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Testis size, peripheral concentrations of testosterone, semen criteria and Sertoli and germ cell numbers in Nelore bulls

Biometria testicular, concentrações periféricas de testosterona, parâmetros seminais e número de células de Sertoli e germinativas em touros Nelore

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Abstract

A study was conducted to evaluate the associations among testis size, testosterone concentrations, semen parameters and aspects of spermatogenesis in Nelore bulls (n = 28). Testis size was measured from 10 to 29 months of age. Bulls were treated with GnRH (12 to 21 months) and semen samples also collected from 25 to 29 months. At 30 months, animals were slaughtered. Correlations were significant when $p < 0.05$. Basal testosterone was highest at 18 months, suggesting that bulls reached puberty at this age. At 30 months, seminiferous tubules represented 77.9 ± 3.8 % of the testicular parenchyma and there were 33.6 ± 8.4 round spermatids/A1 spermatogonium/tubule section, indicating a 47.5% degeneration rate during spermatogenesis. At 30 months, heavier testis correlated with Sertoli cell numbers/testis (r = 0.77), and round spermatids/tubule section, Sertoli cell and A1 spermatogonium (r = 0.50 – 0.60). Scrotal circumference (SC) taken between 10 and 29 months correlated with the percentage of tubules with spermatids (r = 0.42 – 0.59) and number of A1 spermatogonium and round spermatids/Sertoli cell (r = 0.49 – 0.68). Epididymal weight was related to Sertoli cell numbers/testis, and round spermatids/Sertoli cell and A1 spermatogonium (r = 0.51 – 0.61). GnRH-stimulated testosterone from 17 to 21 months correlated with SC between 14 and 29 months (r = 0.48 – 0.60), testis and epididymal weights (r = 0.41 – 0.64) and with parameters of spermatogenesis (r = 0.44 – 0.58). Additionally, sperm motility and vigor from 25 to 29 months correlated with the number of tubules with spermatids (r = 0.42 – 0.59) and GnRH-stimulated testosterone at 12, 13 and 18 months (r = 0.46 – 0.57). In conclusion, testis size during and after the period of pronounced increases in testosterone is an indicator of quantitative parameters of spermatogenesis of post-pubertal bulls.

Key words: Nelore, sperm motility, spermatogenesis, testis, testosterone

Resumo

Conduziu-se o presente estudo com o objetivo de avaliar as associações entre biometria testicular, testosterona, parâmetros seminais e aspectos da espermatogênese em touros Nelore (n = 28). Avaliou-

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se os testículos dos 10 aos 29 meses de idade. Os touros foram tratados com GnRH (12 a 21 meses) e amostras de sêmen coletadas (25 a 29 meses). Aos 30 meses, os animais foram abatidos. Correlações foram consideradas significativas quando $p < 0.05$. Concentrações elevadas de testosterona basal ocorreram aos 18 meses, indicativo da puberdade dos touros. Aos 30 meses, os túbulos seminíferos representaram 77,9 ± 3,8% do parênquima testicular e quantificou-se 33,6 ± 8,4 espermátides arredondadas/espermatogônia A1/secção tubular, referente a 47,5% de degeneração celular durante a espermatogênese. Aos 30 meses, peso testicular correlacionou-se com o número de células de Sertoli/testículo (r = 0,77) e espermátides arredondadas/seção tubular, célula de Sertoli e espermatogônia A1 (r = 0,50 – 0,60). A circunferência escrotal (CE) entre 10 e 29 meses correlacionou-se com a percentagem de túbulos com espermátides (r = 0,42 – 0,59) e número de espermatogônias e espermátides arredondadas/célula de Sertoli (r = 0,49 – 0,68). Detectou-se correlações entre peso epididimário e número de células de Sertoli/testículo e espermátides arredondadas/célula de Sertoli e espermatogônia A1 (r = 0,51 – 0,61). Testosterona pós-GnRH (17 aos 21 meses) apresentou relação com a CE entre 14 e 29 meses (r = 0,48 – 0,60), peso testicular e epididimário (r = 0,41 – 0,64) e parâmetros da espermatogênese (r = 0,44 – 0,58). Motilidade e vigor espermático (25 aos 29 meses) correlacionaram-se com o número de túbulos com espermátides (r = 0,42 – 0,59) e testosterona pós-GnRH aos 12, 13 e 18 meses (r = 0,46 – 0,57). Portanto, a biometria testicular durante e após a ocorrência dos níveis concentrações elevadas de testosterona é um indicador dos parâmetros quantitativos da espermatogênese em touros pós-púberes.
Palavras-chave: Nelore, motilidade espermática, espermatogênese, testículos, testosterona

Introduction

The Brazilian beef cattle herd is one of the world's largest and, within this population, the Nelore and their crosses represent the most significant genetic group (NOGUEIRA, 2004). Despite the importance of the Nelore cattle industry, reproductive performance of herds throughout the country still needs to be improved. This problem is partially caused by inadequate management and/or feeding practices of dams, but the reproductive status of Nelore bulls also affects that scenario. Over the past decades, the breeding capacity of Nelore bulls has been improved considerably as the result of genetic selection and better standards for evaluation of semen quality and service capacity (LOPES et al., 2009). In this regard, knowledge of testicular physiology is crucial for determination of the main factors that affect and the sperm producing capacity of Nelore bulls and strategies for reproductive management.

Studies have shown significant correlations among testis size, number of Sertoli and germ cells (BERNDTSON; IGBOELI, 1989; MOURA; ERICKSON, 1997) and daily sperm production in *Bos taurus* bulls (BERNDTSON; IGBOELI, 1989). Animals with large testis have more spermatids and A1 spermatogonium supported by Sertoli cells and a greater number of round spermatids generated by each Al spermatogonium (MOURA; ERICKSON, 1997). Moreover, as determined by the age at which concentrations of testosterone reach 1 ng/ml in the peripheral circulation, fast-growing bulls have larger testis size at pubertal and post-pubertal ages (MOURA; SOUZA; ERICKSON, 2011). These results suggest that events regulating Leydig and Sertoli cell function early in life are linked to future attributes of the testis. Furthermore, extensive use of research data obtained from studies conducted solely with *Bos taurus* animals seems inappropriate to evaluate *Bos indicus* Nelore bull herds because of differences related to genetic backgrounds and marked variations in management and environment. Thus, the present work was conducted to evaluate the associations among testis criteria, hormonal concentrations, semen parameters and quantitative aspects of the spermatogenesis in Nelore bulls raised in a semi-arid region of the Brazilian Northeast.

Material and Methods

Twenty eight healthy Nelore bulls were raised in pasture with the dams from birth to weaning (10 months) and, from this phase until 30 months

(mo.), animals were kept in pens and fed elephant grass (Pennisetum purpureum), sorghum (Sorghum bicolor) and concentrate. The experiment was carried out in the semi-arid region of the Brazilian Northeast, with a weather type defined as AW, according to Koppen. Animals were managed according to Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching of the Federation of Animal Science Societies.

As previously described in the first publication about this study (MOURA; RODRIGUES; MARTINS FILHO, 2002), scrotal circumference (SC) and both testis diameter and length were recorded at monthly intervals, from 10 to 29 months (mo.) of age. At 30 mo. (439.1 ± 8.1 kg), all animals were slaughtered at a local abattoir, following technical recommendations of the Department of Agriculture of Brazil. At 12, 13, 15, 17, 18, 19, 20 and 21 mo., Nelore bulls were treated with an intramuscular injection of GnRH (D-Ser [Bu]-Pro-LHRH [1-9] nonapeptide ethylamide; Hoechst, Brazil), based on the ratio of 0.05 mg of GnRH per kg of body weight. Blood samples were taken from the jugular vein into tubes containing EDTA at the moment of GnRH injection, 1.5 and 3 hours afterwards. Immediately after collection, samples were centrifuged at 1,500 × *g* for 20 minutes at 4 ºC. Plasma was harvested and stored at –20ºC until determination of testosterone concentrations by radioimmunoassay (Diagnostic Products Corporation, USA; MOURA; RODRIGUES; MARTINS FILHO, 2002; SOUZA et al., 2010). The dose of GnRH per kg of body weight and the approach of blood sampling were based on our previous studies conducted in prepubertal, pubertal and post pubertal beef bulls (MOURA; ERICKSON, 1997; 2001).

Immediately after slaughter, testis and epididymis weight and size were recorded. A small section of 4 mm of testicular tissue from each bull was fixed in Bouin's fluid, followed by three washes in ethanol. Tissue samples were embedded in paraffin, cut at 5 μm and stained with hematoxylin and eosin (MOURA; ERICKSON, 1997; 2001; AGUIAR; ARAÚJO; MOURA, 2006). For each animal, the number of Sertoli and germ cells with an intact nucleolus were counted in 20 cross sections of seminiferous tubules in stage VII of the spermatogenic cycle (BERNDTSON; DESJARDINS, 1974). These numbers, defined as crude counts, were corrected according to Abercrombie's formula (ABERCROMBIE, 1946): True count = N × (5 μm / (5 μm + diameter of the nucleolus, in μm), where N represents the number of cell types per tubule cross section. Based on true counts, we estimated the following cell ratios per seminiferous tubule cross section: number of round spermatids/A1 spermatogonium and per Sertoli cell, A1 spermatogonium/Sertoli cell, primary spermatocytes/Sertoli cell, primary spermatocytes/A1 spermatogonium and number of round spermatids/spermatocyte.

The population of Sertoli cells/testis was estimated as previously reported (MOURA; ERICKSON, 1997; AGUIAR; ARAÚJO; MOURA, 2006). Briefly, testicular volume (V) was determined dividing testis weight (g) by testis density (1.052 g/cm). The volume occupied by 20 seminiferous tubule cross sections (Vst) was calculated as: Vst = π × h × (d2/4), where "h" and "d" represented the section thickness (5 μm) and tubule diameter (mm), respectively. The percentage of testicular volume occupied by seminiferous tubules (% ST) and interstitium was determined by Chalkley's method (CHALKLEY, 1943), which is based on 600 "hits" taken at random within a cross section of the testis. Crude numbers of Sertoli cells/testis was determined by: Crude number = (V × % ST × C) / Vst, where C represented the true numbers of Sertoli cells with an intact nucleolus counted in 20 round cross sections. The resulting crude numbers were corrected according to Abercrombie's formula: True count = crude number × (5 μm / (5 μm + average nucleolar diameter in μm).

At the ages of 25, 27 and 29 mo., semen samples

were collected by eletroejaculation and two samples were obtained at 30-minute intervals at each age-period. For each ejaculate, we determined sperm concentration (using a hemacytometer), percentage of motile sperm, and vigor, as the intensity of the movement of sperm cells as a whole. The percentage of morphologically normal cells was calculated by counting 200 cells per ejaculate (CBRA, 1998). Parameters estimated at the two semen collections were averaged and used as such for the statistical analysis.

Age-related changes in hormone concentrations, testis size and semen criteria, as well as the effect of exogenous GnRH on peripheral concentrations testosterone were evaluated by analysis of variance (repeated measured design) and Duncan's statistical test (SAS, 2003). Within each age period, Pearson's partial correlations were used to determine the strength of the associations involving testis size, numbers of Sertoli and germ cells and testosterone concentrations. In this case, body weight was defined as the partial variable (MOURA; ERICKSON, 1997; SAS, 2003). Correlations were considered significant when $p < 0.05$.

Results and Discussion

Results about age-related changes in scrotal circumference and basal and GnRH-stimulated testosterone have been previously described in detail (MOURA; RODRIGUES; MARTINS FILHO, 2002). In summary, scrotal circumference of the Nelore bulls increased between 10 and 30 mo. of age ($p < 0.05$) and the slow growth rate of testes between 10 and 12 mo. is probably a consequence of the initial phases of germ cell proliferation inside the seminiferous tubules. Larger testis size after 12 mo. was certainly caused by increasing volume of Sertoli cells, number of germ cells and lumen of the seminiferous tubules as known to happen in *Bos taurus* and *Bos indicus* bulls (AMANN; WALKER, 1983; MOURA; ERICKSON, 1997; RAWLINGS et al., 2008; MOURA; SOUZA; ERICKSON, 2011; BRITO et al., 2012b). According to studies conducted in the *Bos taurus* and *Bos indicus*, puberty coincides with the typical peak in basal concentrations of testosterone in the peripheral circulation (AMANN, 1983; ARAVINDAKSHAN et al., 2000; GHOLAMI et al., 2010; MOURA; SOUZA; ERICKSON, 2011). For the Nelore bulls used in the present study, the characteristic peak in basal testosterone occurred at 18 mo., suggesting these animals reached puberty at that age, as we have originally pointed out (MOURA; RODRIGUES; MARTINS FILHO, 2002). Previous reports have shown that Nelore bulls attain puberty at ages from 14.8 to 22.1 mo. (TROCÓNIZ et al., 1991; CHASE JÚNIOR et al., 1997; BRITO et al., 2004) and such variation reflects the large genetic diversity of herds, feeding and management strategies existing in Brazil.

According to what we have discussed (MOURA; RODRIGUES; MARTINS FILHO, 2002), exogenous GnRH caused pronounced increases in testosterone secretion in the Nelore bulls and both basal and GnRH-stimulated testosterone followed the same age-related variations (MOURA; ERICKSON, 2001), as it happens in *Bos taurus* animals (MOURA, ERICKSON, 1997). However, peripheral testosterone (both basal and GnRH-stimulated) reached maximum concentrations much later in the Nelore of the present study (18 mo.) than in *Bos taurus* bulls (at 10 mo.; MOURA; ERICKSON, 1997; MADGWICK et al., 2008). This fact agrees with the general concept that, given the current genetic background of *Bos taurus* and *Bos indicus* herds, the first are more precocious than the later.

Sperm concentration, sperm motility, percentage of cells with progressive motility and vigor significantly increased between 25 and 29 mo. Sperm morphological defects decreased with age, as expected ($p < 0.05$; Table 1). At 30 mo., testis and epididymis of the Nelore bulls weighted 160.7 ± 35.7 and 20.8 ± 2.7 g, respectively, and the average seminiferous tubule cross section had a diameter of

173.1 ± 8.8 μm, representing 77.9 ± 3.8 % of the testicular parenchyma. Also at 30 months, there were 5.1 ± 1.1 × 10□ Sertoli cells/testis, similar to what has been shown in yearling Angus bulls (4.6 ± 0.3 × 10^9 cells/testis; MOURA; ERICKSON, 1997). In a typical tubule cross section of the Nelore animals, each Sertoli cell supported 0.1 ± 0.05 A1 spermatogonium, 1.2 ± 0.4 intermediate or B spermatogonium, 1.2 ± 0.4 primary spermatocytes and 3.5 ± 1 round spermatids. Each seminiferous tubule section had, on average, 11.6 ± 3.4 primary spermatocytes and 33.6 ± 8.4 round spermatids/A1 spermatogonium. Additionally, we show that the Nelore seminiferous tubules contained 3 ± 0.6 round spermatids/spermatocyte, in accordance to the information published by Godinho and Cardozo (1984) and Aponte, De Rooij and Bastidas (2005). This ratio represents 75 % of the expected value (4 spermatids/A1 spermatogonium). Based on the results shown above, we also confirmed that Nelore bulls presented a population of Sertoli cells/testis and Al spermatogonium/Sertoli cell index equivalent to those of European beef bulls, despite the fact that sexual precocity is markedly different in those types of animals. In the bovine species, each A1 spermatogonium generates a theoretical number of sixty-four round spermatids (COUROT; HOCHEREAU-DE RIVIERS; ORTAVANT, 1970; APONTE; DE ROOIJ; BASTIDAS, 2005), indicating that there was a 47.5 % degeneration rate in the spermatogenesis of the Nelore bulls used in our study, playing a critical role in determining total sperm output. A previous study detected a germ cell degeneration rate of 33.6 % in *Bos taurus* bulls (based on the number of round spermatids/type A1 spermatogonium; MOURA; ERICKSON, 1997) and a publication by Santos et al. (1999) reported a 16.5 % germ cell loss in Brazilian Zebu cattle. However, we cannot assume that *Bos indicus* bulls have either higher or lower rates of cell loss than *Bos taurus* bulls because the animals used in the studies cited above had different ages and weights as well as were raised and fed in very different conditions when compared to ours. Cell loss that occurs during spermatogenesis, due to apoptosis, has been reported in several species and it is caused by altered gene expression during mitosis and meiosis and/or external factors such as heat stress, chemical agents and nutritional imbalance (PRINT; LOVELAND, 2000; SOFIKITIS et al., 2008; CHENG et al., 2010). For the Nelore bulls, the average nucleus and nucleolus diameters of Sertoli cells were 8.4 ± 0.4 and 3.5 ± 0.2 μm, and those of spermatogonium reached 7.6 ± 0.5 and 3.3 ± 0.2 μm, respectively. The nucleus of pachytene spermatocytes and round spermatids averaged 7.2 ± 0.2 and 5.7 ± 0.3 μm, respectively. Measurements of Sertoli and germ cell nuclei and nucleoli were equivalent to those determined in yearling *Bos taurus* bulls (MOURA; ERICKSON, 1997).

Table 1. Sperm parameters in Nelore bulls at 25, 27 and 29 months of age.

Criteria	25 months	27 months	29 months
Sperm concentration (x 10^6/ml)	374.7 ± 57.3[a]	398.6 ± 53.7[b]	402.62 ± 54.2[c]
Volume (ml)	9.1 ± 0.5[a]	9.0 ± 0.4[a]	9.9 ± 0.3[b]
Motile sperm (%)	61.1 ± 3.8[a]	70.7 ± 3.3[b]	74.8 ± 3.3[c]
Vigor (1 to 5)	2.8 ± 0.2[a]	3.3 ± 0.2[b]	3.9 ± 0.2[c]

Values represent the average (± SEM) of two semen samples collected at 15-minute intervals at each age period. Different symbols indicate statistical differences across age-periods ($p < 0.05$).
Source: Elaboration of the authors.

At 30 mo. of age, heavier testes of the Nelore bulls were associated with greater seminiferous tubule diameter (r = 0.54) as well as greater number of Sertoli cells/testis (r = 0.77), round spermatids/ tubule cross section (r = 0.53), round spermatids/ Sertoli cell (r = 0.60), A1 spermatogonium (r = 0.50) and primary spermatocyte (r = 0.55; Table 2). Moreover, scrotal circumference measured between 10 and 29 mo. was correlated with the following criteria: seminiferous tubule diameter (r = 0.42 to 0.58), percentage of tubules with round, elongate or mature spermatids (r = 0.42 to 0.59), number of A1 spermatogonium (r = 0.53 to 0.68) and round spermatids/Sertoli cell (r = 0.49 to 0.66) at the age of 30 mo. (Table 2). Animals with greater testicular mass had more tubules with mature spermatids (r = 0.52; Table 2). All correlations between quantitative aspects of spermatogenesis and testis diameter and length were similar to those reported above (Table 2). These associations have been considered as indicators of testicular maturation (APONTE; DE ROOIJ; BASTIDAS, 2005) and agree with previous reports showing that significant correlations exist between testis size and quantitative parameters of spermatogenesis (BERNDTSON; IGBOELI; PARKER, 1987a,b; MOURA; ERICKSON, 1997; BRITO et al., 2012a). In the 30-month old Nelore bull, testis and epididymis weights were closely related (r = 0. 72). Also, epididymal weight was correlated with the number of Sertoli cells/testis (r = 0.61), round spermatids supported by each Sertoli cell (r = 0.56) and round spermatids generated by each A1 spermatogonium (r = 0.51; Table 2). Our findings suggest that the development of epididymis is closely linked to that of the gonad and to events of spermatogenesis. In fact, studies have pointed out that epididymal function not only depends on androgens but also on the influence of luminal components (such as growth factors) from the testis (ABE; TAKANO; ITO, 1984; CORNWALL, 2009).

Table 2. Pearson's correlations among testis weight, diameter and length, epididymal weight and aspects of spermatogenesis in Nelore bulls at 30 months.

Variable	SC/T	DST	RS	RS/SC	RS/A1	RS/SP	MS
Testis weight	0.77	0.54	0.53	0.60	0.50	0.55	0.52
Testis diameter	0.73	0.59	0.58	0.55	0.60	0.56	0.55
Testis length	0.63	0.55	0.52	0.55	0.53	0.58	0.58
Epididymal weight	0.61	NS	NS	0.56	0.51	0.52	0.59

SC/T: number of Sertoli cells/testis; DST: diameter of seminiferous tubules; RS: number of round spermatids per tubule cross section; RS/SC: number of round spermatids per Sertoli cell; RS/A1: number of round spermatids per A1 spermatogonium; RS/PS: number of round spermatids per primary spermatocyte; MS: number of tubule sections with mature spermatids as the most developed germ cell type.
NS: non-significant correlations ($p > 0.05$). All other r-values are statistically significant ($p < 0.05$).
Source: Elaboration of the authors.

The number of tubules with either round, elongate or mature spermatids quantified at 30 mo. was correlated with sperm progressive motility and vigor evaluated from 25 to 29 mo. (r = 0.42 to 0.59). However, links between quantitative aspects of spermatogenesis and sperm morphology did not reach statistical relevance. These observations are corroborated by Berndtson and Igboeli (1989) and Rocha et al. (1996), who reported that the percentage of motile sperm was related to the number of spermatids per gram of testicular parenchyma. Also in accordance with our results, those authors failed to detect associations between the percentage of morphologically normal sperm and any parameters linked to Sertoli and germ cells numbers in the seminiferous tubules. Our findings support the notion that bulls with more seminiferous tubules containing spermatids (which in turn are correlated with testis size) also have better seminal quality as evaluated by the number of motile sperm cells and by the vigor of their movement. To our knowledge, this is the first study to report significant associations between quantitative aspects of the spermatogenesis and sperm motility in *Bos indicus* bulls.

Table 3. Pearson's correlations between testis measurements and peripheral concentrations of testosterone in Nelore bulls.

Testis criteria	Age at which testosterone concentrations were quantified												
	Age	12 mo.		13 mo.		15 mo.		17 mo.		18 mo.	19 mo.		20 mo.	21 mo.
		Basal	GnRH	Basal	GnRH	Basal	GnRH	Basal	GnRH	GnRH	Basal	GnRH	GnRH	GnRH
	10				0.45 [1]	0.45 [1]								
	12		0.51 [1]		0.49 [2]	0.45 [1]								
	13		0.46 [1]		0.46 [1]	0.45 [1]			0.47 [1]					
	14	0.48 [1]	0.50 [1]	0.41 [1]	0.52 [2]	0.48 [1]			0.50 [1]					
	15	0.54[2]	0.49 [2]	0.50 [1]	0.55 [2]	0.54 [2]			0.47 [2]					
	16	0.52 [1]	0.44 [1]	0,42 [1]	0.55 [2]				0.50 [1]					
	17	0.46 [1]	0.52 [1]		0.60 [2]				0.43 [1]					
SC	18	0.47 [1]	0.46 [1]	0.45 [1]	0.54 [2]				0.49 [1]					
	19	0.44 [1]	0.42 [1]		0.56 [2]		0.41 [1]	0.47 [1]	0.52 [2]	0.41 [1]				
	21	0.44 [1]	0.46 [1]		0.59 [2]		0.41 [1]	0.40 [1]	0.52 [2]	0.41 [1]			0.51 [1]	
	22	0.41[1]	0.50 [1]		0.60 [2]		0.46 [1]		0.54 [2]	0.44 [1]	0.46 [1]	0.42 [1]	0.59 [2]	
	23		0.49 [1]			0.43 [2]	0.47 [1]	0.45 [1]	0.54 [2]	0.42 [1]		0.42 [1]	0.58 [2]	
	25		0.48 [1]		0.56 [2]				0.53 [2]	0.43 [1]		0.46 [1]	0.49 [1]	
	27				0.48 [2]				0.54 [2]	0.41 [1]		0.44 [1]	0.50 [2]	
	29								0.48 [2]	0.46 [1]		0.42 [1]	0.52 [1]	
TW	30	NS	0.41 [1]	NS	0.43 [1]	NS	NS	NS	0.50 [2]	0.46 [1]	NS	NS	0.64 [2]	0.53 [1]
EW	30	NS	NS	NS	NS	NS	NS	NS	NS	0.51 [1]	NS	0.46 [1]	0.41 [1]	NS
TD	30	NS	NS	NS	NS	NS	NS	NS	0.51 [2]	0.46 [1]	NS	0.41 [1]	NS	NS
TL	30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.41 [1]	0.47 [1]	NS
L/D	30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.42 [1]	0.41 [1]

SC: scrotal circumference; TW: testis weight; EW: epididymal weight; TD: testis diameter; TL: testis length; L/D: length/diameter. GnRH: average concentrations of testosterone quantified 1.5 and 3 hours. NS: non-significant correlation ($p > 0.05$). All r-values are statistically significant ([1]$p < 0.05$; [2]$p < 0.01$).
Source: Elaboration of the authors.

Basal and GnRH-stimulated testosterone quantified at 12, 13 and 15 mo. were associated with SC measured from 14 to 23 mo. (r = 0.41 to 0.60; Table 3). Also, peripheral concentrations of GnRH-stimulated testosterone at ages from 17 to 21 mo. correlated with scrotal circumference evaluated between 14 and 29 mo. (r = 0.48 to 0.60; Table 3) as well as with testis weight and size (r = 0. 41 to 0.64; Table 3) and epididymal weight estimated in the 30-mo. old bulls (r = 0.51 to 0.57; Table 3). The number of A1 spermatogonium and round spermatids in seminiferous tubule cross sections and number of round spermatids/Sertoli cell, spermatocyte and A1 spermatogonium at the age of 30 mo. were associated with GnRH-stimulated testosterone, but correlations were more consistent when quantified between 17 and 21 mo. (r = 0.44 to 0.58; Table 4). The population of Sertoli/testis was associated with testosterone levels only at the interval from 17 to 21 mo. of age (r = 0.51 to 0.61; Table 4). Additionally, sperm vigor and motility evaluated at ages from 25 to 29 mo. correlated with GnRH-stimulated testosterone quantified at 12 (r = 0.46), 13 (r = 0.47 to 0.55) and 18 mo. (r = 0.48 to 0.57). In the present study, correlations between testosterone and gonad size are likely related to the dependence of testis growth and epididymal development upon androgens (AMANN; WALKER, 1983; CORNWALL, 2009; CHENG et al., 2010; ROBAIRE; HAMZEH, 2011) but these correlations were more significant for GnRH-stimulated than for basal testosterone. As well established, testosterone is dependent on both GnRH and LH, and its secretion occurs in pulses (BRIDGES et al., 1993; BAGU et al., 2006). Thus, the exogenous GnRH used in the present study triggered the maximal capacity of LH secretion and, consequently, pronounced increases in testosterone secretion at a given time. The use of GnRH challenges as an experimental approach overcomes, at least in part, the effect of pulsatile testosterone releases.

Table 4. Pearson's correlations between concentrations of GnRH-stimulated testosterone and Sertoli and germ cell numbers in Nelore bulls.

	Cell counts estimated at 30 months of age					
Testosterone	A1	RS	RE/SC	RS/SP	RS/A1	SC/ Testis
12 –13 mo.	0.48	NS	0.45	NS	NS	NS
15 mo.	0.57	0.43	0.53	0.43	0.43	NS
17 mo.	0.48	NS	0.54	NS	NS	NS
18 mo.	0.50	0.58	NS	0.58	0.58	NS
19 mo.	0.41	0.44	NS	0.44	0.44	0.44
20 mo.	0.45	NS	NS	NS	NS	0.61
21 mo.	NS	NS	NS	NS	NS	0.55

A1: Al spermatogonium; RS: number of round spermatids per tubule cross section; RS/SC: number of round spermatids per Sertoli cell; RS/PS: number of round spermatids per primary spermatocyte; RS/A1: number of round spermatids per A1 spermatogonium; SC/T: number of Sertoli cells/testis.
NS: non-significant correlations ($p > 0.05$). All other r-values are statistically significant ($p < 0.05$).
Source: Elaboration of the authors.

Conclusion

The present study describes the associations among testis size, testosterone concentrations, semen criteria and quantitative aspects of the seminiferous epithelium of Nelore bulls raised in a semi-arid region of the Brazilian Northeast. Pronounced increases in testosterone could be critical to the initiation of testis growth and may be useful as a reliable indicator of the population of Sertoli cells and quantitative parameters of spermatogenesis. Our results also suggest that these associations may be considered as evidence of testicular maturation of

the post-pubertal animals. In this regard, knowledge about the gonad development and reproductive physiology of the tropically-adapted Nelore bulls is crucial for understanding their potential in genetic improvement programs.

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