



# Evaluation of growth performance and reproductive traits at puberty and attainment of sexual maturity in *Bos indicus* Nelore bulls raised in pasture management systems

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**ABSTRACT** - The objective of this study was to characterize the sexual development of 48 Nelore bulls by associating the findings of sperm analyses with growth traits and ultrasound-measured testicular traits. Bulls were evaluated from 14.3 to 24.6 months old at intervals of 28 days, totaling 12 observations per animal. Data were subjected to repeated-measures analyses of variance over time (PROC MIXED, SAS 9.2) and equations for semen traits were developed using stepwise regression (PROC REG, SAS 9.2). Regarding the growth performance on pasture, there was an increase in average body weight (388 to 500±4.59 kg), body condition (5-6), and hip height (139 to 145±0.52 cm) with increasing age. However, a lower average daily gain (0.64 to 0.29±0.05 kg) was observed during the dry season. Reproductive traits such as scrotal circumference (27 to 33.6±0.34 cm), testicular artery diameter (2.14 to 2.96±0.04 mm), blood testosterone concentration (8.3 to 22.7±1.48 mg/mL), sperm motility (total: 42 to 82.8±2.91% and progressive: 26.2 to 62.3±2.61%), percentage of rapid cells (38.1 to 79.8±2.9%), and sperm concentration (43.6 to 645±42.1 × 10<sup>6</sup>/mL) increased with increasing age, while there was a reduction in sperm defects (major: 59.4 to 9.6±3.18% and total: 62.5 to 15.1±2.94%). Ultrasonography and growth traits have a moderate power to predict major and total sperm defects but cannot predict other seminal traits. By 19-20 months of age, Nelore bulls start to exhibit reproductive traits that indicate the attainment of sexual maturity.

**Keywords:** *Bos indicus*, bulls, semen, sexual maturity, ultrasonography

## 1. Introduction

The evaluation of reproductive and growth performance traits of male cattle during puberty and attainment of sexual maturity is of great importance for the selection of young bulls in breeding programs because these traits are important auxiliary parameters when selecting animals with desirable fertility, precocity, and weight gain capacity (Fonseca, 2009).

The onset of puberty is influenced by genetic, environmental, and nutritional factors (Vásquez et al., 2003) and is determined by the onset of spermatogenesis and sexual interest (Vale Filho et al., 1993; Freneau, 1996). Gonadal growth and body development are signs of sexual maturity in bulls. In addition, this stage is characterized by quantitative and qualitative changes in semen (Lunstra and Echternkamp, 1982; Rawlings et al., 2008). Although these characteristic events of sexual maturity are similar for *Bos taurus* and *Bos indicus* breeds, the onset of puberty differs between these genetic groups (Guimarães, 1997).

Reproductive traits develop later in *Bos indicus* breeds than in *Bos taurus* breeds (Fonseca et al., 1975; Vale Filho et al., 1989); however, there is also individual variability, with the observation of late and precocious animals (Eler, 2004). It is therefore necessary and important to identify and select animals that are precocious in terms of puberty and sexual maturity since these animals start their reproductive life earlier, thus rendering the production system more efficient and profitable (Brito, 2006). Within this context, the evaluation of fertility parameters and the reproductive capacity of young bulls may enable the adoption of selection strategies that promote an increase in herd efficiency and advances in genetic improvement mediated by the male effect.

Computerized analyses of ultrasound images allow characterizing some attributes of the testicles and accessory genital glands such as dimensions, echogenicity, and echotexture (Camela et al., 2017). According to Ahmad et al. (2011), testicular echotexture is directly associated with sexual maturity and is also correlated with the spermatogenic capacity of the organ, showing an association with the area of the seminiferous tubules (Coulter and Bailey, 1988; Gábor et al., 1998). The pixel pattern for detecting testicular development can potentially reveal further details of the development of the seminiferous tubules and spermatogenesis (Abdel-Razek and Ali, 2005; Giffin et al., 2014). Ultrasound and sperm quality are attributes for assessing sexual maturity that are extremely important for the selection of younger bulls and can eventually contribute to increasing the reproductive efficiency of the herd. Thus, the present study was developed to evaluate the sexual development of *Bos indicus* Nelore bulls by associating semen parameters with ultrasound-measured testicular traits and growth traits.

## 2. Material and methods

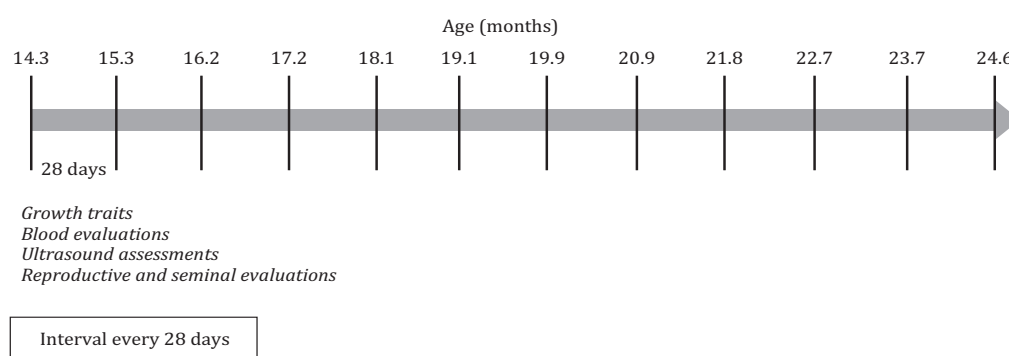
### 2.1. Animals and experimental design

The experiment was validated by the Ethics Committee of UNESP, Jaboticabal, São Paulo, Brazil (Protocol Number 017361/13), and was developed at the Centro Avançado de Pesquisa e Desenvolvimento de Bovinos de Corte, Instituto de Zootecnia (IZ/APTA/SAA), Sertãozinho, São Paulo, Brazil (21°8'16" S and 48°58'22" W, altitude 579 m).

The animals used in this study come from a herd selected for growth since 1980, and for feed efficiency since 2007. Throughout the selection years, there was a 90-day natural breeding season (November 15th to February 15th), and animals were born from September to November (contemporary group). From weaning to one year old, all males were maintained in a performance test for 168 days on a diet for an average daily gain (ADG) of 1.1 kg per day (Benfica et al., 2020). Around 50% of the males were annually selected to be used as bulls. Seven percent were used in the herd to replace bulls, and the rest were sold as breeding stock. Part of these 50% males composed the sample of the bulls evaluated in the present study.

The average initial weight of the 48 Nelore bulls (24 born in 2013 and 24 born in 2014) evaluated in the study was  $388.8 \pm 4.59$  kg (mean  $\pm$  standard error). The animals were kept on *Brachiaria brizantha* cultivar Marandu pasture with mineral supplementation and water *ad libitum*.

The study comprised two replications of 11 months each (replicate 1 [n = 24]: December 2014 to October 2015 and replicate 2 [n = 24]: December 2015 to October 2016). The bulls were evaluated every 28 days (from  $14.3 \pm 0.13$  to  $24.6 \pm 0.13$  months of age) for growth, blood, and reproductive parameters. Figure 1 shows the experimental design of the collections according to the age of the young Nelore bulls.



**Figure 1** - Experimental design of collections according to the age of young Nelore bulls.

## 2.2. Growth traits

Body condition score (BCS), body weight (BW), ADG, and hip height (HH) were obtained every 28 days. Body condition score was ranked on a scale from 1 to 9 [extremely thin and fat animals, respectively, according to Wagner et al. (1988)]. Hip height was taken with the help of a measuring tape, according to Cyrillo et al. (2001).

## 2.3. Blood evaluations

Blood was collected by puncture of the coccygeal vein into tubes (Vacutainer - Labnew® Indústria e Comércio Ltda, Juiz de Fora Brazil) with EDTA or without anticoagulants and was centrifuged at 1,500 *g* for 20 min (Rossi et al., 2022). Serum and plasma samples were aliquoted in microtubes that remained frozen at -20 °C until the hormonal and metabolic analyses.

Plasma testosterone levels were quantified by radioimmunoassay using a commercial kit (Coat-A-Count® Total Testosterone kit, Diagnostic Products Corporation, Los Angeles, CA, USA) as recommended by the manufacturer. The sensitivity of the assay was 0.3 ng/mL and the inter- and intra-assay coefficient of variation was 7.8% and 5.5%, respectively. The Labquest® semi-automatic biochemical analyzer (Labtest Diagnóstica S.A., Lagoa Santa, Brazil) was used to measure serum total cholesterol (Cholesterol Liquiform kits, Labtest Diagnóstica S.A., Lagoa Santa, Brazil) following the manufacturer's instructions.

## 2.4. Ultrasound assessments

A Z5 vet ultrasound (Mindray®, Shenzhen, China) coupled with a 7.5 MHz linear transducer was used for testicular, vesicular gland, and ampulla measurements, as previously described by Rossi et al. (2019). The pixel intensity of the ultrasound images was examined by Image Pro Plus 7.01 software (Media Cybernetics Inc., San Diego, USA) (Ahmadi et al., 2013; Rodrigues et al., 2020).

Mean diameter and blood flow of the testicular artery were measured by color Doppler ultrasound. The blood flow variables analyzed were end-diastolic velocity (EDV), peak systolic velocity (PSV), pulsatility index [ $PI = (PSV - EDV) / \text{mean velocity between PSV and EDV}$ ], and resistive index [ $RI = (PSV - EDV) / PSV$ ] (Wood et al., 2010; Feliciano et al., 2012; Rossi et al., 2019).

## 2.5. Reproductive and seminal evaluations

Scrotal circumference (SC) was measured using a scrotal measuring tape (CBRA, 2013). Semen samples were collected using an electro-ejaculator (Autojac, Neovet®, Uberaba, Brazil). Sperm concentration was determined in a spectrophotometer (SDM1, Minitube®, Tiefenbach, Germany). The sperm variables were determined according to CBRA (2013) recommendations.

Sperm defects (major and total) were analyzed using an Eclipse Ni-U microscope (Nikon®, Tokyo, Japan) equipped with differential interference contrast (DIC) according to the methods of Blom (1973) and CBRA (2013). Semen samples were subjected to computer-assisted sperm analyses (CASA; Ivos-Ultimate device, version 14.0; Hamilton Thorne Biosciences, Beverly, MA, USA). The CASA set-up was adjusted as previously described by Rossi et al. (2019) and Fernandes et al. (2022). The verified sperm kinetic variables were: total sperm motility (TM, %), progressive sperm motility (PM, %), rapid sperm (RAP, %), average path velocity (APV,  $\mu\text{m/s}$ ), straight-line velocity (SLV,  $\mu\text{m/s}$ ), curvilinear velocity (VCL,  $\mu\text{m/s}$ ), amplitude of lateral head displacement (ALH,  $\mu\text{m}$ ), beat cross frequency (BCF, Hz), straightness (STR, %), and linearity (LIN, %).

## 2.6. Statistical analyses

The analysis of variance for repeated measures over time (PROC MIXED) procedure of SAS (Statistical Analyses System, 9.2) was used. Means were fit by the least squares method (LSMEANS) and compared, if necessary, by the probability of difference (PDIF) using a t test. The significance level was set at  $P < 0.05$ .

The statistical model was:

$$Y = \mu + \text{year} + \text{time} + \text{time} \times \text{year} + e,$$

in which  $Y$  is the variable;  $\mu$  is the overall mean; *year* corresponds to the effect of year ( $i = 1, 2$ ), *time* is the effect of sampling time ( $i = 12$ ), *time\*year* is the interaction between repetition and year, and  $e$  is the error. The structure of repeated measurements was considered by modeling residual (co)variances. The best fit statistical model was chosen based on the lowest values for the Bayesian information criterion (BIC) and the Akaike information criterion (AIC).

The linear regression model was:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n + \varepsilon,$$

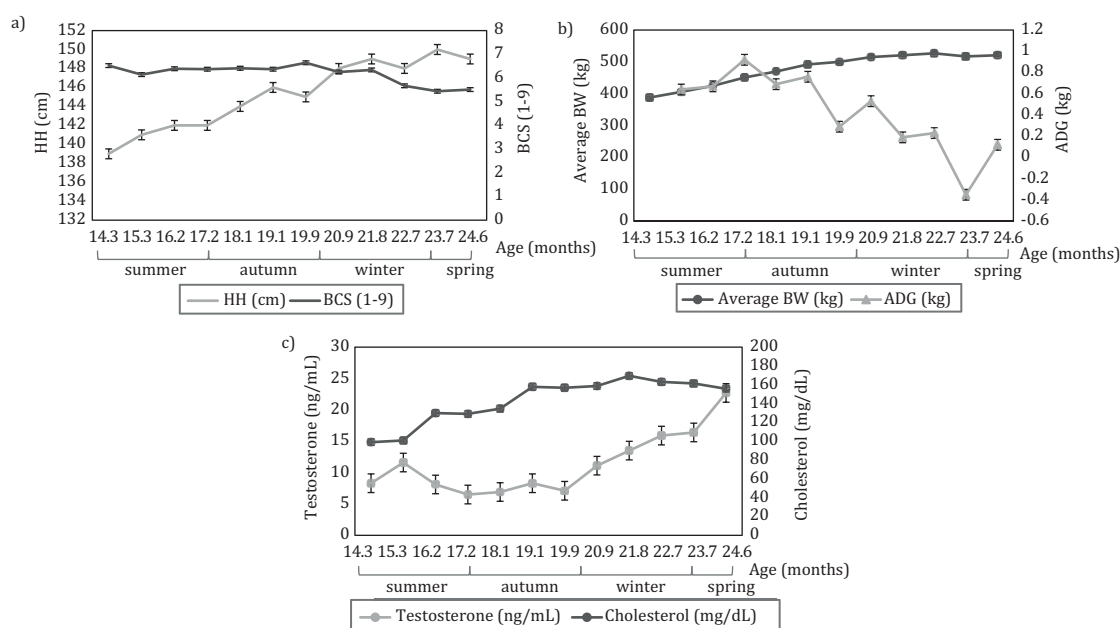
in which  $Y$  is the dependent variable related to the semen traits;  $\alpha$  is the intercept;  $X$  corresponds to the ultrasound, growth, and blood parameters (independent variables);  $\beta$  is the partial regression coefficient of  $Y$  on each independent variable; and  $\varepsilon$  is the residual or error. The equations for each  $Y$  were developed using stepwise regression. The STEPWISE and SLENTY = 0.25 and SLSTAY = 0.10 options of PROC REG were used to select the model.

## 3. Results

An increase in mean BW ( $388 \pm 4.59$  to  $500 \pm 4.59$  kg;  $P < 0.001$ ) and HH ( $139 \pm 0.52$  to  $145 \pm 0.52$  cm;  $P < 0.001$ ) was observed from the beginning of the experiment (14.3 months of age, summer) until 19.9 months of age (autumn) (Figures 2a and 2b). Average daily gain increased until 19.1 months of age ( $0.64 \pm 0.05$  to  $0.76 \pm 0.05$  kg;  $P < 0.001$ ). However, although growth (HH:  $145 \pm 0.52$  cm at 19.9 months of age to  $149 \pm 0.52$  cm at 24.6 months;  $P < 0.001$ ) and ADG ( $0.29 \pm 0.05$  kg at 19.9 months of age to  $0.12 \pm 0.05$  kg at 24.6 months;  $P < 0.001$ ) continued to progress from the end of autumn to the end of the assessments (coinciding with the winter and spring seasons), the rhythm was lower than that observed during the previous months (summer). Nonetheless, the BCS did not change significantly and remained between moderate and good (5-6) throughout the study.

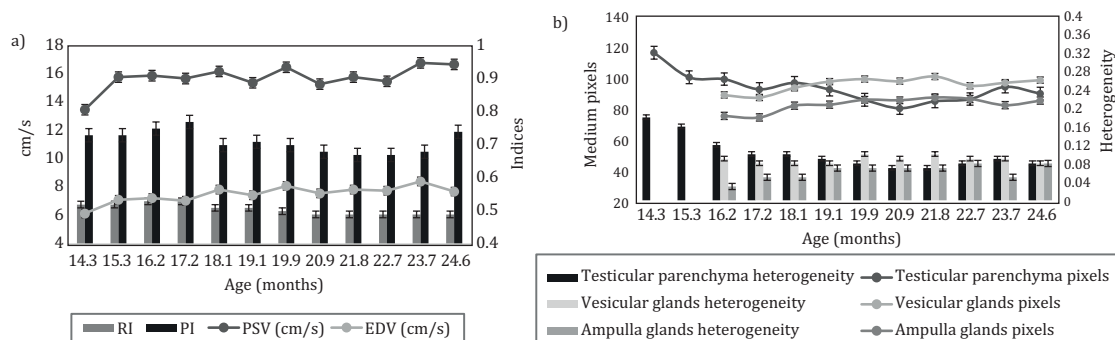
There was a gradual increase in serum cholesterol concentrations ( $P < 0.001$ ) from December ( $99.0 \pm 3.34$  mg/dL at 14.3 months of age) to April ( $157.8 \pm 3.34$  mg/dL at 19.1 months of age), with levels remaining relatively stable thereafter until the end of the experiment ( $155.8 \pm 3.34$  mg/dL at 24.6 months of age) (Figure 2c). Testosterone concentrations varied little from the beginning of the experiment ( $8.3 \pm 1.48$  ng/mL at 14.3 months of age) to May ( $7.1 \pm 1.48$  mg/mL at 19.9 months of age). Thereafter, testosterone levels gradually increased ( $11.1 \pm 1.48$  mg/mL at 20.9 months of age), with peak values being observed in October ( $22.7 \pm 1.48$  mg/mL at 24.6 months of age;  $P < 0.001$ ).

Ultrasonographic assessment of testicular traits showed an increase in testicular artery diameter with increasing age of the animals (from  $2.14 \pm 0.04$  mm at 14.3 months of age to  $2.96 \pm 0.04$  mm at 24.6 months;  $P < 0.001$ ; Figure 4a). Color Doppler analyses showed little variation in the testicular blood flow parameters during the study (PSV:  $13.5 \pm 0.37$  to  $16.7 \pm 0.37$ , EDV:  $6.17 \pm 0.30$  to  $7.72 \pm 0.30$ , RI:  $0.52 \pm 0.01$  to  $0.49 \pm 0.01$ , and PI:  $0.73 \pm 0.02$  to  $0.74 \pm 0.02$ ;  $P < 0.001$ ; Figure 3a). The mean pixel intensity of the testicular parenchyma decreased from 14.3 months of age ( $116.2 \pm 4.12$  pixels) to 17.2 months ( $92.4 \pm 4.12$  pixels), remaining constant thereafter until 24.6 months ( $96.6 \pm 4.12$  pixels;  $P < 0.001$ ; Figure 3b). On the other hand, the mean pixel intensity of the accessory glands increased from



a) BCS - body condition score and HH - hip height; b) BW - body weight and ADG - average daily weight gain; c) testosterone and cholesterol. Seasons of the year = summer (December 21 to March 20), autumn (March 20 to June 20), winter (June 20 to September 22), and spring (September 22 to December 21). P-value between repetitions.

**Figure 2 - Growth traits and blood parameters of young Nelore bulls.**



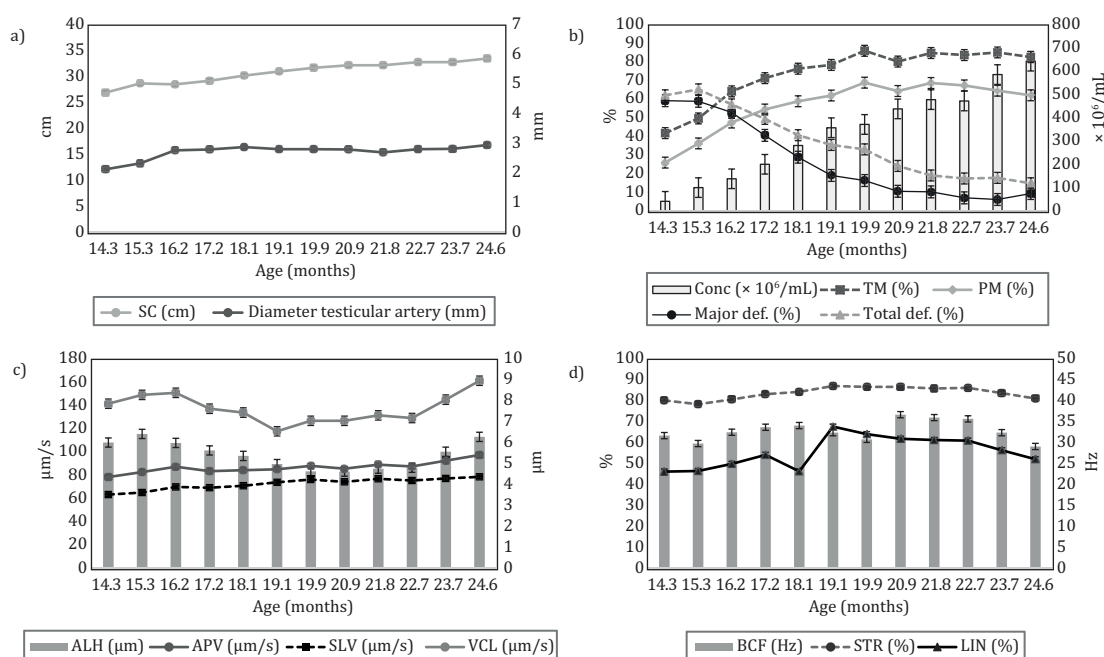
a) Color Doppler ultrasound: RI - resistive index; PI - pulsatility index; PSV - peak systolic velocity; EDV - end-diastolic velocity. b) pixel intensity (testicular parenchyma, vesicular glands, and ampulla glands) and heterogeneity (testicular parenchyma, vesicular glands, and ampulla glands) = heterogeneity of pixels represented by the standard deviation of the image. P-value between repetitions.

**Figure 3 - Ultrasonographic traits of the reproductive system of young Nelore bulls.**

16.2 to 19.9 months of age (vesicular gland:  $88.6 \pm 1.96$  to  $99.0 \pm 1.96$  pixels and ampulla:  $75.1 \pm 2.29$  to  $85.5 \pm 2.29$  pixels), remaining constant thereafter until 24.6 months (vesicular gland:  $98.3 \pm 1.96$  pixels and ampulla:  $84.9 \pm 2.29$ ;  $P < 0.001$ ; Figure 3b).

Reproductive and seminal traits improved with growth and increasing age of the animals. An inverse association was observed among SC, TM, PM, RAP, sperm concentration, and major and total defects, i.e., SC ( $27 \pm 0.34$  to  $33.6 \pm 0.34$  cm), TM ( $42 \pm 2.91$  to  $82.8 \pm 2.91\%$ ), PM ( $26.2 \pm 2.61$  to  $62.3 \pm 2.61\%$ ), RAP ( $38.1 \pm 2.9$  to  $79.8 \pm 2.9\%$ ), and sperm concentration ( $43.6 \pm 42.1$  to  $645 \pm 42.1 \times 10^6/\text{mL}$ ) increased with increasing age of the bulls (14.3 to 26.6 months), while sperm defects decreased (major defects:  $59.4 \pm 3.18$  to  $9.6 \pm 3.18\%$  and total defects:  $62.5 \pm 2.94$  to  $15.1 \pm 2.94\%$ ;  $P < 0.001$ ; Figure 4a and 3b). The other CASA parameters progressed according to the age of the animals (APV:  $78.8 \pm 1.92$  to  $97.8 \pm 1.92 \mu\text{m/s}$ , SLV:  $63.7 \pm 1.46$  to  $79 \pm 1.46 \mu\text{m/s}$ , VCL:  $142 \pm 4.03$  to  $162 \pm 4.03 \mu\text{m/s}$ , ALH:  $6.03 \pm 0.22$  to  $6.3 \pm 0.22 \mu\text{m}$ , BCF:  $31.8 \pm 0.74$  to  $29.2 \pm 0.74$  Hz, STR:  $80.4 \pm 0.71$  to  $81.3 \pm 0.71\%$ , and LIN:  $46.4 \pm 1.2$  to  $52.3 \pm 1.2\%$ ;  $P < 0.001$ ; Figure 4c and 4d).

Scrotal circumference, average pixels, and growth traits have a medium power to predict major ( $R^2 = 0.38$ ) and total sperm defects ( $R^2 = 0.33$ ) (Table 1). When SC and BW increased by one unit, there was a decrease in sperm defects; on the other hand, an increase in average pixels, ADG, and BCS was associated with an increase in sperm defects. It is noteworthy that the independent variables (ultrasound, growth, and blood parameters) had very low power to predict semen traits, except for sperm defects. In addition, no blood parameters had an effect on sperm characteristics and, for this reason, these variables were not selected by the model and are not present in Table 1.



a) Diameter - mean testicular artery diameter; SC - scrotal circumference. b) Conc - sperm concentration ( $\times 10^6/\text{mL}$ ); TM - total motility; PM - progressive motility; Major def. - major sperm defects; Total def. - total sperm defects. c) ALH - amplitude of lateral head displacement; APV - average path velocity, SLV - straight-line velocity; VCL - curvilinear velocity; d) BCF - beat cross frequency; STR - straightness; LIN - linearity. P-value between repetitions.

**Figure 4 - Reproductive and seminal traits of young Nelore bulls.**

**Table 1** - Equations for the prediction of semen trait variables of young Nelore bulls

| Dependent variables and equations  | R <sup>2</sup> | RMSE  | Cp    | Intercept | SC (cm) | Partial regression coefficient |            |      |                          |               |               | Growth trait    |          |           |         |
|------------------------------------|----------------|-------|-------|-----------|---------|--------------------------------|------------|------|--------------------------|---------------|---------------|-----------------|----------|-----------|---------|
|                                    |                |       |       |           |         | Testicular ultrasound          |            |      | Ampulla gland ultrasound |               |               | Average BW (kg) | ADG (kg) | BCS (1-9) | HH (cm) |
|                                    |                |       |       |           |         | Diameter (mm)                  | PSV (cm/s) | RI   | Average pixels           | Heterogeneity | Heterogeneity |                 |          |           |         |
| Concentration ( $\times 10^6$ /mL) | 0.24           | 339.0 | 11.6  | -1508.8   | 48.9    | -                              | -          | -    | -                        | -             | 1722.2        | -               | -105.9   | -         | -       |
| Major def. (%)                     | 0.38           | 16.4  | 9.74  | 68.5      | -1.56   | -                              | -          | -    | 0.38                     | -             | -             | -0.11           | 6.58     | 4.77      | -       |
| Total def. (%)                     | 0.33           | 17.8  | 9.23  | 65.4      | -2.42   | -                              | -          | -    | 0.26                     | -             | -             | -0.08           | -        | 9.86      | -       |
| TM (%)                             | 0.22           | 13.5  | -0.95 | 41.6      | -       | 7.21                           | -          | 9.33 | -                        | -151.8        | -52.5         | 0.06            | -4.86    | -         | -       |
| PM (%)                             | 0.12           | 12.8  | 6.66  | 54.4      | -       | -                              | -          | -    | -                        | -111.2        | -             | 0.03            | -3.17    | -         | -       |
| RAP (%)                            | 0.19           | 14.0  | 5.99  | 43.2      | -       | 8.36                           | -          | -    | -                        | -136.1        | -             | 0.04            | -5.38    | -         | -       |
| APV ( $\mu$ m/s)                   | 0.08           | 12.3  | 0.43  | 73.6      | -       | 9.85                           | -          | -    | -0.13                    | -             | -             | -               | -3.44    | -         | -       |
| SLV ( $\mu$ m/s)                   | 0.05           | 9.29  | 6.17  | 31.8      | -       | -                              | -          | -    | -0.10                    | -             | -             | -               | -        | -         | 0.35    |
| VCL ( $\mu$ m/s)                   | 0.10           | 27.8  | 8.43  | 88.6      | -       | 24.2                           | -          | -    | -                        | -             | -             | -               | -        | -         | -       |
| ALH ( $\mu$ m)                     | 0.05           | 1.22  | 14.5  | 1.82      | -       | 1.16                           | -          | -    | -                        | -             | -             | -               | -        | -         | -       |
| BCF (Hz)                           | 0.08           | 5.07  | 16.4  | 51.6      | -       | -4.69                          | -0.28      | -    | -                        | -             | -             | -               | -        | -         | -       |
| STR (%)                            | 0.09           | 4.50  | 12.8  | 92.3      | -       | -3.80                          | -          | -    | -                        | -             | -             | -               | -        | -         | -       |
| LIN (%)                            | 0.21           | 8.31  | 14.9  | 56.2      | 0.65    | -9.23                          | -          | -    | -                        | -             | -             | -               | -        | -         | -       |

Major def. - major sperm defects; total def. - total sperm defects; TM - total motility; PM - progressive motility; RAP - rapid sperm; APV - average path velocity; SLV - straight-line velocity; VCL - curvilinear velocity; ALH - amplitude of lateral head displacement; BCF - beat cross frequency; STR - straightness; LIN - linearity.

RMSE - root mean square error; Cp - Mallows's Cp statistic; SC - scrotal circumference; diameter - mean testicular artery diameter; PSV - peak systolic velocity; RI - resistive index; average pixels - average pixel intensity; heterogeneity - heterogeneity of pixels represented by the standard deviation of the image; BW - body weight; ADG - average daily weight gain; BCS - body condition score; HH - hip height.

The linear regression model was  $Y = \alpha + \beta_1X_1 + \beta_2X_2 + \dots + \beta_nX_n + \epsilon$ , in which  $Y$  is the dependent variable related to the semen traits;  $\alpha$  is the intercept;  $X$  corresponds to the ultrasound, growth, and blood parameters (independent variables);  $\beta$  is the partial regression coefficient of  $Y$  on each independent variable; and  $\epsilon$  is the residual or error. Example: Major def. =  $68.5 - 1.56 \text{ SC} + 0.38 \text{ average pixels} - 0.11 \text{ average BW} + 6.58 \text{ ADG} + 4.77 \text{ BCS}$ .

## 4. Discussion

The evaluation of growth traits is essential to select young bulls with high genetic potential for meat production. In the present study, a positive association was observed among age, weight, and some reproductive parameters in Nelore bulls. In general, an increase in BW, HH, SC, testicular artery diameter, blood testosterone concentration, TM, PM, RAP, and sperm concentration was observed, while there was a decrease in sperm defects with increasing age. These results are confirmed by the prediction equations (Table 1), demonstrating a greater power of SC and growth traits to predict sperm defects. An increase in SC and BW was associated with a reduction in major and total defects.

At the beginning of the present study, the Nelore bulls (14.3 months old) already produced sperm, in agreement with previous results that showed the onset of puberty in *Bos indicus* bulls between 14 and 16 months old (Fonseca et al., 1975; Vale Filho et al., 1989; Freneau et al., 2006). Indicators of the onset of puberty include the occurrence of the first spermatozoa in semen and displaying sexual interest (Vale Filho et al., 1993; Freneau, 1996; Freneau et al., 2006). Puberty is, then, confirmed when the animals show 10% progressive motility and  $50 \times 10^6$  total sperm in the ejaculate (Wolf et al., 1965).

On the other hand, sexual maturity is reached a few months later when the growth of the body and reproductive organs stabilizes, accompanied by high plasma testosterone levels (Austin and Short, 1984). In the present study, an increase in blood testosterone concentration was observed after 19 months old and coincided with some reproductive parameters such as SC, testicular artery diameter, sperm motility, and sperm concentration. This finding can be explained by the fact that testosterone plays a key role in the maintenance of spermatogenesis (Zirkin, 1998).

However, most studies establish sexual maturity as the age at which bulls exhibit seminal traits of at least 50% progressive sperm motility and sperm morphology with a maximum of 20% minor sperm defects and 10% major sperm defects (Blom, 1973; Lunstra and Echternkamp, 1982; Rawlings et al., 2008). These seminal indicators of sexual maturity were observed in the present study when Nelore bulls raised on pasture reached the age of 19 to 20 months. These results corroborate the literature (Lunstra and Echternkamp, 1982), according to which sexual maturity of males of different cattle breeds usually occurs between four and five months after puberty, although variations may be observed as a result of the influence of nutrition and environment, which directly affect body development and consequently the reproductive performance of bulls (Brito, 2006; Barth et al., 2008; Fernandes Junior and Franceschini, 2007; Sethi et al., 2022). However, there was no delay in the attainment of sexual maturity after puberty in the present study, suggesting the absence of environmental and nutritional effects, although ADG was lower in winter (June to August) and spring (September to November). The latter can be explained by the low productive and nutritional quality of pastures during these periods of the year, due to the lack of rain and low night temperatures (Rossi et al., 2019). Nonetheless, BCS remained between moderate and good (5-6) during the study, and the animals continued to gain weight, although at a slower rate than that observed during the rainy seasons (summer: December to February; autumn: March to May).

In the present study, an increase in serum cholesterol concentrations was observed with increasing age of the bulls, which may be related to weight gain since cholesterol concentrations vary depending on the physiological condition of the animal (Bagatell and Bremner, 1998). Another factor that may explain the variation in cholesterol concentration is the occurrence of puberty and attainment of sexual maturity in the animals, with studies reporting a positive association between cholesterol levels and testosterone concentration (Gebara et al., 2002).

The ultrasound analysis of the echotexture and echogenicity of the reproductive organs of bulls is an important tool for evaluating their sexual development (Evans et al., 1996; Chandolia et al., 1997; Brito et al., 2012). An increase in echogenicity and testicular development occurred at the beginning of the present study, similar to the previously reported by Ahmad et al. (2011) and Brito et al. (2012). This finding can be explained by morphological modifications of Sertoli cells (Eurell and Frappier, 2012; Rodrigues et al., 2020) and variations in the diameter of the seminiferous tubules at the onset of puberty (Evans et al., 1996). However, after puberty and as sexual maturity is attained, there is an

increase in the secretion of testicular fluids (Eurell and Frappier, 2012; Rodrigues et al., 2020), which might explain the lower echogenicity observed in the present study in sexually mature bulls at about 19-20 months old.

Rectal ultrasound also permitted to assess the bulls' accessory sex glands located in the pelvic region. As seminal plasma is produced by these sex glands (Gonçalves et al., 2008), it is therefore important to evaluate these organs in reproductive examinations of bulls (Rodrigues et al., 2020). The echogenicity of the parenchyma of the vesicular glands and ampullae was lower in the initial evaluations when the bulls were younger and during the post-pubertal period (16.2 and 17.2 months of age) and subsequently increased with the attainment of sexual maturity. These results can be explained by the fact that the vesicular glands contain small vesicles that store seminal plasma dispersed in the parenchyma and change their size and shape according to age and the attainment of sexual maturity, factors that influence echogenicity (Camela et al., 2017; Rodrigues et al., 2020).

## 5. Conclusions

Young *Bos indicus* Nelore bulls raised on pasture show improvement in reproductive and seminal traits with increasing age and weight gain. By 19-20 months old, Nelore animals exhibit the reproductive traits desirable for sexually mature bulls. Ultrasonographic assessments and growth traits have a moderate power to predict major and total sperm defects but cannot predict other seminal traits.

## Data availability

The entire dataset supporting the results of this study was published in the article itself.

## Author contributions

**Conceptualization:** Rossi, G. F.; Garcia, J. M. and Mingoti, G. Z. **Data curation:** Rossi, G. F. and Mingoti, G. Z. **Formal analysis:** Rossi, G. F. and Mercadante, M. E. Z. **Investigation:** Rossi, G. F.; Rodrigues, N. N.; Dias, E. A. R.; Bastos, N. M.; Vrisman, D. P.; Simili, F. F.; Garcia, J. M.; Monteiro, F. M. and Mingoti, G. Z. **Methodology:** Rossi, G. F.; Simili, F. F.; Mercadante, M. E. Z.; Monteiro, F. M. and Mingoti, G. Z. **Project administration:** Rossi, G. F.; Monteiro, F. M. and Mingoti, G. Z. **Resources:** Rossi, G. F.; Rodrigues, N. N.; Dias, E. A. R.; Bastos, N. M.; Vrisman, D. P.; Simili, F. F.; Gimenes, L. U.; Garcia, J. M.; Mercadante, M. E. Z.; Monteiro, F. M. and Mingoti, G. Z. **Supervision:** Rossi, G. F.; Monteiro, F. M.; and Mingoti, G. Z. **Writing – original draft:** Rossi, G. F.; Mercadante, M. E. Z.; Monteiro, F. M. and Mingoti, G. Z. **Writing – review & editing:** Rossi, G. F.; Gimenes, L. U.; Mercadante, M. E. Z.; Monteiro, F. M. and Mingoti, G. Z.

## Conflict of interest

The authors declare no conflict of interest.

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