain the effect of AMH on the onset of puberty. In humans, it is hypothesized that changes in the anatomical, cellular, and endocrinological organization of the testicles during puberty may be responsible for the differential AMH concentrations. These changes that occur at puberty include the maturation of the blood-testis barrier, composed of junctions of Sertoli and myoid cells. The functions of the specialized blood testis barrier include permeability only to chemical substances between the seminiferous tubules of the testicles and blood. This prevents AMH from entering the peripheral circulation, which may explain the low peripheral concentration once males reach puberty. As a result, AMH synthesized in Sertoli cells is channeled into the lumen of the basal compartment of the seminiferous tubules, thereby increasing its concentration in seminal plasma (Rey et al., 2003; Fénichel et al., 1999; Fujisawa et al., 2002).

In turn, Rey et al. (1993) reported that peripheral testosterone concentrations increase during puberty, while AMH concentrations decrease. In addition, it has been observed that meiotic divisions in primary spermatocytes in the seminiferous tubules during spermatogenesis are associated with decreased AMH expression upon attainment of puberty (Hirobe et al., 1992; Rey et al., 1996).

This pattern of negative regulation can be explained by a study in which a significant reduction in the testosterone concentration was observed in Sertoli-cell-androgen-receptor-knockout mice. Consequently, this led to a transient increase in both mRNA and protein levels of AMH expression in Sertoli cells (Xu et al., 2019). This indicates the intricate mechanism that may explain this negative regulation. Additionally, there are transcriptional activities in the SP1, GATA, and Sox9 binding sites in the proximal promoter regions, the binding in the NF-κB site in the distal promoter region of AMH that lead to greater transcriptional activity (Chang et al., 2004). The AMH promoter region does not have androgen receptor binding sites (Lukas-Croisier et al., 2003); however, there are NF-κB binding sites that are negatively regulated by androgen receptors. Accordingly, testosterone does not directly regulate the transcription of AMH. Instead, it inhibits NF-κB, which in turn suppresses the transcriptional activation of AMH, leading to this negative regulation (McKay and Cidlowski, 1998; McKay and Cidlowski, 1999).

In cattle, the attainment of puberty in males is not accompanied by an increase in the number of Sertoli cells, which are fixed (Johnson et al., 2008). However, there is a positive association between the number of Sertoli cells and sperm quality, i.e., physical, hormonal, and nutritional support of spermatogenesis (Rajak et al., 2016). A negative correlation was found between the number of Sertoli cells in bulls at puberty and plasma AMH concentrations (Rajak et al., 2017). This finding suggests that bulls with lower peripheral AMH concentrations may have a higher number of Sertoli cells, which could contribute to better semen quality. This suggests that Sertoli cell status can be assessed by measuring AMH, a biomarker of fertility at puberty in bulls. The integration of crossbreeding strategies can also be pivotal in reducing reproductive losses, as noted in studies that highlight genetic group influences on fertility outcomes in extensive production systems (Vivián Paradizo et al., 2024). This underscores the potential benefits of crossbreeding in enhancing reproductive efficiency and reducing losses across different production scenarios.

In this study, a single sample taken at pre-puberty was a good indicator of semen quality in Simmental and Brahman bulls. This suggests that the pre-pubertal AMH concentration may serve as a predictive marker for semen quality in these breeds. In horses, lower AMH concentration has been observed in foals with precocious puberty (Ball et al., 2008; Claes et al., 2013).

Similarly, concentrations below normal pre-pubertal levels have been noted in boys with precocious puberty due to activating mutations of the LH-CG receptor, leading to gonadotropin-independent precocious puberty (testotoxicosis), or due to the presence of androgen-secreting Leydig cells (Rey et al., 1993). These observations highlight the potential role of AMH in the regulation of pubertal development across different species.

In a study by Queiroz (2014), 37 bulls underwent three blood collections three months apart to investigate whether changes in AMH concentrations could predict puberty. The study concluded that AMH can serve as an effective endocrine marker to distinguish young and older animals with just a single blood collection. Thus, AMH may provide information on the development and reproductive potential of bulls. In contrast to these concept, Costa Filho et al. (2017) found no association between AMH concentrations measured in Nellore males at weaning, at 12 months of age, and at 16 months of age and the occurrence of super-early, early, or late puberty. The lack of association between the findings of this study and those of the above-mentioned report may be related to the period during which the animals underwent the andrological examinations. In addition, in the same study, AMH concentration was measured at weaning and at 12 and 16 months of age, when the animals were also subjected to semen collection. Because the animals used were zebu, the age at onset of puberty is expected to be somewhat later (approximately 22 months), which may be reflected in the lack of relationship with AMH concentration.

In this study, a single dose of AMH in pre-puberty proved effective in predicting the onset of puberty in both Brahman and Simmental bulls. Therefore, the use of this marker aiming at early prediction of the onset of puberty can be recommended.

5. Conclusions

Anti-Müllerian hormone concentration is correlated with the scrotal circumference and semen quality, turbulence, motility, and vigor. Scrotal circumference correlates with AMH concentrations and semen parameters, making its measurement essential for assessing bulls before puberty. The pre-pubertal concentration of AMH was identified as a reliable predictor of sexual precocity, as it shows significant negative correlations with scrotal circumference and semen quality. Lower concentrations of AMH are associated with better fertility potential, suggesting that AMH should be considered in selection programs aimed at improving reproductive performance.

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Conflict of interest

The authors declare no conflict of interest.

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