

RESEARCH ARTICLE

Testicular Development in Holstein, F1 Crossbred Holstein-Gyr, Gyr, and Nellore Bulls During Puberty and Sexual Maturity in Tropical Conditions

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Abstract: Context: Puberty and sexual maturity are very important economic aspects in the beef industry and bull production. The aim of this study was to characterize and determine the sexual development of *Bos taurus*, *Bos indicus*, and F1 crossbreeds of these two subspecies of bulls and their testicular growth and shape. We observed the evolution of testicular development characteristics and seminal aspects from puberty to sexual maturity in bulls, and we verified differences between seminal characteristics, scrotal circumference, and testicular volume between puberty and sexual maturity. We used 17 Holstein, 21 Gyr, and 29 crossbred F1 Holstein-Gyr bulls from 7 to 25 months of age in a semi-extensive system, as well as 18 Nellore from 10 to 30 months of age in an extensive pasture range. Every four weeks, we measured testicular growth (length, width, and volume) and scrotal circumference. Semen samples were collected every two weeks, and physical and morphological aspects of ejaculate were observed. Seminal puberty and sexual maturity were defined according to seminal characteristics. Age at puberty and sexual maturity differed between Holstein, Gyr, and crossbred F1 Holstein-Gyr bulls. These ages were similar between the Nellore and Gyr zebu bulls. The period from puberty to sexual maturity was similar between the genetic groups in the study. The testicular shape was moderately long in zebus, but ovoid in taurine and F1 crossbred bulls. The relationship between testicular width and length was larger between taurine and F1 crossbred bulls than zebu bulls. Scrotal circumference and testicular volume development were represented by curves logistic functions. Scrotal circumference as a measure of sexual development at puberty and sexual maturity differed between taurine, crossbred F1, and zebu bulls. However, at sexual maturity, testicular size in bulls of different zebu and taurine subspecies was equivalent in all dimensions assessed. In addition to scrotal circumference, measurement of testicular length could be included as a means to calculate testicular volume when working with the production of zebu bulls in a tropical climate, as a method of monitoring sexual development.

Keywords: Cattle, Sexual Development, Testicular Biometry, Semen, Sperm Morphology

Received: 23-06-2025 | **Revised:** 25-07-2025 | **Accepted:** 17-10-2025 | **DOI:** 10.3844/ajavsp.2026.21.02.013

Introduction

Puberty is the starting point of fertility, and the period during which rapid reproductive and somatic development is verified. Its onset, according to several lines of research, comprises changes in hypothalamus sensitivity to the inhibitory effects of gonadal and extra-gonadal factors [1]. The greatest differences regarding the chronology of reproductive development are related to bovines of *Bos taurus taurus* and *Bos taurus indicus* subspecies, as well as the products of their crossbreeding. From this perspective, taurines exhibit great precocity [2-5].

Once puberty is established in a bull, qualitative and quantitative changes in the seminal characteristics of the ejaculate can be observed: increases in volume, progressive motility, vigor, and sperm concentration, as well as a concomitant decrease in the percentage of sperm defects [6-9].

Increase in testicular size represents one of the most well-characterized evolutionary responses to sperm competition, and accelerated development is an important characteristic of puberty and sexual maturity. As the relative testicular size of a male increases, the proportion of seminiferous tubules in the testicular parenchyma increases. Consequently, in order to select for greater investment in testicular tissue, increased sperm competition levels can be expected [10]. Testicular size depends upon sperm demand and is molded by a series of selective forces: sperm competition, mating rate, and sperm count, typically identified as the three most powerful evolutionary forces [11].

Scrotal circumference is highly correlated with the age of bulls, with r values ranging between 0.54-0.95, depending upon the age interval reported [5, 12-16]. Linear relationships of testicular growth with age have been reported for young Holstein bulls aged between 6 and 24 months [12], as well as curvilinear and quasilinear relationships [14].

Breed is another factor that influences testicular size and scrotal circumference. Few differences have been reported among young bulls of taurine breeds, although a study reported that the scrotal circumference of Angus bulls was greater than that of Holstein bulls of the same age, and that this difference was reversed at 36 months of age [17]. The young bulls, aged 18 to 24 months, reached 100% and 89% of the scrotal circumference of an adult of each breed, respectively. A study on breeds of beef bulls reported differences, although not significant, in the scrotal circumference, the highest of which was that of the Brown Swiss and the lowest of which was that of the Hereford [13]. In breeding soundness evaluation of taurine bulls at 24 months of age, a scrotal circumference greater than or equal to 34 cm is considered acceptable [18].

In order to better understand testicular growth, it is important to characterize its type, and the logistic function has been found to be the one that best approaches reality for several breeds [19, 20]. This function presents itself as adequate for the description of scrotal circumference growth as a function of age in Nellore bulls raised in pasture. The high positive correlations between weight, scrotal circumference, and age allow the selection of Nellore bulls raised in pasture to be indicated at 12 and 18 months, with approximate measurements of 20.1 cm and 25.4 cm, respectively [20, 21].

The concept of scrotal circumference as a practical measurement of testicular size in taurine breeds has been revised by studies that consider shape as well as the relationship between testicular length and width [22, 23]. In Zebu breeds, these differences in testicular shape are more striking and, based on studies in Brazil, the scrotal circumference as a measurement of size has been questioned in favor of testicular volume as a better option for this subspecies [24].

Based on these observations, studies have estimated the genetic relationship between testicular volume and other production characteristics in Nellore bulls [25, 26], as well as breeding soundness evaluation [27], and the results suggest genetic progress. Consequently, the use of this characteristic as a selection criterion may be expected [26].

We tested the hypothesis that testicular size is equivalent at puberty and sexual maturity (according to several seminal definitions) in *Bos taurus taurus*, *Bos taurus indicus*, and crossbred F1 bulls of these two subspecies.

Our goal was to verify and test the differences in testicular biometry at puberty and sexual maturity (according to several definitions of seminal characteristics), in the case of Holstein, Gyr, and crossbred Holstein-Gyr F1 bulls, and simply to verify, in the case of Nellore.

Materials and Methods

At the time this work was done, we did not have the mandatory IACUC (CEUA) in Brazil, as it is only since passage of Law 11794/08 that these have become mandatory (CEUA/UFV (IACUC) started in 2011). However, we seek to develop the

work in such a way as to observe the Principle of the three R's (replacement, reduction, refinement) and the Guide for Care and Use of Laboratory Animals/NIH-USA.

Animals and Handling Conditions

This study of testicular and sexual development in bulls was conducted in different locations. All data collection procedures were equivalent and carried out by the same staff. We used 17 Holstein bulls (registered), from 6 to 24 months of age, as well as 21 Gyr (*Bos taurus indicus*), and 29 F1 crossbreds Holstein-Gyr bulls, both from 7 to 24 months of age. This experiment was conducted in Minas Gerais State, Southeast Region of Brazil. All three groups were handled in a semi-extensive system. Bulls were provided with a balanced commercial high-energy feed for growing animals. The feed was accompanied by the administration of corn silage, once per day, in collective troughs. During the rest of the time, the young bulls remained in *Brachiaria decumbens* pasture. The amount of feed provided was adjusted during the course of the experiment in order to satisfy general growth and development requirements. We also used 18 Nellore bulls (*Bos taurus indicus*, nonregistered) from 10 to 30 months of age in Tocantins, Amazonian Legal State, Brazil. Animals were kept in an extensive pasture system with *ad libitum* mineral supplementation through the use of commercial salt. Given regional characteristics, the pastures (composed of several forage species) remained almost always green during the course of field experimentation.

Testicular Biometry

We measured the scrotal circumference (mm) with a plastic measuring tape [12]. A metal pachymeter was used to aid in the measurement of testicular length (mm) and width (mm). For the purpose of calculation and analysis, we considered the mean values of the length and width of the left and right testicles. Testicular length measures include the head and exclude the tail of the epididymus.

The testicular volume (cm³) was calculated according to the following formula [28]:

$$VOL = 2 [(r^2) \times \pi \times h]$$

Where: r = radius calculated by width/2, h = testicular length.

Testicular shape was calculated as the ratio of testicular width to length [23]. We adopted the scale of 1 to 0.5, in which 1 means WIDTH = LENGTH and 0.5, WIDTH = 1/2 LENGTH. According to this scale, the following shapes were established:

Ratio 1 \leq 0.5 = long; Ratio 2 = 0.51 to 0.625 = moderately long; Ratio 3 = 0.626 to 0.750 = long/ovoid; Ratio 4 = 0.751 to 0.875 = ovoid/spherical; Ratio 5 $>$ 0.875 = spherical.

For each of the four groups, we analyzed testicular growth curves represented by scrotal circumference and testicular volume. A logistic function was employed to accomplish this. Previous studies have suggested that this function is the best representative for testicular growth in several breeds [20, 29, 30]. This is represented by the logistic equation:

$$Y = A / (1 + B^{kt})$$

Where A is the testicular volume at maturity, B is the scale parameter, k is the maturity constant, and t is the days of age. Y = SC (scrotal circumference at t days of age) or TV (testicular volume at t days of age). TV a = 1300 b = 4 c = 0.10; SC a = 45 b = 4 c = 0.10; A = SC or TV estimated at maturity; B = proportion of SC or TV at asymptotic maturity; k = constant of maturation, given by the ratio between the maximum growth rate and SC or VT at maturity.

Seminal Characteristics

We collected ejaculates from bulls every four weeks until the appearance of the first sperms; subsequently, collections were taken every two weeks until the end of the experiment. Electroejaculation was applied for sample collection, with animals adequately and safely contained. The procedures for collection and evaluation of physical and morphologic aspects of ejaculate were performed in accordance with CBRA [31] recommendations. The proportion of progressively motile sperm was estimated under 400 \times microscopy. For assessment of sperm morphology, a semen sample was preserved in formol-citrate, a wet-mount was prepared, and 200 spermatozoa per sample were examined with interference phase-contrast microscopy under 1000 \times magnification. Sperm abnormalities were classified according to Blom [32], based on major and minor defects.

In order to study the sexual development process in bulls, we established definitions for the sexual development events of puberty and sexual maturity according to seminal characteristics. Puberty (APUB) was defined as the age at which an

ejaculate with at least 10% progressive motility and 50×10^6 sperm/ejaculate was first collected [33]. Three different definitions were employed in determining sexual maturity:

1. Age at freezable ejaculate (ejaculate with at least 50% progressive motility and 500×10^6 sperm/ejaculate [3])
2. (AMATC).2. Age at which sexual maturity was characterized by ejaculate containing 70% morphologically normal sperm and a maximum of 15% major sperm defects and 30% total sperm defects (AMAT1) [34]
3. Age at which ejaculate displayed a maximum of 15% major sperm defects and 30% total sperm defects with at least 500×10^6 sperm/mL (AMAT2)

Statistical Analysis

We analyzed quantitative and qualitative data with the use of [35]. Data analysis registered an output of 2163 observations with 242 characteristics analyzed. From total observations collected, 458, 757, 588, and 360 were associated with Holstein, crossbred F1 Holstein-Gyr, Gyr, and Nellore bulls, respectively. Analyses of the logistic function for scrotal circumference and testicular volume were performed using the procedure for nonlinear regression Proc Nlin [35].

For each event of sexual development, we calculated the means and standard deviations of the scrotal circumference (mm), testicular volume (cm³), and testicular length and width (mm). The means of the quantitative characteristics of age, scrotal circumference, testicular volume, testicular length and width, and the relationship between testicular width and length were compared through analysis of variance with the Tukey-Kramer test (where 0.05 was the cutoff for significance). Comparisons of testicular shapes were conducted through analysis with the chi-square test.

Statistical comparison was made between Holstein, Gyr, and F1 crossbred Hol-Gyr bulls. The Nellore bull group was presented in descriptive form. The two data sets are not comparable because the experiments were not carried out in the same region or with the same nutrition.

Results and Discussion

Table 1 displays ages at puberty and sexual maturity, as well as the testicular biometry (scrotal circumference and testicular volume, length, and width) of *Bos taurus taurus* and *Bos taurus indicus* bulls at different events of sexual development.

Table 1: Testicular size at puberty and sexual maturity in Holstein, Crossbred F1, Gyr and Nellore bulls

	Holstein (n = 17)			Crossbred F1 (n = 28)			Gyr (n = 24)			Nellore (n = 19)	
	Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM
Age and seminal puberty (APUB) (10% PM and 50×10^6 sperm/ejaculate)											
Age (months)	10.1	0.3	c	12.3	0.4	b	14.3	0.4	a	14.6	0.4
Scrotal circumf. (cm)	27.8	0.52	a	27.4	0.49	a	23.5	0.35	b	22.5	0.53
Testicular volume (cm ³)	422.5	27.2	a	383.2	18.5	a	267.1	13.2	b	254.7	17.1
Testicular length (cm)	8.84	0.22	a	8.52	0.16	ab	8.07	0.15	bc	7.64	0.19
Testicular width (cm)	5.47	0.14	a	5.30	0.09	a	4.55	0.08	b	4.55	0.11
Age of 1st freezable ejac. (AMATC) (50% PM, 500×10^6 sperm/ejaculate)											
Age (months)	12.5	0.2	c	15.3	0.4	b	18.0	0.5	a	19.8	0.4
Scrotal circumf. (mm)	31.2	0.39	a	30.5	0.45	a	28.2	0.48	b	28.6	0.34
Testicular volume (cm ³)	546.6	26.7	a	559.5	22.2	a	511.9	31.4	a	547.9	22.5
Testicular length (cm)	9.40	0.24	a	9.82	0.16	a	10.0	0.24	a	10.5	0.17
Testicular width (cm)	6.06	0.12	a	5.99	0.10	ab	5.62	0.13	b	5.87	0.08
Age at 70% normal sperm (AMAT1) (<30 TD, <15 MAD)											
Age (months)	13.2	0.2	c	16.1	0.4	b	18.9	0.4	a	19.6	0.3

Scrotal circumf. (cm)	32.4	0.45	a	31.1	0.55	a	28.2	0.43	b	28.44	0.87
Testicular volume (cm ³)	564.9	29.2	a	580.0	37.5	a	514.2	21.9	a	555.6	31.1
Testicular length (cm)	9.56	0.25	a	9.95	0.13	a	9.86	0.16	a	10.0	0.16
Testicular width (cm)	6.09	0.10	a	6.00	0.26	a	5.49	0.09	b	5.89	0.12
Age at full maturity (AMAT2) (<30 TD, <15 MAD, 500 x10 ⁶ [sperms])											
Age (months)	16.6	0.3	c	18.9	0.4	b	20.8	0.5	a	21.8	0.4
Scrotal circumf. (cm)	34.1	0.47	a	32.7	0.47	a	29.5	0.57	d	30.6	0.64
Testicular volume (cm ³)	755.4	34.7	a	709.3	29.0	a	677.5	31.2	a	694.6	20.4
Testicular length (cm)	10.88	0.15	a	10.71	0.17	a	11.02	0.16	a	10.77	0.17
Testicular width (cm)	6.61	0.12	a	6.45	0.09	a	6.21	0.11	d	6.39	0.14

circumf. = circumference PM = progressive sperm motility MAD = Major sperm defects, Values followed by distinct letter indicate differences between columns P<0.05 Tukey-Kramer test. Nellore bulls are not compared only descriptive propose

Table 2: Data associated with logistic growth curve of scrotal circumference in Holstein, Crossbred F1, Gyr and Nellore bulls during sexual development

	A	Confidence interval of A	B	K	Confidence interval of K	R ²	N.I
Holstein	374.2	381.3 367.2	4.35	0.25	0.27 0.22	0.95	6
Crossbred F1	348.6	357.9 339.2	4.07	0.22	0.25 0.19	0.93	5
Gyr	353.2	379.8 326.5	3.41	0.13	0.16 0.11	0.92	6
Nellore	357.8	371.5 344.1	5.93	0.15	0.17 0.13	0.97	5

A: scrotal circumference at maturity; B: scale parameter; k: maturity constant, R2 = coefficient of determination, N.I = number of interactions

At puberty, testicular size (scrotal circumference and testicular volume) was greater in Holstein and crossbred F1 bulls than in zebu bulls (P<0.05). At all events studied here, sexual maturity and scrotal circumference were greater in Holstein and crossbred F1 bulls than in zebu bulls (P<0.05). However, when comparisons were made using testicular volume, there were no differences between groups (P>0.05). Therefore, testicular volume at sexual maturity demonstrates that zebu and taurine bulls have the same testicular size, supporting the hypothesis proposed. This difference between scrotal circumference and testicular volume is attributed to different testicular shapes displayed by the bull groups under study (Tables 4 and 5).

These results are comparable to those reported previously. Holstein bulls presented testicular volumes equivalent to those described in other studies [36], and also in beef bulls [37], at sexual maturity. Regarding the zebu bulls in this study, testicular volumes are equivalent to those reported by previous research on Gyr [38] and Nellore [26, 39] bulls, respectively.

Scrotal circumference at puberty observed during this study was in accordance with pioneering studies that determined 27 cm as the measurement at which seminal puberty may be expected in beef cattle [13]. This measurement of scrotal circumference was subsequently recommended as an approximation for age at puberty [40] and was recently confirmed in Holstein bulls [36]. Likewise, testicular volume observed in this study is equivalent to that reported by recent studies applying the same formula [36].

Figure 1 displays the logistic growth curves of scrotal circumference for the four genetic groups.

All curves associated with each genetic group exhibited high coefficients of determination. The curves associated with the Holstein and crossbred F1 bulls are displayed in parallel form. Table 3 shows the data regarding the logistic curves of scrotal circumferences associated with each genetic group.

Scrotal circumference was greater at maturity in Holstein bulls. The growth rate presented by the constant of maturation (k) was smaller in zebu than in Holstein or crossbred F1 bulls.

In this study, scrotal circumference of Nelore bulls at maturity was assessed according to previous research focused on Nelore bulls [20, 41] as well as Guzerá bulls [30], both of which are zebu breeds. However, the bulls in our study exhibited a higher constant of maturation (k), which suggests a faster growth speed.

The crossbred F1 bulls in this study displayed a smaller scrotal circumference at maturity than reported for Canchim bulls, which are considered a cross between zebu and taurine bulls [29].

Figure 2 shows the logistic curves for testicular volume of each of the four genetic groups studied. The testicular volume curves associated with zebu bulls during the puberty period of 9 to 18 months of age are parallel. Afterwards, at sexual maturity post-21 months of age, these curves lose this behavior in favor of higher growth rates.

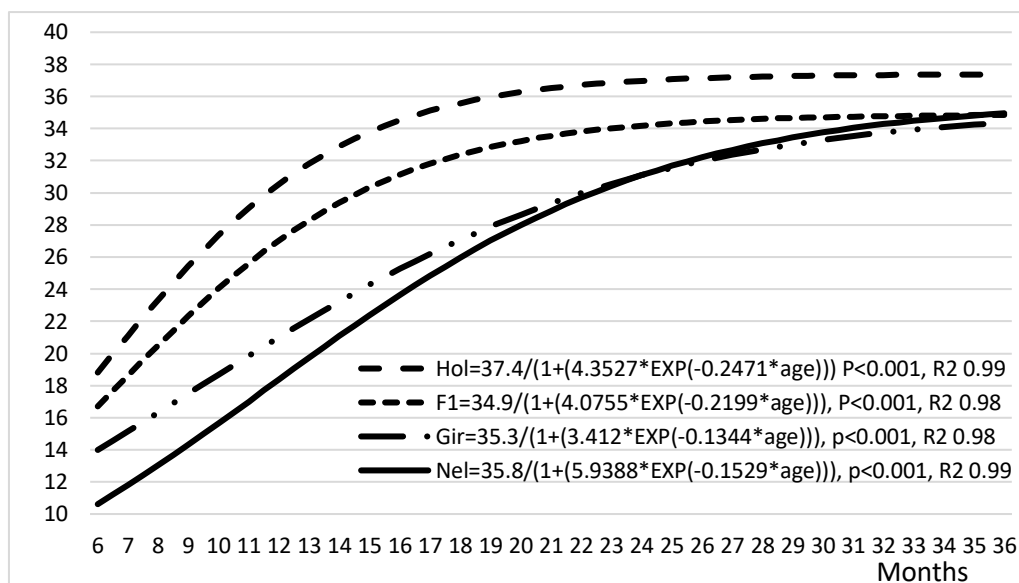


Fig. 1: Testicular growth represented by the scrotal circumference logistic curves of in Holstein, Crossbred F1, Gyr and Nelore bulls during sexual development

Table 3 presents the data associated with the logistic curves of each genetic group.

This particular characteristic of testicular growth displays different behaviors over time. Hostltein and crossbred F1 bulls exhibited parallel curves similar to those for scrotal circumference (Figure 2). Zebu bulls showed a curve that resembles the testicular growth of bulls studied in previous reports [40]. It is interesting to observe that, at ages close to the sexual maturity of the genetic groups, these curves converge, pointing to an equivalent testicular volume. Therefore, these results concur with those of [24] in Brazil, who reported that the testicular size of zebu bulls should also consider the testicular volume as a function of their more elongated testicles relative to taurine breeds.

Table 3: Data associated with logistic growth curve of testicular volume in Holstein, Crossbred F1, Gyr and Nelore bulls during sexual development

	A	Confidence interval of A	B	K	Confidence interval of K	R ²	N.I
Holstein	907.7	1040.2 901.2	22.2	0.26	0.31 0.22	0.95	10
Crossbred F1	888.5	998.8 828.1	35.35	0.27	0.30 0.23	0.93	8
Gyr	901.0	1005.2 796.8	101.9	0.27	0.30 0.23	0.92	9
Nelore	1055.6	1114.3 996,8	98.63	0.23	0.26 0,21	0.97	8

A: testicular volume at maturity; B: scale parameter; k: maturity constant, R²= coefficient of determination, N.I = number of interactions

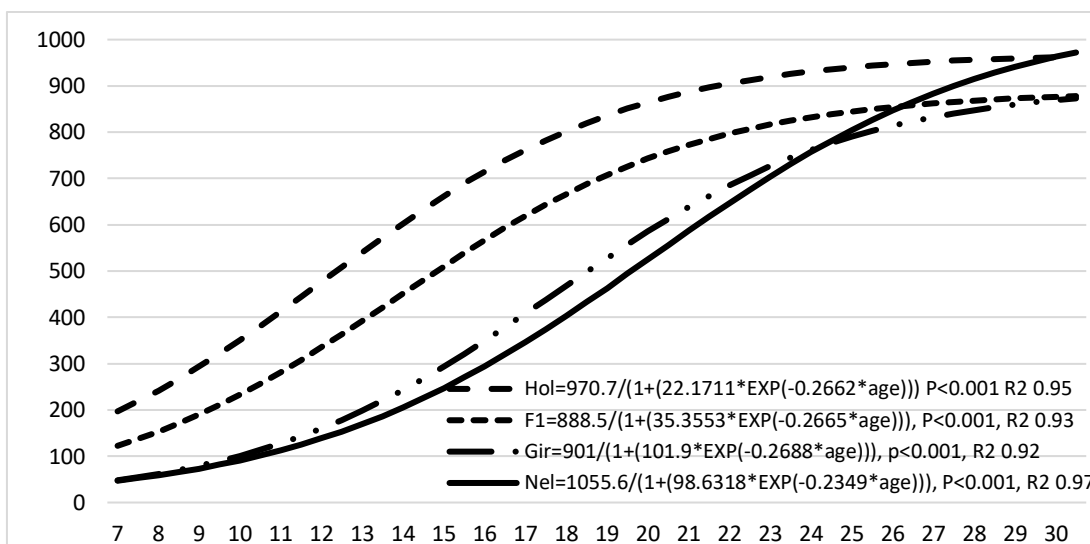


Fig. 2: Testicular growth represented by the testicular volume (cm³) logistic curves of in Holstein, Crossbred F1 Holstein-Gyr, Gyr and Nellore bulls during sexual development

The testicular volume at maturity and rate of maturation were equivalent in all genetic groups analyzed. These data confirm the findings identified through comparing mean events of sexual maturity at which the testicles of each genetic group display the same sizes, as predicted by this characteristic (Table 1).

Table 4 presents testicular shape frequencies at puberty and sexual maturity [22]. Based on a descriptive approach, we observed that zebu bulls displayed testicles mainly of moderately long shape in higher proportion than taurine bulls, which presented a preponderance of the ovoid shape. These shape frequencies might be responsible for the differences observed while comparing between genetic group scrotal circumference at sexual maturity, but not when using testicular volume to compare.

The moderately long testicular shape was predominant in Nellore bulls aged 21.3 months, in agreement with a previous report [42].

There was a correlation between testicular volume and scrotal circumference (0.92, p<0.001). Selection by scrotal circumference would be an adequate measurement for forecasting testicular volume in Nellore bulls, even given the greater frequency of animals with long testicles.

Table 5 displays the ratio between mean testicular width and length associated with each genetic group. This ratio was greater (P<0.05) in Holstein and crossbred F1 bulls than in Gyr bulls, confirming the more elongated testicular shape in the zebu bull. The mean proportions observed in Nellore bulls are similar to the ones observed in Gyr bulls.

Table 4: Class frequency of testicular shape in Holstein, Crossbred F1 Holstein-Gyr, Gyr and Nellore bulls, according to Bailey’s criteria [23] at puberty and at sexual maturity

	Puberty			
	Moderately Long % (n)	Moderately Ovoid % (n)	Ovoid Spherical % (n)	Total (n-%)
Holstein	58.8% (10)	23.5 % (4)	17.7 % (3)	17–20.0
Crossbred F1 Hol-Gyr	55.2 % (16)	31.0 % (9)	13.7% (4)	29-34.1
Gyr	85.7 % (18)	14.3 % (3)	-	21-24.7
Nellore	72.2 % (13)	27.8% (5)	-	18-21.2
Total	67.1	24.7	8.2	85-100
	Sexual maturity			
	Moderately Long % (n)	Moderately Ovoid % (n)	Ovoid Spherical % (n)	Total (n-%)

Holstein	47.1 % (8)	41.2 % (7)	11.7 % (2)	17–20.0
Crossbred F1 Hol-Gyr	58.6 % (17)	32.0 % (9)	10.3 % (3)	29-34.1
Gyr	80.9 % (17)	19.1 % (4)	-	21-24.7
Nellore	83.3 % (15)	16.7 % (3)	-	18-21.2
Total	67.0 %	27.1 %	5.9	85-100

P<0.001 chi squared (50% of expected cells are <5 obs.)

Table 5: Mean relationship between testicular width and length in Holstein, Crossbred F1 Holstein-Gyr, Gyr and Nellore bulls at puberty and sexual maturity

	Puberty (APUB)		Age at 70% normal sperm (AMAT1)	
	Mean	SD	Mean	SD
Holstein	0.62	0.08 a	0.65	0.08 a
Crossbred F1 Hol-Gyr	0.62	0.06 a	0.61	0.08 a
Gyr	0.57	0.04 b	0.56	0.03 b
Nellore	0.59	0.04	0.58	0.04

Values followed by distinct letters indicate differences between columns within each event of sexual development P<0.05 variance adjusted by Tukey-Kramer. Nellore bulls are not compared, only descriptive propose

These results corroborate previous research on the predominance of the more elongated testicular shape in zebu bulls [42-46]. This condition is credited to the adaptive capacity of this subspecies to tropical warmer conditions. In a study conducted with 1124 Nellore bulls, the predominance of animals with moderately long testicles was reported to be 72.5% and 68.2% in animals two and three years of age, respectively, which also demonstrates the predominance of elongated shapes. The same authors reported no animals with spherical testicular shape, and only five animals with ovoid-spherical shape at two and three years of age [44]. The percentage of moderately long testicles of approximately 75% observed in this study coincides with other research [47].

Studies have shown that the model of testicular thermoregulation consists of a constant temperature in the entire testicular parenchyma and depends upon the combination of a positive and negative gradient in different parts of the testicle [48]. In zebu bulls, the scrotum and testicular tissue under the tunic have positive temperature gradients, which contrasts with the negative temperature gradient of the tunic in taurine and crossbred bulls [4, 48, 49].

The relationship between testicular artery length and testicular volume is greater in Nellore, intermediate for crossbred, and smaller in Angus bulls [50]. If we also considered the thickness of the testicular artery wall and the distance between arterial-venous blood in the testicular vascular cone, the relationship was smaller in Nellore, intermediate in crossbred, and higher in Angus bulls [4]. All of the anatomical differences among zebu and taurine bulls discussed above could also influence testicular shape.

Scrotal shape exhibits a negative relationship with arterial temperature in the testicular ventral pole and at the point at which the testicular artery penetrates the parenchyma in taurine and zebu bulls, respectively, and with intratesticular and epididymal temperatures in crossbred bulls [50]. The scrotum, which is more elongated, favors a greater surface for exposure of the spermatic cord region and the vascular structures contained in it, which may raise heat loss by irradiation in this area. This may contribute to arterial temperature decrease, and therefore to the decrease of testicular and epididymal temperatures, since arterial blood is the main testicular heat source [50].

In general, testicular thermoregulation depends upon the interaction between opposite gradients associated with scrotal and testicular temperatures. The morphologies of the spermatic cord and vascular cone may contribute to the greater resistance to high-temperature environments displayed by zebu bulls. The influence of scrotal, vascular cord, and testicular morphology increases the capacity of testicular thermoregulation and positively affects semen quality and sperm production in bull breeds adapted to the tropical climate [51].

Therefore, relative testicular size will probably continue to be a useful method for estimating male investment in sperm production [52]. The testicle is doubtlessly exposed to some of the most variable and fluctuating selective pressures among

organ systems, such as the demand for sperm production quantities (and their specific morphology), which change frequently and rapidly in response to variations in the mating system, resulting in post-mating sexual selection [52].

Zebu and taurine subspecies were separately subjected to evolution for several hundreds of thousands of years. Analysis of mitochondrial DNA and microsatellite loci sequences suggest that zebu breeds diverged from taurine breeds between 110,000 and 850,000 years ago [53]. The evolutionary adaptation of zebu breeds to tropical conditions implies, among other mechanisms discussed above, that the exchange (variation) in the testicular shape is an additional tool for the maintenance of sperm production, increasing testicular size, and more efficient thermoregulation. It should also be pointed out that, according to the laws of thermodynamics, heat loss in a cylindrical body is greater than in a spheroidal equivalent volume [54].

Thermal stress causes fewer effects on semen quality in zebu than in taurine bulls, and this phenomenon reflects not only adaptations that affect whole-body thermoregulation, but also specific adaptations that improve local cooling of blood entering the testicles. This conclusion is based on the results of a study comparing the anatomic characteristics of the testicular thermoregulation system in Nellore, crossbred (5/8 or 5/16 Charolês × zebu), and Angus bulls. Among the findings was the observation that the proportion of testicular length to testicular artery volume is greater in Nellore, intermediate in crossbred, and smaller in Angus bulls. The thickness of the testicular artery wall and the distance between arterial-venous blood in the testicular vascular cone was smaller in Nellore, intermediate in crossbred, and higher in Angus bulls. As expected, given these anatomical differences, intra-arterial testicular temperature was smaller in Nellore, intermediate in crossbred, and greater in Angus bulls [4].

Testicles elongated in zebu breeds displayed better vascular architecture, with a greater surface area and better venous and arterial distributions, improving heat loss from the testicular core. A higher temperature gradient exists in the dorsal and ventral poles of the testicle, as well.

The morphological testicular characteristics that may influence testicular thermoregulation, semen quality, and sperm production include an elongated scrotal shape, which affects the distance between the testicles and the body, as well as heat loss by irradiation from the spermatic cord region [4]. Variations in the morphology of the spermatic cord, which represents a region of major importance for heat transfer between the arterial and venous blood in the testicle, may influence the temperature of the arterial blood that reaches the testicles.

Higher capacity for testicular thermoregulation is associated with increased elongation of scrotal shape, length and volume of the testicular artery, and distance between the artery wall and the veins of the testicular cord [4]. Morphological differences in the scrotum, in the spermatic cord, and in testicular shape could also contribute to improving the capacity of testicular thermoregulation in zebu bulls.

Testicular thermoregulation depends upon opposite interactions between scrotal and testicular temperature gradients in taurine and crossbred bulls. In zebu bulls, the morphology of the spermatic cord contributes to greater resistance to high environmental temperatures, and testicular, scrotal, and spermatic cord morphologies influence the capacity of testicular thermoregulation, affecting semen quality and sperm production in bulls [4].

From the cited studies, we assume that more elongated testicular shapes display morpho-physiological advantages, which are seen as favorable for reproduction in tropical climates. Given these observations and considering the testicular shape in zebu bulls, which are normally elongated, we believe that this characteristic may have great value in preserving adaptive characteristics.

We conclude that scrotal circumference as a measure of sexual development at puberty and sexual maturity was different between the taurine, crossbred F1, and zebu groups. However, at sexual maturity, the testicular size in bulls of different zebu and taurine subspecies was equivalent in all events assessed.

Acknowledgment

Funding support Nestle S.A. and Vale do Boi farm by with animals, transport and facilities. Scholarships by CNPq and CAPES.

Authors Contributions

Gustavo Eduardo Freneau: Conceptual project, participated in all experiments Analysis and interpretation of data, final approval of the version to be submitted and any revised version.

Jose Domingos Guimaraes: Conceptual project, participated in all experiments, analysis and interpretation of data.

Vicente Ribeiro Vale Filho: Conceptual project, financial support.

Arnhold Emmanuel: Statistical support.

Conflicts of Interest

There is no conflict of interest.

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