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—Original Article—

The Correlation between Age, Body Weight and Testicular Parameters in Murrah Buffalo Bulls Raised in Brazil

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Abstract. Buffalo are an economically important source for meat and milk production, especially in Brazil. However, important aspects of their biology remain unknown thus far. Herein, we describe the reproductive characteristics of male Murrah buffalo (*Bubalus bubalis*) raised under extensive management conditions by applying biometrics associated with testicular weight. We analyzed seven males, divided into two groups: G1, which consisted of four 18-month-old animals, and G2, which consisted of three 24-month-old animals. Testicular development occurs slowly in Murrah buffalo, suggesting a delay of sexual maturity. The biometric testicular parameters analyzed were scrotal circumference, testicular weight, testicular length, testicular width, testicular thickness and testicular circumference. Our data indicate strong correlations between SC, age and body weight, and additional significant relationships were identified between body weight, age and other testicular parameters. Thus, these parameters are suitable indicators when selecting bulls for breeding purposes.
Key words: Buffalo reproduction, Feed management, Testis

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Interest in buffalo species has grown in recent years, especially in Brazil, mainly due to the adaptive capacity of these animals to tropical and subtropical climatic conditions and their ability to thrive in areas unsuitable for cattle and other domestic animals. Currently, buffalo are economically important due to their excellent fertility, longevity, feed conversion efficiency and productivity in comparison to cattle [1]. The productivity of buffalo with respect to milk, meat and work output is currently better than that of cattle living under Brazilian conditions [2]. However, important aspects of their biology remain unknown thus far.

In contrast to cattle, buffalo may remain reproductively inactive even during the favorable reproductive season. Perhaps often occurs in relation to stressful environmental factors; obviously buffalo are at a risk for low levels of reproduction under such conditions [3]. Several factors can act as stressors and, strongly influence the reproductive activity of the animals [4]: nutrition, climate and management are among the main factors that generally contribute to the differences in the reproductive performance of buffalo and cattle (*Bos taurus*), which may both be reared in extensive management systems characterized by nutritional deficiencies that affect the development of reproductive aspects [5]. In addition, male buffalo may be affected by environmental heat and express a greater desire to copulate during cooler times of the day. According to Ohashi [6], male buffalo showed the best libido during the night, after sunset or before dawn. Another important factor is the normality of spermatogenesis. For mammals that are maintained in captivity, including buffalo bulls, spermatogenesis depends on the normal maintenance of testicular temperature between 2 and 6 C lower than body temperature [4].

To better understand the reproductive characteristics of male buffalo [7], we conducted a study on the testicular development of Murrah buffalo in Brazil.

The biometric parameters of buffalo testicles can be established using comparative studies with other species. Investigations into the mammalian body and testicular biometrics are important for various aspects of reproduction; these studies help characterize puberty and sexual maturity and enable inferences about spermatogenesis [8]. Testicular biometry is an important component of monitoring the testis for normality and gauging potential sperm production [9].

Biometric parameters, such as scrotal circumference (SC), testicular weight (TW) and testicular length (TL), are essential measurements in the andrological evaluation of a breeding animal. Among these parameters, SC is used most often because it is easy to measure and displays a high correlation with body weight and reproductive capacity (libido), particularly sperm production [10]. While the biometric data related to SC help define the reproductive parameters for a species, SC alone should not be used for the selection of breeders. Rather, a complete andrological evaluation (a breeding soundness examination), including an evaluation of semen quality, should be performed to certify the reproductive capacity of a male [7].

The aim of this study was to identify the reproductive characteristics of Murrah buffalo bulls using testicular biometric parameters and

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their correlations with body weight at two different ages.

Materials and Methods

Animals

Murrah buffalo bulls were divided into two groups: G1 contained four 18-month-old males, and G2 contained three 24-month-old males. The animals were obtained from the Department of Animal Production, Faculty of Veterinary Medicine, UNESP-Botucatu/SP, which is located at 22°53'09" S, 48º26'42" W and is 804 m above sea level. The city's climate is tropical, with mild winters and hot summers. The buffalo were fed *Brachiaria sp*. and mineral salt ad libitum and supplemented with corn silage during the dry periods. Endoparasites control was performed with levamisole phosphate, applied 1 ml per 40 kg of liveweight, and the vermifuge (Ripercol, Fort Dodge Animal Health, Campinas, São Paulo, Brazil) was administered to the animals at the beginning of the wet season (November) and early dry season (June). The vaccines were applied to the animals: the first dose of clostridial was given at four months, with a booster after one month followed by subsequent annual applications; FMD was administered in May and November.

The buffalo used in this study were the same age at the beginning of the experiment. The testes were collected at two time points because the specific age of onset of puberty and spermatogenesis in Murrah buffalo bulls raised under the conditions described was unknown.

The project was conducted in accordance with the ethical principles for animal experimentation of the Animal Experimentation Ethics Committee of the School of Animal Science of Unesp Dracena (Protocol No. 017/2011).

Testicular parameters

Prior to collecting the testes, the animals were physically restrained and anesthetized [11] according to the ethical principles for animal experimentation as recommended by the Brazilian College of Animal Experimentation [12], and they were weighed using a mechanical balance (ACR 1500, 4,000 kg, Balanças Açôres, Cambé, PR, Brazil).

After collection, the epididymis was separated from the testis, and the testicular weight (TW), testicular length (TL), testicular width (TWD), testicular thickness (TT) and testicular circumference (TC) were determined for each testis. To obtain the TW (g), an analytical balance was used (BK 3000; range 0.2–3100 g). The length, width and thickness were measured using calipers with millimeter divisions. The TC and SC were measured using a metric tape.

Histological processing

Each testis was sectioned into three regions (the capitata, middle and end caudata portions) for tissue fixation. The middle portions of the testicular parenchyma were placed in Bouin's solution for at least 24 h. After dehydration in increasing concentrations of alcohol, followed by infiltration with xylenes, the tissue samples were embedded in paraffin [13]. A microtome (Leica RM2145, Leica, Berlin, Germany) was used to section the tissue into 5-mm-thick slices, and these sections were stained with hematoxylin- and eosin (HE). The samples were analyzed under a light microscope (Leica DM 2500, Leica).

Statistical analysis

Descriptive analyses of the mean and standard deviation for each testicular biometric parameter were performed with the GraphPad Prism4 software (GraphPad Software, La Jolla, CA, USA). Age, body weight and testicular biometric parameters were tested for correlations using R statistical software (R Development Core Team 2009) [14].

Results and Discussion

Histological analyses

At 18 months, the testicular parenchyma contained testicular cords and internal cord compartments associated with the presence of Leydig cells, vessels and nerves. Within the testicular cords, gonocytes, undifferentiated supporting cells and luminal processes were observed. Gonocytes and spermatogonia in the differentiation process were identified near the basal membrane (Fig. 1). Although spermatids were present in high numbers at 18 months in Nili-Ravi buffalo testes [15], spermatozoa were not present in the tubule lumens analyzed here.

The testicular parenchyma at 24 months contained seminiferous tubules with lumens and germinative epithelia present at different stages of the seminiferous epithelial cycle (Fig. 2). The following cell types were identified: Sertoli cells, spermatogonia, pre-leptotene/leptotene spermatocytes, pachytene spermatocytes, zygotene spermatocytes, diplotene spermatocytes, round spermatids and luminal spermatozoa (Fig. 2). These cells could be classified into eight stages of cellular associations according to the tubular morphology method [16]. Bongso [17] noted that the spermatozoa in the tubular lumen of swamp buffalo had the same morphology at 16 months. In crossbred buffalo (Mediterranean × Jaffarabadi), this morphology was observed at 13 months [18]. Ohashi [19] showed the onset of the spermatogenic process in buffalo at 9 months. At 24 months, our animals were considered delayed when compared with crossbreeds reared in Brazilian conditions. Our histological analysis of the spermatogenic process indicated that these animals were not sexually mature.

Testicular parameters

The data for SC and body weight are listed in Table 1. Although the SC and body weight differed significantly between the two age groups analyzed, there was an increase in the SC with increasing body weight. Additional testicular biometric parameters are often assessed in domestic animals, particularly those animals used for livestock production because testicular parameters are an important part of the andrological evaluation. Among the available biometrics for testes, SC is one of the most commonly employed because it is easy to measure and highly correlated with body weight [19].

As shown in Table 2, there was a significant correlation between SC, age and body weight. This result has been described previously in river [20], swamp [21] and Murrah [22] buffalo.

The data presented here differ from the findings in cattle [23], for which the SC is particularly important in identifying bulls with high reproductive capacity. Neither scrotal circumference nor body weight should be used alone as a routine and reliable parameter to predict the reproductive capacity in buffalo [24].

The mean values and standard deviation for TW, TL, TWD, TT

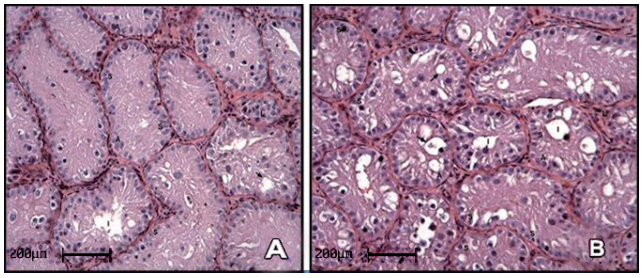

Fig. 1. Testis of Murrah buffalo bulls at 18 months of age characterized by an immature sexual phase (A and B). We observed solid testicular cords with gonocytes (A) and undifferentiated supporting cells (S). Leydig cells, vessels and nerves were present in the interstitium. Photomicrograph, hematoxylin-eosin method.

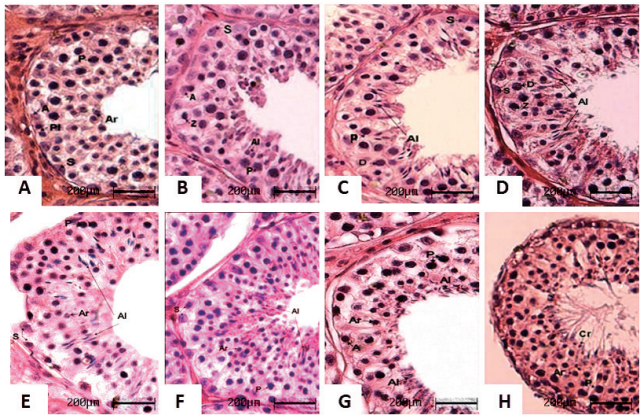

Fig. 2. Different cell types in the seminiferous epithelium of the Murrah buffalo at 24 months of age. Photomicrographs of cross sections of the seminiferous tubules represent the stages of the cycle of the seminiferous epithelium. Stage one (A), two (B), three (C), four (D), five (E), six (F), seven (G) and eight (H) represent the following groups of cells: primary spermatocytes at the pre-leptoteno/leptoteno (PL) stage, primary spermatocytes at the pachytene (P) stage, round spermatids (Ar), elongated spermatids (Al), primary spermatocytes at the zygotene (Z) stage, primary spermatocytes at the diplotene (D) stage, residual bodies (CR) and spermatozoa (E). All the stages presented spermatogonia type A (A) and Sertoli cells (S). Hematoxylin-eosin staining.

and TC, relative to buffalo age, are shown in Table 1. Although the average TW did not differ between the two groups, significant differences were observed for TL, TWD, TT, and TC ($P<0.05$). Furthermore, these variables exhibited high correlation coefficients, as noted in Table 2. Body weight, age, SC, TW, TL, TWD, TT and TC were positively correlated ($P<0.05$). TW was not related to the other biometric parameters. This finding differs from observations made in Piau pigs [25], rams and cattle [26], in which a high correlation was described for all parameters. The strength of the correlations obtained suggests that TL, TWD, TC and TT are useful parameters for the selection of breeding animals.

Under the husbandry conditions described here, both body and testicular development of buffalo occurred more slowly than the typical growth rates for *Bos taurus* [27]. The data for *Bos indicus* [28], however, suggest a similar rate of body and testicular development to the rates observed for buffalo in the present study. These similarities can be attributed to the fact that Zebu cattle are also raised under extensive farming conditions, and therefore suffer from the same nutritional consequences, particularly dietary protein deficiencies, which can lead to developmental delays.

In conclusion, our analysis of biometric testicular parameters in Murrah buffalo revealed that the TL, TWD, TC and TT are useful

Table 1. Mean* body and testicular biometric parameters in Murrah buffalo bulls at 18 and 24 months of age

Age group	BW (kg)	SC (cm)	TW (cm)	TL (cm)	TWD (cm)	TT (cm)	TC (cm)
G1 (18 mo)	270.25 ± 14.59^a	18.75 ± 1.5^a	21.11 ± 2.79^a	5.07 ± 0.36^a	2.72 ± 0.24^a	2.48 ± 0.25^a	8.09 ± 0.60^a
G2 (24 mo)	446.70 ± 32.56^b	25.30 ± 3.21^b	77.66 ± 38.5^b	7.13 ± 1.25^a	3.73 ± 0.69^b	3.55 ± 0.51^b	12.58 ± 1.71^b

*Means with different superscript letters are significantly different ($P<0.05$) according to analysis of variance and the Student's *t* test (5%). BW=body weight, SC=scrotal circumference, TW=testicular weight, TL=testicular length, TWD=testicular width, TT=testicular thickness, TC=testicular circumference.

Table 2. Correlations between age, body weight and testicular parameters in Murrah buffalo bulls at 18 and 24 months of age

	Age	BW	SC	TW	TL	TWD	TT	TC
Age	–	0.975*	0.854*	0.776*	0.808*	0.777*	0.847*	0.904*
BW	–	–	0.843*	0.851*	0.827*	0.775*	0.838*	0.904*
SC	–	–	–	0.517	0.963*	0.932*	0.898*	0.931*
TW	–	–	–	–	0.495	0.379	0.479	0.572
TL	–	–	–	–	–	0.982*	0.949*	0.963*
TWD	–	–	–	–	–	–	0.971*	0.965*
TT	–	–	–	–	–	–	–	0.978*
TC	–	–	–	–	–	–	–	–

BW=body weight, SC=scrotal circumference, TW=testicular weight, TL=testicular length, TWD=testicular width, TT=testicular thickness, TC=testicular circumference. *Significant ($P<0.05$).

indicators for the selection of bulls for breeding.

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References

1. **Bernardes O.** Buffaloes breeding in Brasil: position and economic relevancy. *Rev Bras Reprod Anim* 2007; **31**: 293–298. (In Portuguese).
2. **Nascimento CNB, Carvalho LODM.** Creation of Buffaloes: feeding, handling and improved facilities. *EMBRAPA-SPI* 1993; 403 (In Portuguese).
3. **Vale WG, Ribeiro HFL, Sousa JS, Silva AOA, Barbosa EM, Rolim Filho ST.** Selection and breeding soundness evaluation in the male buffalo. *Ver Bras Reprod Anim* 2008; **32**: 141–155. (In Portuguese).
4. **Garcia AR.** Influência de fatores ambientais sobre as características reprodutivas de búfalos do rio (*Bubalus bubalis*). *Rev Ciênc Agrar* 2006; **45**: 1–13. (In Portuguese).
5. **Van Demark NL, Mauger RE.** Effect of energy intake on reproductive performance of dairy bulls. I. Growth, reproductive organs, and puberty. *J Dairy Sci* 1964; **47**: 798–802. [CrossRef]
6. **Ohashi OM, Sousa J, Ribeiro HFL, Vale WG.** Distúrbios reprodutivos em touros *Bos indicus, Bos taurus* e mestiços, criados em clima amazônico. *Pesq Vet Bras* 1988; **8**: 31–35. (In Portuguese).
7. **Ohashi OM, Miranda MS, Cordeiro MS, Socorro SS, Santos D.** Reproductive development of male buffalo: testicular biometry, espermatic activity and endocrinology. *Rev Bras Repr Anim* 2007; **31**: 299–306. (In Portuguese).
8. **Assis Neto AC, Carvalho MAM, Melo MIV, Miglino MA, Oliveira MF, Almeida MM, Papa PC, Kfoury Jr JR.** Biometric aspects of the testicular and corporal development of Agoutis (Dasyprocta aguti) rised in captivity. *Braz J Vet Res Anim Sci* 2003; **40**: 154–160. (In Portuguese).
9. **Paula TAR, Navarro RD.** Testicular componentes of peccaries (*Tayassu pecari*) and collared peccary (*Tayassu tajacu*). *Rev Bras Reprod Anim* 2001; **25**: 206–207. (In Portuguese).
10. **Brito LF, Silva AE, Unanian MM, Dode MA, Barbosa RT, Kastelic JP.** Sexual development in early and late maturing Bos indicus and Bos indicus x Bos taurus crossbred bulls in Brazil. *Theriogenology* 2004; **62**: 1198–1217. [Medline] [CrossRef]
11. **Massone F.** Anestesiologia veterinária farmacologia e técnicas. Rio de Janeiro: Guanabara Koogan; 2003: 344.
12. **Colégio Brasileiro de Experimentação Animal COBEA.** Princípios éticos na experimentação animal. Disponível em: <http://www.cobea.org.br/etica.htm#3>.
13. **Luna LG.** Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. New York: McGraw-Hill; 1968: 258.
14. **R Foundation for Statistical Computing** R: A language and environment for statistical computing. Vienna, Austria 2009; ISBN 3-900051-07-0, URL http://www.R-project.org.
15. **Ahmad N, Umair S, Shahab M, Arslan M.** Testicular development and establishment of spermatogenesis in Nili-Ravi buffalobulls. *Theriogenology* 2010; **73**: 20–25. [Medline] [CrossRef]
16. **Curtis SK, Amann RP.** Testicular development and establishment of spermatogenesis in Holtein Bull. *J Anim Sci* 1981; **53**: 1645–1657. [Medline]
17. **Bongso TA, Hassan MD, Nordin W.** Relationship of scrotal. Circumference and testicular volume to age and body weight in the swamp buffalo (Bubalus bubalis). *Theriogenology* 1984; **22**: 127–134. [Medline] [CrossRef]
18. **Melo MIV, Vale Filho VR.** Puberty in crossbred buffaloes. *Arq Bras Med Vet Zoot* 1992; **44**: 537–542. (In Portuguese).
19. **Ohashi OM, Oba E, Nogueira JR, Souza SS, Silva AOA.** Characteristics of the reproductive development of male buffaloes: testicular biometrics, puberty and sexual maturity. *R Bras Med Vet* 2001; **23**: 103–107. (In Portuguese).
20. **Viana RB, Baruselli PS, Cardoso EC, Araujo CV, Monteiro BM, Gomes V.** Effect of mineral supplementation minerals level in seminal. *In*: Proceedings of the XXV Jubilee World Buiatrics Congress; Budapest, Hungary. 2008: 213–213.
21. **Mccool CJ, Entwistle KW, Gilham MP.** Testis growth rates in the australian Swamp buffalo. *Buffalo J* 1985; **1**: 29–36.
22. **Pant HC, Sharma RK, Patel SH, Mittal AK, Kasiraj R, Misra AK, Prabhakar JH.** Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriogenology* 2003; **60**: 27–34. [Medline] [CrossRef]
23. **Chenoweth PJ.** Breedings soudness evalution in bulls. *In*: Morrow DA (ed.), Current Therapy in Theriogenology. Philadelphia: W.B. Saunders; 1980: 330–339.
24. **Carter AP, Wood PDP, Wright PA.** Association between scrotal circumference, live wight and sperm output in cattle. *J. Reprod Fertil* 1980; **59**: 447–451. [Medline] [CrossRef]
25. **Silva SM, Melo MIV, Scheid IR, Nascimento EF, Cassali GD.** Establishment of spermatogenesis in pigs of Large White and Landrace breeds with different growth with emphasis on puberty. *In*: Congresso Brasileiro de Veterinários Especialistas em Suínos; Belo Horizonte, Brazil. 1999: 301–302 (In Portuguese).
26. **Courot M, Hochereau-De Revivers MT, Ortavant R.** Spermatogenesis. *In*: Johnson AD, Gomes WR, Vandemark NL (eds.), The Testis. New York: Academic Press; 1970: 339–432.
27. **Coulter GH.** Escrotal circumference —a review. *In*: Annual Meeting of the Society for Theriogenology; 1991; San Diego, USA. Proceedings 330–339.
28. **Silva AED, Dode MAN, Rosa AAM, Unanian MM, Caldas MCS.** Establishment of male sexual activity in Nellore in Central Brazil. *In*: Cong. Bras. Reprod. Animal; 1991; Belo Horizonte, Brazil. 425 (In Portuguese).