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Testicular Biometry, Semen Quality and Sexual Maturity of Nellore Young Bulls Raised in the Brazilian Tropical Savannah

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Abstract: This study was designed to evaluate the relationship between testicular biometry and semen quality and determine the sexual maturity age of selected yearling Nellore bulls extensively raised in the Brazilian tropical savannah. Three hundred sixty-one bulls participating in a performance test were divided in age classes (17, 18, 19 and 20 months) and submitted to Breeding Soundness Evaluation (BSE). Measurements of Scrotal Circumference (SC), testicular length and testicular weight were used to study the testicular biometry including the calculation of Testicular Weight and Volume (TW, TV) by means of previously described formulas. Progressive sperm motility (MOT), sperm vigor, mass movement and sperm morphology (major, minor and total defects) were evaluated and results used to classify the bulls as apt (n = 202) or not apt (n = 159). SC ranged between 29.1 and 30.5 cm with differences for age classes ($p<0.05$). TW and TV increased with age from 198.4-327.2 g and 187.7-309.3 cm^3, respectively with differences. MOT differed in all age classes ($p<0.05$). Proportion of major and minor sperm defects did not differ but there was a significance for tail defects between the age classes. There was a positive and moderate correlation between body weight and SC; a negative and moderate correlation with total sperm defects. The BSE was an effective tool to evaluate the bulls and the results permitted to conclude that selection of Nellore bulls raised under extensively conditions in the Brazilian tropical savannah can be indicated from 17 months of age.

Key words: Semen traits, zebu, environment, beef cattle, MOT

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO) classification, livestock systems in which >90% of dry matter fed to animals comes from rangelands, pastures or annual forages is named as Solely Livestock Production Systems and is present in the tropics and sub-tropics. In the Brazilian, tropical savanah beef cattle is produced mainly in this system and about 70% of the cows are subjected to a multiple mating system which requires the constant selection of bulls that can simultaneously act as diffusers of genetic material and improving the quality of the herd. Under these conditions the management of bulls has to be understood as a way to reduce the risk of losses in the herd as a result of male infertility. Reproductive efficiency is the main factor to determine the economic feasibility of beef cattle production system. Sexually precocious herds and with high fertility rates show a higher yield rate, resulting in a higher amount of animals for marketing and selection. Even though, there is a trend to assign reproductive failure only to cows while the herd's female represents a loss of one calf/year, the bull, according to the bull/cow ratio used, causes a loss of 25-80 calves/year (Duarte *et al*., 2005). The Breeding Soundness Evaluation (BSE) must be used not only to detect bulls bearing reproductive deficiencies but also those more precocious regarding sexual maturity. One out of every five bulls shows subfertility and the rate of questionable bulls regarding testicular biometric, physical and morphological aspects of the ejaculate and behavioral aspects is high. Sperm morphology reflects the functional condition of testicles and the increase in the abnormal spermatozoon

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ration is associated to the conception rates decrease. This subject was reviewed by Kastelic and Thundathil (2008) which state that no single measurement reliably predicts bull fertility.

The selection of bulls for sexual precocity contributes a great deal to the genetic progress. Under field conditions the selection intensity applied to the males may be much higher than in females which justifies their use as young as possible, also bringing production-related gains and lower costs to the system. To assess the reproductive capability of young bulls, many parameters have been proposed, among which is the measurement of the Scrotal Circumference (SC), easily obtained and with a high rate of repeatability among evaluators. The SC is frequently used due to the easiness to measure and due to its association to the volume of testicular tissue responsible for producing androgens and spermatozoa. Such feature has moderate to high inheritability and is related to weight gain, sperm production and seminal quality of the bulls and sperm abnormalities have been related to a small SC (Burns *et al*., 2011; Viu *et al*., 2006). When the SC increases, puberty age decreases and subsequent production is improved in female calves; measuring the SC at weaning allows foreseeing the opportunity the male will have to reach the desirable SC as yearling and decrease feed costs due to the precocious slaughter of male calves (Engelken, 2008). Scrotal circumference of Nellore bulls at 18 months of age was found to be the most advantageous testicular trait to use before andrological assessment with an heritability of 0.42 and a genetic correlation of 0.56 with satisfactory andrological evaluation (Silva *et al*., 2011).

In order to improve the selection of young zebu bulls as early as possible, one alternative would be using the method that weighs the testicular biometry and the physical-morphological aspects of the ejaculated spermatozoa, generating an index to foresee the fertility of such bulls. Sperm evaluation is extremely important during BSE of zebu bulls raised extensively in the tropics, since, in such conditions, assessing the fertility of a bull based on the conception rate may be deceiving because the breeding season is usually based on a multiple-sire matting system (Chacon, 2001).

Zebu is well adapted to the tropical savannah environment where the presence of well defined dry and rainy seasons favors the mating season in the beef cattle production commercial systems between November and March. The introduction of bulls as potential breeders in the herd at 24 months old harms those from later conceptions which will be added to the original herd or solder older as breeders. Economic projections indicated that the use of yearling bulls has a better cost-benefit effect than the practice of waiting until the age of 24 months (Kasari *et al*., 1996). In that context, studies that improve the knowledge about the timeline of reproductive events, mainly those related to sexual maturity, especially under field conditions must contribute to the setting of variables which permit the recognition of pos pubertal events to introduce younger bulls as sires in the herd, causing a major impact on the system's productive and economic efficiency.

This study was designed in order to assess the correlation between the testicular biometry and BSE results of young Nellore bulls selected to be used under field conditions in the tropical savannah, cerrado biome and to search tools to determine the sexual maturity status, making possible to use them earlier during the breeding season.

MATERIALS AND METHODS

The study was carried out in the Brazilian cerrado biome (Middle West region, 16°28' South and 49°17' West, 823 m.a.s.l.), presenting a tropical Savannah climate with temperatures ranging from 11-21°C (minimum) and 28-34°C (maximum). Relative humidity during the period of the study ranged from 70-85% in rainy months and 40% in dry months. Evaluations were carried out during the 3 1st months of the dry season (April, May and June) in 3 consecutive years.

The data set used refers to 361 BSE performed in Nellore bulls aged 17-20 months raised in renewed pastures under the integrated livestock-agriculture program in the National Rice and Beans Research Center of the Brazilian Agricultural Research Corporation (CNPAF-EMBRAPA) in the state of Goias, Brazil. The animals were selected in beef cattle farms located in the same geographic region of CNPAF-EMBRAPA, participating in the Genetic Improvement Program for Brazilian Nellore breed with a strict control over genetic traits individual performance and arrangement of contemporary groups. All the animals were offspring of bulls with foreseen expected progeny difference provided by the Nellore Brazil Program. The animals arrived at CNPAF-EMBRAPA with ages between 215 and 305 days and remained for 18 months or throughout the duration of the performance test.

During the performance test run at CNPAF-EMBRAPA the bulls were maintained exclusively under a rotating grazing system in Brachiaria brizantha Hochst A. Rich. Stapf. cv. Marandu e Brachiaria brizantha A. Rich. Stapf. cv. Xaraes pastures, receiving mineral supplementation containing 88 g of phosphor *ad libitum* during the rainy period and protein-energy mix with 46% of crude protein and 0.16 kg average daily consumption during the dry period.

At the Breeding Soundness Evaluation (BSE) the bulls were divided into classes according to their age, being C17 (17 months, n = 54); C18 (18 months, n = 135); C19 (19 months, n = 111) and C20 (20 months, n = 61).

Bulls were weighed and underwent a clinical and genital exam. Scrotal Circumference (SC) was obtained using a scrotal tape lane; Testicular Length (TL) and Width (TW) were measured by a digital caliper, covering the biggest planes in each testis. Semen samples were obtained by electroejaculation. Sperm progressive Motility (MOT), Vigor (VIG) and Mass Movement (MM) were evaluated immediately after semen collection and only samples with progressive sperm motility higher than 50% were fixed in 10% buffered formalin to assess sperm morphology using contrast microscopy. The sperm defects were grouping into head, midpiece and tail defects, sorting them into Major (MjD), Minor (MiD) and Total (TD) defects, as recommended by the Brazilian College of Animal Reproduction. According the results bulls were classified as: apt (MOT = 50%; VIG = 3 and TD<30%) or not apt (MOT<50%, VIG<3 and TD>30%).

Testicular Volume (TV) and Testicular Weight (TW) were calculated according the following previously described formulas (Bailey *et al*., 1998): $TV = 4/3(\pi)\times(L/2)\times(W/2)^2$ where: TV = Testicular Volume; L = testicular Length; W = testicular Width and π = correction factor (3.14). $TW = 0.5533\ (L)\times(W)^2$ where TW = Testicular Weight; L = testicular Length and W = testicular Width.

The Average Testicular Volume (ATV) and the Average Testicular Weight (ATW) were calculated by the arithmetic mean between the volume and weight of the left and right testicles. In order to determine the Testicular Shape (TS), a ratio between testicular width and length (width/length) was used at a 1.00-0.50 scale where 1.00 means width = length and 0.50 means width = ½ of length. Due to such scale, the following shape nomenclature was set: ratio 1:≤0.50 = long; ratio 2:0.51-0.625 = long/moderate; ratio 3:0.626-0.750 = long/oval; ratio 4:0.751-0.875 = oval/spherical; ratio 5:>0.875 = spherical (Unanian *et al*., 2000).

The data set generated was subjected to critical and consistency analysis using the Univariate procedure. Analysis of variance was performed using the GLM procedure. The adjusted means were obtained using the LSMEANS statement option and analyses were performed using the following model:

$$Y_{ij} = \mu + I_j + e_{ij}$$

Where:

- Y = j bull observation
- μ = The intercept
- I_j = The age class effect (17, ..., 20)
- e_{ij} = The random error associated to each ijk observation, assuming an independent and normal distribution with zero mean and variance σ^2

The frequency of apt or not apt bulls by age classes was subjected to the frequency dispersion study using the χ^2-test by means of the FREQ procedure. Pearson correlation coefficient was applied to calculate the intensity obtained when associating two variables through the CORR procedure.

RESULTS

Table 1 presents results regarding body weight, testicular biometry and sperm traits in the age classes. There was no significant variation (p>0.05) between animal weight, whose averages were 445.3±11.2 kg (C17) until 445.7±6.7 kg (C20). There was no difference (p>0.05) in SC regarding the age classes with variation ranging from 29.1-30.5 cm. ATV ranged from 150-500 cm^3 whereas the ATW ranged from 0.198-0.327 kg. There were differences (p<0.05) between ages for tail defects with high proximal droplet rate in the seminal samples of immature bulls.

Table 1: Weight, testicular biometry, physical seminal traits and sperm defects of Nellore young bulls raised under extensive conditions in the Brazilian cerrado biome

	Bull age* (μ±SE)			
Variables	C17	C18	C19	C20
Weight (kg)	445.3±3.3	441.9±4.00	442.6±5.50	445.8±4.10
SC (cm)	29.1±1.3^a	30.0±1.40^b	30.2±1.50^b	30.5±1.40^b
Testicular volume (cm^3)	187.7±8.5^a	252.4±10.9^b	259.8±11.4^b	309.6±10.9^c
Testicular weight (g)	198.4±8.7^a	266.7±11.2^b	274.6±11.7^b	327.2±11.3^c
Sperm Prog. Motility (%)	41.2±3.4^a	49.1±3.30^b	58.3±3.20^c	64.6±3.30^d
Vigor (1-5)	3.5±0.6	3.4±0.70	3.5±0.70	3.4±0.80
Mass movement (1-5)	3.2±0.5	3.1±0.80	3.2±0.70	3.2±0.90
Major defects (%)	20.7±4.1	19.6±3.80	17.3±3.50	15.2±1.30
Minor defects (%)	6.0±2.2	5.2±2.20	4.9±2.00	6.3±2.90
Total defects (%)	26.7±4.2	24.8±4.00	22.3±3.70	21.5±3.70

*Classes: C17 = 17 months; C18 = 18 months; C19 = 19 months, C20 = 20 months. Within a row, means without a common superscript differ (p<0.05)

Table 2: Correlations between weight, testicular biometry, physical seminal traits and sperm defects of Nellore young bulls raised under extensive conditions in the Brazilian tropical savannah

			Testicular biometry			Semen physical traits			Sperm defects		
Variables	BW	SC	ATW	ATV	TS	MOT	VIG	MM	MjD	MiD	TD
BW		0.400	0.110[b]	0.120[b*]	0.020	-0.010	0.050	0.020	0.080	0.040	0.08
SC	0.42		0.490[a]	0.530[a]	-0.020	0.220[a]	0.230[a]	0.220[a]	-0.280[a]	0.080	-0.39[a]
ATW	0.03	<0.001		0.890[a]	-0.290[a]	0.190[a]	0.080	-0.200[a]	-0.100	0.200[a]	-0. 0 2
ATV	0.03	<0.001	<0.001		-0.290[a]	-0.190[a]	-0.080	-0.200[a]	-0.090	0.200[a]	0.02
TS	0.68	0.660	<0.001	<0.001		0.100[b]	0.090	0.110[a]	0.020	-0.150[b]	-0.04
MOT	0.63	<0.001	0.001	0.001	0.550		0.770[a]	0.740[a]	-0.260[a]	-0.050	-0.25[a]
VIG	0.41	<0.001	0.140	0.140	0.100	<0.001		0.800[a]	-0.230[a]	-0.060	-0.24[a]
MM	0.65	<0.001	<0.001	<0.001	0.050	<0.001	<0.001		-0.240[a]	-0.170[a]	-0.28[a]
MjD	0.14	<0.001	0.080	0.080	0.710	<0.001	<0.001	<0.001		0.080	0.94[a]
MiD	0.44	0.150	<0.001	<0.001	0.003	<0.001	0.240	<0.001	0.140		0.43[a]
TD	0.11	<0.001	0.770	0.770	0.490	<0.001	<0.001	<0.001	<0.001	<0.001	

Within the lower diagonal the p-value. [a]p<0.01; [b]p<0.05. BW: Bull Weight; SC: Scrotal Circunference; ATW: Average Testicular Weight; ATV: Average Testicular Volume; TS: Testicular Shape Score; MOT: Sperm Motility; VIG: Sperm Vigor; MM: Massal Movement; MjD: sperm Major Defects; MiD: sperm Minor Defects; TD: sperm Total Defects

From all evaluated bulls, 56% were considered apt while 44% were not apt or immature. The rate of apt or not apt at each age range was respectively, 46 and 54% at 17, 52 and 48% at 18, 60 and 40% at 19, 66 and 34% at 20 months old with differences ($p<0.05$) for older bulls.

The ejaculate of the bulls with 150 cm^3 TV showed the highest rate of tail defects with predominance of the proximal droplet. The bulls with 200 cm^3 presented the lowest rate of TD and 70% of them were classified as apt.

Long-oval (ratio 3:0.626-0.750) TS prevailed, especially at the 17 months old range, followed by the long/moderate (ratio 2:0.51-0.625). Few animals showed other TS without differences. Table 2 summarizes the correlations between body weight, testicular biometry and seminal traits. There was a moderate positive correlation ($p<0.01$) between BW and SC and a low positive correlation ($p>0.05$) between BW and sperm defects. The SC showed a moderate negative correlation ($p<0.01$) with TD.

DISCUSSION

Low sperm motility and high number of defects is a common finding in the ejaculate of young bulls around puberty. The improvement of semen traits occurs from 12-16 months of age in Bos taurus and 14-18 months in Bos indicus and testicular growth is faster from 25 until approximately, 37-50 weeks old. Such growth consists in a significant increase in diameter and length of the seminiferous tubules and great proliferation and differentiation of germinative cells (Rawlings *et al*., 2008). Gonad growth is related to the increase of both, germinative and Sertoli cells, resulting in an increase of the seminiferous tubules diameter (Madgwick *et al*., 2008).

In Nellore bulls, testicular growth assessed by SC varies slightly between the 10th and 12th month of life and later, between the 17th and 25th month. Such ongoing gonad growth process matches the rise in serum testosterone concentrations by 18 months of life and precedes the appearance of the first spermatozoa in the ejaculate (Moura *et al*., 2002). The earlier young bulls are assessed, considered superior regarding their contemporaries and used as replacement animals, the higher the genetic progress per time unit for the variable under selection, also enabling to meet the high demand for selected bulls. The body weight may influence when determining and anticipating the sexual maturity of Nellore bulls raised extensively, probably reflecting effects of deficient feeding-pre and post-weaning which results in the improper development of testicles and delaying puberty and sexual maturity (Dias *et al*., 2008) but the nutritional improvement affects LH secretion positively and increases testicular growth (Brito *et al*., 2007). All animals assessed in this study weighed more than 440 kg, showing that the feeding at weaning was proper and probably helped puberty and sexual maturity ages to get closer to each other.

The SC has been directly linked to multiple fertility measures in bulls and may be used as a tool to foresee fertility. Pubertal age decreases when SC increases and the subsequent productivity of female offspring is improved (Engelken, 2008). The SC did not show a significant increase before 20 months old (29-30 cm) and was higher than reported in previous studies (Ortiz *et al*., 2001) with yearling Nellore bulls (25.7 cm). The SC is an indicator of testicular size, sperm production capacity, physical seminal traits, time at which the first spermatozoa appears in the ejaculate and fertility of males, in addition to related females, mainly half sisters and daughters (Arteaga *et al*., 2001; Engelken, 2008). Studies on the genetic correlations between the SC and reproductive traits of Nellore cows have shown that the selection for SC at 12 months old is even more effective than at 18 months old when the purpose is to improve such

variable in replacement heifers (Gressler *et al*., 2000). The difference between the SC from C17 and the other age classes (Table 1) shows that having reached the puberty the bulls underwent a period of reproductive development until the sexual maturity, resulting in increases in SC and improvement in the physical and morphological semen traits.

The ATW was higher than previously reported for Nellore bulls at the same age and extensively raised but under different environmental conditions (Moura *et al*., 2002) as in the semiarid area of Brazil offering evidence of the environmental factors related to the testicular development.

Bulls with a 200 cm^3 ATV presented lower TD than the average of studied animals and more than half (70%) of them were considered apt younger than the others, allowing to hypothesize that they showed high phenotypic trait for sexual precocity, since they had a SC of 29 cm at 17 months old.

Regarding TS, there was a predominance of long oval shape (0.626>ratio<0.750) and the higher rate was verified in the bulls 17 months old (61%), decreasing until 20 months (42%). This result suggests that such testicular shape is more adequate to sperm production and may be an indicator of sexual precocity for Nellore bulls created under the studied environmental conditions. The second most common testicular shape was the long/moderate (0.626>ratio<0.750) with an increase from 22% in bulls at 17 months to 49% at 20 months which indicates a change in testicular shape as age progresses, resulting in rounder testicles.

The study of TS is important because it facilitates the thermoregulation process. More elongated testicles show a bigger surface of interaction with environment, making thermoregulation easier and permitting a more uniform distribution of the blood vessels and sperm tissue, thus favoring the qualitative and quantitative traits of the ejaculate (Bailey *et al*., 1998). The persistence of the long oval TS as age progresses shall result in a higher SC. However, the ejaculates obtained from the bulls with longer testicles and smaller SC showed higher concentration of spermatozoa/mL than the ejaculates of bulls with oval TS which had higher SC. In a retrospective study in Brazil using data from 5903 Nellore bulls averaging 21 months submitted to BSE (Silveira *et al*., 2010), correlations between SC and reproductive traits were positive and >70% of the animals were considered sexually mature at that age. Although, differences were verified for testicular length, no differences were present regarding TS among the bulls classified as apt or not.

Zebu bulls show a delay in the start of puberty (Aponte *et al*., 2005) but the observation that the spermatogenesis starts at nine months old and that there no longer are immature sertoli cells at 14 months old suggests that there is a chance such bulls reach sexual maturity between 17 and 18 months old, since sperm concentration increases between the pubertal phase and sexual maturity (Burns *et al*., 2011). In the present study, half of the bulls already had adequate semen quality to be used as sires at 18 months old, reflecting the maturation status of the epididymal epithelium and spermatozoa. Changes in the seminal quality after puberty in Nellore bulls were characterized by an increase of motility and ratio of morphologically normal spermatozoa with decrease in the major defects ratio (Viu *et al*., 2006). In similar environmental conditions, the assessment of yearling Zebu bulls demonstrated that the animals with healthy testicles did not show differences in the number of sperm abnormalities vs. older bulls with the same testicular condition.

The moderate and positive correlation between BW and SC (Table 2) suggests that part of the genes involved in the expression of such trait is the same. The ATW and the ATV showed a low correlation with the morphological aspects of the semen. As to Bos taurus, the SC in zebu cattle shows moderate to high inheritability and a high genetic correlation with other testicular and qualitative semen traits.

The Nellore breed is well adapted to the Brazilian tropical savannah in the cerrado biome and the high demand for breeders in the beef cattle production systems favors studies to safely evaluate and select young bulls and introduce them earlier in the herd. However, in most studies, the 20 months old age is adopted to start assessments (Dias *et al*., 2008), whereas the results presented herein show that Nellore bulls may already be sexually mature from 18 months old. The role of the bull in the fertility of cows has to be understood in terms of the overall constrains to fertility in beef cattle and the selection of bulls before the breeding season is a way to minimizing the risk of infertility in the herd (Parkinson, 2004).

Another interesting point in such strategy is related to the time of birth of future breeders. In temperate climate areas, where there is a clear difference between the seasons, male calves born in Autumn reached puberty later than those born in Spring (Tatman *et al*., 2004), showing a longer rise in LH concentration with higher amplitude peaks (Aravindakshan *et al*., 2000), very likely due to the effect of photoperiod. In the tropical savannah,

the year may be divided in two different seasons rainy and dry-making the quality of the available food be more important than the photoperiod in the critical stages of the calf development. In such conditions, identifying those animals which despite the environmental conditions, may reach sexual maturity earlier and introduce them as breeders in the production system may contribute to reducing the puberty age of female offspring with the subsequent increase in their productivity.

In addition to that, the precocious separation of new breeders helps to decrease production costs, since it permits the development of a confinement and slaughter program for males and the reduction in the bull:female mating ratios.

CONCLUSION

This study clearly demonstrates that the correlation of BSE and testicular biometry is a very effective tool to evaluate reproductive ability in young bulls and that the selection of Nellore bulls raised under extensively conditions tropical savannah can be indicated from 17 months of age.

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