Sexual development in early- and late-maturing Bos indicus and Bos indicus × Bos taurus crossbred bulls in Brazil

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Abstract

Two experiments were conducted to evaluate sexual development in early- and late-maturing Nelore (Bos indicus) and Canchim (3/8 Bos indicus × 5/8 Bos taurus crossbred) bulls and to determine predictors of sexual precocity, and pubertal and maturity status. In Experiment 1, 12 Nelore bulls where examined from 300 to 900 days of age. Puberty was characterized by an ejaculate containing ≥50 million sperm with ≥10% motile sperm, and maturity by an ejaculate containing ≥70% morphologically normal sperm. In Experiment 2, 28 Canchim bulls where examined from 295 to 488 days of age and puberty was characterized by an ejaculate containing ≥30% motile sperm. In both experiments, bulls were classified as early- or late-maturing based on age at puberty. Early-maturing bulls were younger (P < 0.05) than late-maturing bulls at puberty (527 days versus 673 days in Experiment 1 and 360 days versus 461 days in Experiment 2) and at maturity (660 days versus 768 days in Experiment 1). In general, early-maturing bulls were heavier and had greater scrotal circumference (SC), testes, and testicular vascular cone diameter than late-maturing bulls during the experimental period. Scrotal circumference adjusted for 365 days of age was a good predictor of sexual precocity; minimum yearling SC of 19 and 24 cm for Nelore and Canchim bulls, respectively, had the best predictive values. Early-maturing bulls were lighter and had smaller SC at puberty than late-maturing bulls; therefore, sexual precocity was not related to the attainment of a threshold body weight or testicular size earlier, but to lower thresholds in early-maturing bulls. When predictors of pubertal status were evaluated, SC had the best sensitivity/specificity relationship in Nelore bulls, and high sensitivity and specificity in Canchim bulls. When predictors of sexual maturity were
evaluated in Nelore bulls, age, weight, and SC had similar sensitivity, specificity, and predictive values. At puberty, approximately 60% of the sperm present in the ejaculate were morphologically defective. Changes in semen quality after puberty in Nelore bulls were characterized by increased motility and proportion of morphologically normal sperm, with a decrease in the proportion of major sperm defects. In conclusion, early-maturing bulls were more developed in the pre-pubertal period and attained puberty at earlier stages of body and testicular development than late-maturing bulls. Yearling SC could be used to select bulls for sexual precocity and SC was the best predictor of pubertal status. Age, weight, and SC were equally good predictors of sexual maturity in *B. indicus* bulls.

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**Keywords:** *Bos indicus*; Bull; Puberty; Scrotal circumference; Semen

### 1. Introduction

The majority of world’s cattle population is located in tropical and subtropical regions, where *Bos indicus* breeds and *Bos indicus* × *Bos taurus* crossbreds predominate. *B. indicus* breeds are better adapted to the tropics and are more resistant to heat stress than are *B. taurus* breeds because they usually have a smaller frame, greater skin surface to body size ratio, more sweat glands and lower thermogenesis [1]. Moreover, *B. indicus* cattle are productive on low-quality forage diets and are more resistant to both ecto and endoparasites. However, cattle productivity in the tropics is generally lower than in temperate regions and delayed age at puberty is a substantial contributor to the low efficiency of meat and milk production in tropical regions. In contrast to *B. taurus* bulls that attain puberty around 8–10 months of age [2–4] and are used for breeding purposes as yearlings [5,6], *B. indicus* bulls generally do not reach puberty until 16–18 months of age [7–9] and can only be used as breeding bulls when 2 years old [10,11]. Selection for precocious sexual maturation in *B. indicus* and crossbred bulls can decrease production costs, reduce generation interval, and increase genetic gains and overall productivity.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations increase between 2 and 5 months after birth (early gonadotropin rise), and precede the testicular cellular events that accompany initiation of spermatogenesis in bulls [12–14]. Age at puberty is related to the timing and magnitude of the early gonadotropin rise; LH pulse frequency increases earlier and mean concentration is higher in early- than in late-maturing bulls [15,16]. Although intensive blood samplings to determine LH secretory pattern is not practical for selection of precocious bulls, certain characteristics that can be readily measured, like body weight and scrotal circumference, also differ between early- and late-maturing bulls. Some recent studies have shown that the ultrasonographic appearance of the testes and accessory sexual glands change during the pre-pubertal period in bulls [17–19], suggesting a potential use of ultrasonography for evaluating sexual maturation. Moreover, testicular echodensity also differs between early- and late-maturing bulls [16].

The objectives of this study were to evaluate sexual development in *B. indicus* and *B. indicus* × *B. taurus* crossbred bulls and to determine differences between early- and
late-maturing bulls. In addition, predictors of sexual precocity and pubertal and sexual maturity status were also determined.

2. Materials and methods

2.1. Experiment 1

This experiment was conducted at the Brazilian National Beef Cattle Research Center (EMBRAPA/CNPGC), located in Campo Grande, Mato Grosso do Sul State (latitude: 20°26′34″ S; longitude: 54°38′47″W). Twelve Nelore bulls (*B. indicus*) born in November and weaned with 7.5 months were used. From birth to the conclusion of the experiment, the bulls were extensively managed in *Brachiaria brizantha* pasture with mineral supplementation ad libitum. Average monthly temperature, relative humidity, and precipitation were 22.9 °C, 69.1%, and 109.5 mm, respectively.

Weight and SC were measured and semen was collected by electroejaculation at approximately 20 days intervals from 300 to 900 days of age (age S.D. varied from ±2 to 7 days). Ejaculate volume was estimated in a graduated tube and sperm concentration was determined by the hemocytometer method. The proportion of progressively motile sperm was estimated under 400× magnification and phase-contrast microscopy. For assessment of sperm morphology, a semen sample was preserved in formol-citrate, a wet-mount was prepared, and 100 spermatozoa per sample were examined with phase-contrast microscopy under 1200× magnification and phase-contrast microscopy. Sperm abnormalities were classified according to Barth and Oko [20]; major defects included acrosome defects, abnormal head shape, vacuoles, double heads, abnormal loose heads, proximal cytoplasmic droplet, abnormal midpiece, accessory tails, and strongly folded or coiled principal piece, while minor defects included distal cytoplasmic droplet, coiled principal piece, and normal loose heads. Puberty was characterized by an ejaculate containing ≥50 × 10⁶ sperm with ≥10% motile sperm [2] and sexual maturity was characterized by an ejaculate containing ≥70% morphologically normal sperm. Seventy percent normal sperm was selected as the threshold to define sexual maturity because it is the minimum for a bull to be considered satisfactory after a breeding soundness evaluation [21]. In other words, if puberty is defined as when a bull is first capable of reproducing, than sexual maturity could be defined as when a bull can be first used for reproduction and produce results that are considered satisfactory in the industry, whether breeding cows at pasture or producing semen for artificial insemination. Bulls were classified as early- or late-maturing according to the distribution of age at puberty (Fig. 1).

2.2. Experiment 2

This experiment was conducted at the Brazilian Southeast Agriculture and Livestock Research Center (EMBRAPA/CPPSE), located in Sao Carlos, Sao Paulo State (latitude: 21°57′42″ S; longitude: 47°50′28″ W). Twenty-eight Canchim bulls born in July and weaned with 8 months were evaluated. Canchim is a beef breed developed by crossing *B. indicus* breeds (mainly Indubrasil, Guzera, and Nelore) with Charolais; in 1983, it was
financially recognized as a breed composed by 3/8 *B. indicus* and 5/8 *B. taurus*. From birth to the conclusion of the experiment, the bulls were extensively managed in *Cynodon dactilon* pasture with mineral supplementation ad libitum. Average monthly temperature, relative humidity, and precipitation were 20.9 °C, 75.2%, and 112.1 mm, respectively.

Weight, SC, testes dimensions, testicular vascular cone (TVC) diameter, and testicular and vesicular glands (VG) pixel-intensity were determined at 19–30 days intervals from 295 to 488 days of age (age S.D. varied from ±8 to 9 days). Testes width and length were measured with calipers and a B-mode ultrasound scanner equipped with a 7.5 MHz linear-array transducer (Scanner 450 VET Echograph; Piemedical, Maastricht, The Netherlands) was used to image the TVC, testes, and VG. Images were frozen, recorded on a VHS recorder, and subsequently digitized and analyzed with image analysis software (Image 1.58; National Institutes of Health, Bethesda, MD, USA). For imaging the TVC, the transducer was held horizontally on the caudal surface of the scrotal neck and a cross-sectional image of each cone was recorded. For imaging the testes, the transducer was held vertically (parallel to the long axis of the testes) on the caudal surface of the scrotum, aligned so that the mediastinum was readily apparent before an image of each testis was recorded. A transversal–sectional image of each VG was recorded during a transrectal exam. Testicular and VG pixel-intensity were determined on a selected area of the parenchyma on a scale of 1 (white) to 255 (black); therefore, reduced pixel-intensity correspond to increased tissue echodensity (brightness). The area for analysis was selected by drawing a rectangular figure 0.5 to 1 cm deep into the parenchyma, where it looked homogeneous (the edges of the image were avoided and, for the testes, the area was selected above the mediastinum). The average area analyzed consisted of 4165 pixels for testes and 2428 pixels for VG. For TVC diameter and testicular and VG pixel-intensity, the averages of left and right measurements were used for analysis. Semen was collected by electroejaculation after each exam until puberty was detected and sperm motility and morphology were determined as described in Experiment 1. In this experiment ejaculate volume and sperm concentration were not evaluated and puberty was defined by an ejaculate containing ≥30% motile sperm. When the definition of puberty described in Experiment 1 is used, the interval between appearance of first sperm in the ejaculate and

![Fig. 1. Age at puberty among early- and late-maturing Nelore bulls (*Bos indicus*; Experiment 1, *n* = 6 per group).](image-url)
puberty is relatively short (2–3 weeks) and usually corresponds to the interval between exams used in the study [2,4,22]. Therefore, the majority of bulls are expected to be pubertal in the very next collection after sperm are firstly observed in the ejaculate. Raising the threshold value of sperm motility could compensate the differences between the two criteria (i.e. ≥50 million sperm and ≥10% motility versus ≥30% sperm motility) that could be attributed to sperm output. Moreover, ~30% sperm motility is a common figure observed at puberty in bulls of different breeds [2,23–25]. Bulls were classified as early- or late-maturing according to the distribution of age at puberty (Fig. 2).

2.3. Statistical analyses

Sperm motility and morphology data were arcsine-transformed before analysis. Two-sample Student’s t-test (Statix Analytical Software; Tallahassee, FL, USA) was used to determine group (early- versus late-maturing) differences in weight, SC and testes characteristics adjusted for 365 days of age, to determine group differences in age, weight, SC, testes, TVC and VG characteristics, sperm production (sperm concentration and total sperm output), and semen quality (sperm motility and morphology) at puberty and at maturity, and to determine group differences in the interval between puberty and maturity in Experiment 1. Mixed model analysis with Tukey’s test (Statistical Analysis System; SAS Institute, Cary, NC, USA) was used to determine and locate group, age, and group-by-age effects on weight, SC, testes, TVC and VG characteristics, sperm production, and semen quality using the covariate structure that best fitted each end-point [26]; data were evaluated both according to chronological age and according to age normalized to puberty in Experiment 1. Paired t-test (Statix Analytical Software) was used to determine differences in sperm production and semen quality between puberty and maturity in Experiment 1. Threshold values of SC and testes pixel-intensity adjusted for 365 days of age were selected as best predictors of sexual precocity (i.e. early- or late-maturing) by evaluating ROC curves (WinEpiscope; http://www.clive.ed.ac.uk/winepiscope). Threshold values of age, weight, SC, and testes pixel-intensity were selected as best predictors of pubertal and sexual maturity status also by evaluating ROC curves. In addition, regression coefficients (Statix Analytical Software) were determined using age at puberty and
maturity as dependent variables and weight, SC, and testes pixel-intensity as independent variables.

3. Results

3.1. Experiment 1

Two Nelore bulls (one early- and one late-maturing) had not reached maturity by the end of the experimental period; the proportion of normal sperm in the last three ejaculates averaged 55 and 65% in these bulls, respectively. There were no differences in weight or SC adjusted for 365 days of age between early- and late-maturing bulls. Early-maturing Nelore bulls were younger (−147 days; −197 to −96 days, 95% CI; P < 0.0005) and had smaller SC (−3.7 cm; −5.5 to −1.8 cm; P < 0.001) than late-maturing bulls at puberty. Early-maturing bulls were also younger than late-maturing bulls at maturity (−108 days; −208 to −9 days; P < 0.05; Table 1). There was an age effect (P < 0.05) and a tendency (P = 0.07) of a group-by-age effect on weight when data were analyzed according to age. The rate of growth was somewhat reduced between 560 and 640 days of age in late-maturing bulls, while it was constant in early-maturing bulls. Early-maturing bulls were heavier (P < 0.05) than late-maturing bulls from 580 to 680 days, which corresponded approximately to the period between the attainments of puberty by the two groups (Fig. 3A). When weight was analyzed according to age at puberty, there were group and age effects (P < 0.05). Overall, late-maturing bulls were heavier than early-maturing bulls (327.5 ± 11.8 kg versus 279.4 ± 11.9 kg, respectively; mean ± S.E.M.) from 240 days before to 240 days after puberty (Fig. 3C). There was an age effect (P < 0.05) and a tendency (P = 0.07) of a group effect on SC when data were analyzed according to age. Overall, early-maturing bulls tended to have greater SC than late-maturing bulls (24.7 ± 0.4 cm versus 23.5 ± 0.4 cm, respectively). It seemed that after 400 days of age SC increased faster in early-maturing bulls (Fig. 3B). When SC was analyzed according to age at puberty, there were group, age and group-by-age interaction effects

Table 1
Mean (±S.E.M.) age, weight, and scrotal circumference (SC) at puberty (≥50 × 10⁶ sperm and ≥10% motile sperm) and maturity (≥70% normal sperm) in early- and late-maturing Nelore bulls (*Bos indicus*; Experiment 1)

<table>
<thead>
<tr>
<th></th>
<th>Early (n = 6)</th>
<th>Late (n = 6)</th>
<th>Overall (n = 12)</th>
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<tbody>
<tr>
<td><strong>Puberty</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>526.7 ± 12.3ᵃ</td>
<td>673.3 ± 19.1ᵇ</td>
<td>600.0 ± 24.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>282.2 ± 9.7</td>
<td>313.3 ± 14.8</td>
<td>297.8 ± 9.7</td>
</tr>
<tr>
<td>SC (cm)</td>
<td>22.5 ± 0.6ᶜ</td>
<td>26.2 ± 0.6ᵈ</td>
<td>24.3 ± 0.7</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 10)</td>
<td></td>
</tr>
<tr>
<td><strong>Maturity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>660.0 ± 36.3ᵉ</td>
<td>768.0 ± 23.3ᶠ</td>
<td>714.0 ± 27.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>332.0 ± 14.1</td>
<td>365.4 ± 15.8</td>
<td>348.7 ± 11.5</td>
</tr>
<tr>
<td>SC (cm)</td>
<td>27.6 ± 1.7</td>
<td>29.4 ± 0.5</td>
<td>28.5 ± 0.9</td>
</tr>
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</table>

Columns with different superscripts differ (a, b: P < 0.0005; c, d: P < 0.001; e, f: P < 0.05).
Fig. 3. Mean (±S.E.M.) scrotal circumference (SC) and body weight according to age (A and B) and age at puberty (C and D) in early- and late-maturing Nelore bulls (Experiment 1; n = 6 per group). Arrows indicate mean age at puberty (≥50 × 10⁶ sperm and ≥10% motile sperm; ▲ early, ▼ late). G, A, G*A: group, age, and group-by-age effects, respectively. Means with superscripts indicate differences between groups within age (a: P < 0.05; b: P < 0.005; c: P < 0.01).
Late-maturing bulls had greater ($P < 0.005$ to <0.05) SC than early-maturing bulls from 200 to 160 days before puberty and from 40 days before to 80 days after puberty. Scrotal circumference growth rate decreased after puberty in late-maturing bulls, while it was maintained constant in early-maturing bulls (Fig. 3D).

There was no difference between early- and late-maturing bulls in the interval between puberty and maturity (110 ± 15.8 days), and in sperm production and semen quality at these sampling dates. Sperm concentration and total sperm output increased ($P < 0.05$) between puberty and maturity. Semen quality also improved during this period; sperm motility and proportion of normal sperm increased ($P < 0.05$ and <0.0005, respectively), while the proportion of major sperm defects decreased ($P < 0.0005$; Table 2). There were group, age and group-by-age interaction effects ($P < 0.05$) on sperm motility and major defects when data were analyzed according to age. When compared to late-maturing, early-maturing bulls had a greater ($P < 0.001$ to <0.05) proportion of motile sperm from 560 to 660 days of age (not significant at 600 days), and a smaller ($P < 0.01$ to <0.05) proportion of major sperm defects from 600 to 740 days of age (Fig. 4A and B). There were also age effects ($P < 0.01$) on ejaculate volume, sperm concentration, total sperm number, and proportion of normal sperm; all increased with age (data not shown). There were group, age, and group-by-age interaction effects ($P < 0.05$) on sperm concentration, and group and age effects ($P < 0.05$) on total sperm number in the ejaculate when data were analyzed according to age at puberty. Sperm concentration was greater ($P < 0.001$ to <0.1) in late-than in early-maturing bulls from 160 to 200 days after puberty (Fig. 4C). Overall, late-maturing bulls had greater total sperm number than early-maturing bulls ($1.58 ± 0.22$ sperm versus $0.82 ± 0.21 \times 10^9$ sperm, respectively) and sperm production increased after puberty in both groups (data not shown). There were also age effects ($P < 0.0005$) on sperm motility, proportion of normal sperm and major sperm defects. Both sperm motility and proportion of normal sperm increased after puberty, while major sperm defects decreased (Fig. 4D).

Despite the lack of significant difference in SC adjusted for 365 days of age between early- and late-maturing bulls, a cut-off value of 19 cm had 66.7% sensitivity, specificity, and positive and negative predictive values for identifying early-maturing bulls. When predictors of puberty were evaluated, cut-off values of 560 days, 300 kg, and 24 cm for age, weight, and SC were selected as best predictors. Age and SC had greater sensitivity and

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mean (±S.E.M.) sperm production and semen quality at puberty ($≥50 \times 10^6$ sperm and $≥10%$ motile sperm) and maturity ($≥70%$ normal sperm) in Nelore bulls ($Bos indicus$; Experiment 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty ($n=12$)</td>
<td>Maturity ($n=10$)</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>$2.7 ± 0.5$</td>
</tr>
<tr>
<td>Concentration ($×10^9$ sperm/ml)</td>
<td>$82.9 ± 15.9^a$</td>
</tr>
<tr>
<td>Total sperm number ($×10^9$)</td>
<td>$179.0 ± 34.7^a$</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>$36.4 ± 5.1^a$</td>
</tr>
<tr>
<td>Normal sperm (%)</td>
<td>$39.5 ± 4.6^c$</td>
</tr>
<tr>
<td>Major sperm defects (%)</td>
<td>$51.7 ± 5.1^c$</td>
</tr>
<tr>
<td>Minor sperm defects (%)</td>
<td>$8.8 ± 1.8$</td>
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</tbody>
</table>

Columns with different superscripts differ (a, b: $P < 0.05$; c, d: $P < 0.0005$).
Fig. 4. Mean (±S.E.M.) sperm motility (A) and major sperm defects (B) according to age, and sperm concentration according to age at puberty (C) in early- and late-maturing Nelore bulls (Experiment 1; n = 6 per group). Arrows indicate mean age at puberty (≥50 × 10⁶ sperm and ≥10% motile sperm; ▽ early, ▲ late). Changes in semen quality are shown according to age at puberty for both groups combined (D). G, A, G*A: group, age, and group-by-age effects, respectively. Means with superscripts indicate differences between groups within age (a: P < 0.001; b: P < 0.05; c: P < 0.1; d: P < 0.01).
negative predictive values, while weight had the greatest specificity and positive predictive value. Overall, SC had the best sensitivity/specificity relationship. When predictors of sexual maturity were evaluated, cut-off values of 740 days, 350 kg, and 29 cm for age, weight, and SC were selected as best predictors with similar sensitivity, specificity, and predictive values. Weight and SC had significant moderate to high positive regressions with age at puberty and maturity (Fig. 5).

3.2. Experiment 2

When adjusted for 365 days of age, SC in early-maturing Canchim bulls was greater (3.2 cm; 2.1–4.2 cm; \( P < 0.0001 \)) and testicular pixel-intensity was less (−19.0 points; −35.3 to −2.7 points; \( P < 0.05 \)) than in late-maturing bulls. Early-maturing bulls were also younger (−101 days; −121 to −81 days; \( P < 0.0001 \)) and lighter (−22.5 kg; −45.4 to −0.5 kg; \( P < 0.05 \)) than late-maturing bulls at puberty. Moreover, VG pixel-intensity was greater (16.6 points; 1.2–32.2 points; \( P < 0.05 \)) in early- than in late-maturing bulls at puberty (Table 3). There were age effects (\( P < 0.0001 \)) on weight, SC, TVC diameter, VG pixel-intensity, and testes length, width and pixel-intensity, while group effects (\( P < 0.05 \)) were observed on SC, TVC diameter, and testes length, width and pixel-intensity. There were also group-by-age effects (\( P < 0.05 \)) on testes length and pixel-intensity (Figs. 6 and 7). Weight did not change significantly between 295 and 320 days of age, then it decrease until 342 days before increasing again (Fig. 6A). Overall, early-maturing bulls had greater SC (25.7 ± 0.4 cm versus 22.3 ± 0.3 cm, respectively; \( P < 0.0001 \)) and TVC diameter (16.7 ± 0.4 cm versus 15.6 ± 0.3 cm, respectively; \( P < 0.05 \)) than late-maturing bulls; SC increased continuously with age, while there was more variation in TVC diameter despite the general tendency of increase (Fig. 6B and C). Vesicular gland pixel-intensity decreased (image became more echodense, brighter) between 295 and 320 days of age, but did not change significantly thereafter (Fig. 6D). Testes length and width increased with age; length was greater \( (P < 0.005 \) to \( <0.05 \)) in early-maturing bulls from 320 to 404 days of age (i.e. until before late-maturing bulls reached puberty), while width was greater \( (P < 0.0001 \) during the entire period of the study (overall testes width was 45.7 ± 1.1 mm

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Mean (±S.E.M.) age, weight, scrotal circumference (SC), and characteristics of the testes, testicular vascular cones (TVC) and vesicular glands (VG) at puberty (≥30% motile sperm) in early- and late-maturing Canchim bulls (Bos indicus × Bos taurus crossbred; Experiment 2)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Early ( (n = 11) )</td>
</tr>
<tr>
<td>Age (days)</td>
<td>360.1 ± 9.4a</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>254.9 ± 8.4c</td>
</tr>
<tr>
<td>SC (cm)</td>
<td>24.5 ± 0.4</td>
</tr>
<tr>
<td>Testes length (mm)</td>
<td>71.1 ± 1.4</td>
</tr>
<tr>
<td>Testes width (mm)</td>
<td>43.2 ± 1.5</td>
</tr>
<tr>
<td>Testes pixel-intensity1</td>
<td>161.9 ± 3.8</td>
</tr>
<tr>
<td>TVC diameter (mm)</td>
<td>16.8 ± 1.0</td>
</tr>
<tr>
<td>VG pixel-intensity1</td>
<td>198.4 ± 6.7c</td>
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Columns with different superscripts differ \( (a, b: P < 0.005; c, d: P < 0.05) \).

1 Pixel-intensity scale: 1: white; 255: black.
Fig. 5. Relationship of pubertal status (≥50 × 10^6 sperm and ≥10% motile sperm; left) and mature status (≥70% normal sperm; right) with age (top), weight (middle), and SC (bottom) in early- and late-maturing Nelore bulls (Bos indicus, Experiment 1; n = 6 per group). PV (+) and PV (−): positive and negative predictive values, respectively.
Fig. 6. Mean (±S.E.M.) body weight (A), scrotal circumference (SC; B), testicular vascular cone (TVC) diameter (C), and vesicular gland (VG) pixel-intensity (D) according to age in early- \( n=11 \) and late-maturing \( n=17 \) Canchim bulls (Experiment 2). Arrows indicate mean age at puberty (≥30% motile sperm; † early, ‡ late). G, A: group and age effects, respectively. Pixel-intensity scale: 1: white; 255: black.
Fig. 7. Mean (±S.E.M.) testes length (A), width (B), and pixel-intensity (C) according to age in early- (n = 11) and late-maturing (n = 17) Canchim bulls (Experiment 2). Arrows indicate mean age at puberty (≥30% motile sperm; • early; ↑ late). G, A, G*A: group, age, and group-by-age effects, respectively. Pixel-intensity scale: 1: white; 255: black. Means with superscripts indicate differences between groups within age (a: \( P < 0.005 \); b: \( P < 0.05 \); c: \( P < 0.01 \)).
versus 38.0 ± 0.9 mm in early- and late-maturing bulls, respectively; Fig. 7A and B). Testes pixel-intensity decreased (image became more echodense, brighter) with age in both groups, but was not similar between early- and late-maturing bulls until before late-maturing bulls reached puberty, though significant differences were observed only at 295 and 342 days of age, when testes pixel-intensity was greater ($P < 0.005$) in late-maturing bulls (Fig. 7C). The most prevalent sperm defect at puberty was proximal cytoplasmic droplet (28% of the spermatozoa were affected), followed by midpiece defects and abnormal sperm head shape (Table 4).

When predictors of sexual precocity at 365 days of age were evaluated, cut-off values of 24 cm and 170 points for SC and testicular pixel-intensity were selected as best predictors. Scrotal circumference had 72.7% sensitivity, 100% specificity, 100% positive predictive value, and 85% negative predictive value for identifying early-maturing bulls, while

![Graphs showing relationship between age, weight, scrotal circumference, and testes pixel-intensity with age at puberty.](image)

**Fig. 8.** Relationship of age (top-left), weight (bottom-left), scrotal circumference (SC; top-right), and testes pixel-intensity (bottom-right) with age at puberty (≥30% motile sperm) in early- ($n = 11$) and late-maturing ($n = 17$) Canchim bulls ($Bos indicus \times Bos taurus$ crossbred, Experiment 2). PV (+) and PV (-): positive and negative predictive values, respectively.
testicular pixel-intensity had 36.3% sensitivity, 29.4% specificity, 25.0% positive predictive value, and 41.7% negative predictive value. When predictors of pubertal status were evaluated, cut-off values of 430 days, 285 kg, and 25 cm for age, weight, and SC were selected as best predictors. Weight and SC had greater sensitivity, specificity, and positive predictive values than age, but negative predictive values were similar. Weight and SC had significant but small positive regressions with age at puberty. Testicular pixel-intensity had small sensitivity, specificity, and predictive values with small insignificant regression with age at puberty (Fig. 8).

4. Discussion

Nelore bulls seemed slightly older at puberty than previously reported in several B. indicus breeds [7–9,27–29]. Canchim bulls were younger at puberty than 1/2 and 3/4 Brahman or Shaliwal × Shorthorn crossbred bulls in Australia [23], but were older than 1/2 Brahman × Angus bulls in Florida [30]; differences in breed composition and management were probably responsible for these differences. In both experiments, age at puberty was greater than reported in B. taurus breeds [2–4,22,24,28,31]. In addition to genotype, environment has a major effect on age at puberty. Angus and Hereford bulls raised in subtropical Florida [28] and Holstein and Brown-Swiss bulls raised in tropical Mexico [24] were older at puberty than bulls of the same breeds raised in temperate regions [2–4,31]. Therefore, management, climate, and photoperiod may also contribute to the delayed puberty observed in bulls in the tropics.

Scrotal circumference and weight at puberty in Nelore bulls were greater than that reported in Bunaji and Sokoto bulls [7], similar to bulls of the same breed [9], and smaller than in Guzerat and Brahman bulls [8,9,27–29], possibly reflecting breed and management differences among studies. In Canchim bulls, SC and weight at puberty were similar to Brahman or Shaliwal × Shorthorn crossbred bulls [23], and smaller than that reported in Brahman × Angus bulls [30]. Scrotal circumference and body weight at puberty in both experiments were smaller than that described in B. taurus beef bulls [2,4,22,28,31]. Greater age and smaller SC and weight at puberty in B. indicus and crossbred bulls indicated that
both body and sexual development in these bulls were slower than in \textit{B. taurus} bulls. Despite their slower developmental rate, \textit{B. indicus} bulls usually reach the same mature SC and weight as \textit{B. taurus} bulls raised in the same conditions \cite{8,10,28,32}. Moreover, studies conducted with mature AI bulls in Brazil demonstrated that sperm production is similar or even greater in \textit{B. indicus} than in \textit{B. taurus} bulls \cite{32,33}. Therefore, delayed puberty seems to be the main factor affecting the lower reproductive efficiency of \textit{B. indicus} and crossbred bulls when compared to \textit{B. taurus} bulls; there is no evidence to support inherently lower fertility.

In general, sperm motility and total sperm number at puberty in Nelore and Canchim bulls were similar to other breeds \cite{2,9,23–25}, but less than reported in Brahman bulls \cite{8}. Total sperm defects at puberty (approximately 60\% in both experiments) was similar to some reports in \textit{B. taurus} and crossbred bulls \cite{2,23,25}, but higher than others studies in \textit{B. indicus} bulls, which reported the total proportion of defective sperm at puberty as only 11–15\% \cite{8,9}; the main sperm abnormality observed at puberty was proximal cytoplasmic droplets. Consistent with the present observations, a study conducted with 130 Nelore bulls between 12 and 18 months old in Brazil indicated that the proportion of total sperm defects was approximately 57\% \cite{34}. In Nelore bulls, sperm concentration, motility, and normal morphology increased after puberty, while major sperm defects decreased, in agreement with a study in \textit{B. taurus} bulls \cite{25}. Sexual maturity in Nelore bulls was not attained until approximately 24 months of age, consistent with observations in Florida, where Brahman and Brahman × Nelore bulls only had ≥70\% normal sperm after 24 months of age, and observations in Brazil, where 20\% of 178 Nelore bulls were still considered immature at 2 years of age based on their spermiogram \cite{11}.

Initiation of spermatogenesis is related to the increased gonadotropin secretion that occurs between 2 and 5 months after birth in \textit{B. taurus} bulls \cite{12–14}. It is not know if delayed puberty in \textit{B. indicus} and crossbred bulls in relation to \textit{B. taurus} bulls is a result of a delayed gonadotropin rise or slower establishment of spermatogenesis, since evaluation of gonadotropins secretory patterns during calfhood have not been reported in bulls with those genotypes. In two studies evaluating testicular histological changes in \textit{B. indicus} calves \cite{35,36}, seminiferous tubules diameter began to increase approximately 4–5 months after birth, similar to \textit{B. taurus} bulls \cite{37}. However, spermatocytes and mature spermatozoa were not observed until approximately 9 and 14 months, respectively, in contrast to 4 and 8 months in \textit{B. taurus} bulls. The relationship of age at puberty with the timing and magnitude of the early gonadotropin raise has been recently demonstrated; early-maturing bulls had increased LH pulse frequency and mean concentration at younger ages than late-maturing bulls \cite{15,16}. Moreover, inhibition of gonadotropin secretion before 5 months of age delayed puberty, while hastening gonadotropin secretion (with GnRH treatment) hastened puberty \cite{38,39}. Despite the lack of supporting data, differences in gonadotropins secretory pattern are probably also responsible for differences in age at puberty between early- and late-maturing \textit{B. indicus} and crossbred bulls.

Early-maturing bulls were 3.3–4.8 months younger than late-maturing bulls at puberty, clearly demonstrating the opportunity for selection of precocious bulls. In general, early-maturing bulls were heavier and had greater SC, testes, and TVC diameter than late-maturing bulls during the entire experimental period, in agreement with previous reports in \textit{B. taurus} bulls \cite{15,16}. Another study with \textit{B. taurus} bulls also demonstrated that average
SC during the 14-weeks before puberty had high negative correlation with age at puberty, indicating that bulls with larger SC reached puberty earlier [25]. Scrotal circumference adjusted for 365 days of age was a good predictor of sexual precocity and minimum yearling SC of 19 and 24 cm for Nelore and Canchim bulls, respectively, are suggested for selection of early-maturing bulls. Also in agreement with previous experiments [16–19], both VG and testicular parenchyma became increasingly more echodense (brighter) with age (in this study it was demonstrated by a decrease in pixel-intensity value). The lack of difference in VG pixel-intensity between early- and late-maturing bulls was somewhat surprising as differences in the stage of sexual development and possibly in serum testosterone concentrations were expected to have affected VG function and ultrasonic appearance. Early-maturing Canchim bulls had more echodense testes than late-maturing bulls until before the latter group reached puberty, similar to previous observations in B. taurus bulls [16]. However, yearling testicular echodensity was not a good predictor of sexual precocity.

In general, early-maturing bulls were lighter and had smaller SC at puberty than late-maturing bulls, indicating that sexual precocity was not related to attainment of a threshold body or testicular development earlier. In fact, the present study suggests that these thresholds are lower in early-maturing bulls, i.e. late-maturing bulls must reach a more advanced stage of body and testicular development before puberty is attained. This was especially evident when weight and SC were evaluated according to age at puberty in Nelore bulls and is consistent with observations in early- and late-maturing B. taurus bulls [15]. Furthermore, early-maturing Nelore bulls attained puberty during the phase of rapid SC growth, while late-maturing bulls attained puberty immediately before entering a plateau phase of SC development. As SC developmental rate was kept constant in early-maturing bulls, but was reduced in late-maturing bulls after puberty, SC at maturity was not significantly different between the groups (despite the numerical difference). Testes pixel-intensity at puberty did not differ between early- and late-maturing Canchim bulls, indicating that a certain developmental stage of the testicular parenchyma must be reached before puberty. Previous studies also demonstrated that testicular pixel-intensity differ between pre-pubertal and pubertal Nelore and Canchim bulls [19], but there are no age related changes in mature B. indicus, B. taurus, and crossbred bulls [32]. The differences in sperm motility and major sperm defects between early- and late-maturing bulls at given ages were consistent with the delayed puberty in the latter group. Greater total sperm number in late-maturing bulls when compared to early-maturing bulls after puberty was associated with greater sperm concentration in the ejaculate and probably reflected differences in SC between the two groups.

Scrotal circumference was the best predictor of pubertal status as indicated by the best sensitivity/specificity relationship in Nelore bulls, and by the high sensitivity and specificity in Canchim bulls; SC accounted for 74 and 13% of the variation in age at puberty in Nelore and Canchim bulls, respectively. Similarly, Lunstra et al. [4] observed that across several B. taurus breeds, puberty was attained consistently when SC reached 27.9 cm, despite great differences in age and weight among breeds. Based on the results of the present experiment, Nelore and Canchim bulls were expected to be pubertal when SC was ≥24 and 25 cm, respectively. Age was also a good predictor of pubertal status in both Nelore and Canchim bulls, but while weight was as good as SC for prediction of pubertal
status in Canchim bulls, weight had high specificity, but low sensitivity in Nelore bulls. Nonetheless, weight accounted for 56 and 15% of the variation in age at puberty in Nelore and Canchim bulls, respectively. Therefore, Nelore and Canchim bulls were expected to be pubertal after 560 and 430 days of age, respectively, Nelore bulls with less than 300 kg were unlikely pubertal, and Canchim bulls were expected to be pubertal with \( \geq 325 \) kg. Testicular pixel-intensity however, was not a good predictor of pubertal status. Age, weight, and SC were equally good predictors of sexual maturity status in Nelore bulls; bulls older than 740 days, heavier than 350 kg, and with SC \( \geq 29 \) cm were expected to be mature.

In conclusion, sexual development in \( B. \textit{indicus} \) and \( B. \textit{indicus} \times B. \textit{taurus} \) crossbred bulls seemed delayed in comparison to \( B. \textit{taurus} \) bulls. Early-maturing bulls were better developed (heavier and greater SC) in the pre-pubertal period and attained puberty at earlier stages of body and testicular development than late-maturing bulls. Yearling SC could be used to select bulls for sexual precocity and SC was the best indicator of pubertal status. Age, weight, and SC were equally predictive of sexual maturity status in \( B. \textit{indicus} \) bulls.

References


