Association of testicular echogenicity, scrotal circumference, testicular volume and testosterone concentration in buffaloes*

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ABSTRACT. Ayala H.D.M., Ribeiro H.F.L., Rolim Filho S.T., Silva E.V.C. & Vale W.G. **Association of testicular echogenicity, scrotal circumference, testicular volume and testosterone concentration in buffaloes.** [Associação entre a ecogenicidade, circunferência escrotal, volume testicular e concentração de testosterona em búfalos.] *Revista Brasileira de Medicina Veterinária, 38(4):334-340, 2016.* Programa de Pós-Graduação em Ciencia Animal, Universidade Federal do Pará, Rua Augusto Corrêa 1, Campus Universitário do Guamá, Belém, PA 66075-110, Brazil. E-mail wm.vale@hotmail.com

This article aimed to discuss the changes in the testicular parenchyma, analyzed by the use of ultrasonography, and correlates them with the testicular biometric parameters and testosterone concentration in crossed Murrah x Mediterranean buffaloes. Nineteen buffaloes, with initial ages between 11 and 59 months,were submitted to fortnightly collections of semen for a period of six months. At each collection the testicular biometry and testicular echogenicity were evaluated as well as blood samples were also collected to measure the plasma testosterone levels. The data were submitted to analysis of variance by the GLM procedure, considering the age group fixed effect. The average data obtained were compared by the Duncan test, at 5% significance. There was a significant growth ($P<0.05$) of the scrotal circumference, which varied from 12.88±0.51 cmto 31.18±0.75 cm among animals aged 12 to 60 months, as well as testicular volume, which ranged from 30.28±17.37 to 611.96±38.69 cm³ among the animals. The echogenic intensity of the testicular parenchyma varied in pixels from 78.67±6.36 to 109.24±3.13 in animals aged 12 to 60 months respectively. In the animals with ages between 12 and 19 months was observed levels of testosterone considered being low, whereas in the animals from 20 to 21 months there was a progressive increase in the testosterone levels, which showed their peak in the animals older than 60 months. Therefore, through ultrasonography it was possible to detect differences in echogenicity among different phases of pre-puberty, puberty and sexual maturity in the animals, and this procedure seems to be an important tool in the selection of this characteristic.

KEYWORDS. Amazon, *Bubalus bubalis*, buffalo, testicular biometry, ultrasonography.

RESUMO. O presente artigo teve como objetivo discutir as alterações no parênquima testicular, analisados através da ultra-sonografia, e correlacionar com os parâmetros biométricos testiculares e concentração de testosterona em búfalos mestiços. Dezenove touros, mestiços das raças Murrah x Me-

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diterranea, com idade inicial entre 11 e 59 meses, foram submetidos a coletas quinzenais de sêmen por um período de seis meses. A cada coleta foram avaliados dados sobre a biometria e ecogenicidade testicular, quando amostras de sangue foram coletadas para a determinaçãodos níveis de testosterona no plasma. Os dados foram submetidos à análise de variância pelo procedimento GLM, considerando o efeito fixo faixa etária. Os dados médios obtidos foram comparados pelo teste de Duncan, a 5% de significância. Houve uma diferença, P <0,05) em causa a circunferência escrotal, que variou de 12,88 ± 0,51 cm a 31,18 ± 0,75 centímetros entre os animais com idade entre 12 a 60 meses, bem como o volume testicular, que variou de 30,28 ± 17,37 para 611,96 ± 38,69 cm³ entre os animais. A intensidade ecogênica do parênquima testicular variou em pixels a partir de 78,67 ± 6,36-109,24 ± 3,13 em animais com idade de 12 a 60 meses, respectivamente. Nos animais com idades entre 12 e 19 meses foi observado níveis de testosterona considerados diminuídos, enquanto que nos animais de 20 a 21 meses, houve um aumento progressivo nos níveis de testosterona, que tem seu pico nos animais com idade superior a 60 meses. Portanto, por meio de ultrassonografia, foi possível detectar diferenças na ecogenicidade entre animais pré-púberes e sexualmente maduros, podendo-se predizer que a ultrassonografia testicularé uma ferramenta importante na seleção destas características.

PALAVRAS-CHAVE. Amazonia, *Bubalus bubalis*, búfalo, biometria testicular, ultrasonografia.

INTRODUCTION

The latest statistics available shows that buffaloes account for more than 708 million tons which means of 14 per cent of global milk (FAO 2011). The growing demanded for buffalo milk has led the herd's breeding of this species an increasingly attractive economic activity. Therefore, the growth of the buffalo herd needs to be associated with enhanced productivity, like for other producing animals, by identifying animals that have genetic merit to allow multiplication and distribution of genetically improved animals which can be achieved by various reproduction biotechnologies (Vale et al. 2002, Vale et al. 2009).

The advent of biotechnology applied to animal reproduction has greatly facilitated the diffusion of genetic material from sires and cows, permitting the generation of thousandsof progeny animals from a single sire and thereafter to leverage the diffusion of the paternal genome (Ohashi et al. 2007).

For this reason, accurate examinations like clinical and reproductive health of sires and evaluation of the progeny (progeny testing) are necessary, as well as complementary examinations of potential semen donors to prevent dissemination of undesirable traits in the herd are equally essential. These needs are specially required to the Brazilian buffalo herd, in which the inbreeding seems to be high (Vale et al. 2002, Vale et al. 2008).

Concerning the breeding soundness examination (BSE) of a sire, such procedures is fundamental to know the normal testicular biometric parameters, especially the scrotal circumference, which is one of the most widely used biometric measures for evaluation of reproductive development because it is easy to obtain and highly repeatable, besides presenting a strong positive correlation with body weight and reproductive capacity as well as in semen production (Vale and Ribeiro 2009, Ahmad et al. 2010).

During the sexual maturation period, the testicular parenchyma at first goes through modifications in the lumen of the seminiferous tubules. These changes occur due to the start of secretion of fluids and ultrasonography allows characterizing these testicular alterations during sexual maturation (Curtis & Amann 1981, Abdel-Razek & Ali 2005).

Besides ultrasonography is a complementary technique to clinical examination in reproduction of female and male (Moura & Merkt 1996). It is very practical and provides the veterinarian with concrete information on the herd's reproductive state, helping the solution of problems as well infertility through rapid decisions and application of the most suitable treatments. Testicular echography is a diagnostic tool that is more sensitive and reliable than examination by palpation (Chapwanya et al. 2008, Gnemmi & Lefebvre 2008, Pinho et al. 2013). For these reasons, it has considerable potential for assessment of the testicular functioning of bulls (Gábor et al. 1998).

Animals between 8 and 22 weeks of age have a large part of the increase in the pixel intensity presented by this technique. This occurs in a context of elevation of testosterone serum concentrations. After spermatogenesis, the pixel intensity levels equalize (Chandolia et al. 1997a).

Thereby, the aim of this study was to evaluate the testicular parenchyma's echotexture of bufalloes in different age ranges and associate these observations with testicular biometry and testosterone concentrations.

MATERIAL AND METHODS

The study was carried out on the campus of Federal Rural University of Amazônia (UFRA) with animals of the Experimental Dairy Buffalo Unit, located in Belém, Pará, Brazil, at 01º 27′ 21′′ South latitude, 48º 30′ 16′′ West longitude and 10 meters altitude. The region has an average annual temperature of 26.4 ºC, average relative humidity of 84% and average yearly insulation of 2,338.3 hours. The climate is classified as Afi on the Köppen scale (Bastos et al. 2002).

A total of (n=19) male crossed buffaloes (Murrah x Mediterranean) were used in this experiment, with initial ages between 11 and 59 months. The animals were raised extensively in cultivated pastures (*Brachiaria humidicola*) with commercial mineral supplement and water *ad libitum* associated to health schedule for vaccination against common regional diseases which affect buffalo herds. All animals underwent a regime andrological routine examinations breeding soundness examination (BSE) and fortnightly blood collections for a period of six months, from 15th May to 15th November 2010.The determination of testosterone was conducted by immunoassay for the quantitative in vitro in serum and plasma using the immunoassay technique of electrochemiluminescence (ECLIA), Sanchez-Carbayo et al. (1998) designed to be used in the immunoassay analyzer from Roche Diagnostics Elecsys 1010/2010 which was the section of the National Primate Center Laboratories (CENP). During each collection, data were recorded regarding testicular biometry and testicular ultrasound images were obtained. The scrotal circumference (SC) was assessed with a tape measure, and testicular volume (VOL) was calculated according to the method described by Bailey et al. (1998). The ultrasound device used was a Mindray model DP-2200 VET (São Paulo, Brazil) coupled to a linear 7.5 MHz frequency transducer. During each collection two images were captured, in the longitudinal-lateral and transverse-lateral planes.

The gray scale values of the ultrasound images were determined from the echographic images of the testicular parenchyma, registered for the respective age ranges and expressed in pixel intensity units. The pixel intensity range was defined by numerical values from 0 to 255, where 0 represented dark (anechoic) and 255 represented white (hyperechoic). The testicular echodensity (ECHOt) was determined from these images, expressed in number of pixels/area, using the public domain Image JSoftware, based on an image processing program developed by the National Institutes of Health in the United States. This program calculates the area and pixel statistics in values defined by the user (Rasband 2009).

The average number of pixel units of each testis in the two planes was obtained from five areas measuring approximately 1 cm^2, selected at random within the testicular parenchyma. Then these two mean values were used to compute the overall mean testicular echodensity (ECHOt) for each animal.

The variables expressed as percentage were transformed into arcsine (x/100), the blood testosterone levels (T) into log(x), as suggested by Sampaio (1998). The transformed data were then subjected to analysis of variance by the GLM procedure, with age range as the fixed effect. The means were compared with the Duncan test. The significance level in all the tests was 5%.

For the regression analysis, the dependent variable was the pixel intensity and the independent variables were: age in days (AGEd) and months (AGEm), age range (AGEr), total testicular volume (VOLt). The REG process of the SAS program was used with the stepwise method (SAS, 1999).

RESULTS AND DISCUSSION

Testicular biometry

The scrotal circumference and testicular volume results are shown in Table 1 whereas in the Figure 1 shows the pattern of general increasing scrotal circumference and testicular volume according to rising age.

The mean circumferences (± standard deviation) ranged from 12.88±0.51 cm to 31.18±0.75 cm in animals from 12 to 60 months year old, respec-

Table 1. Scrotal circumference and testicular volume (mean ± standard deviation) of male crossed buffaloes (Murrah x Mediterranean) according to age range, evaluated during six consecutive months, raise in extensive management (n=19).

Age range (month)	Scrotal circumference (cm)	Testicular volume (cm^3)
12	12.88±0.51^{a}	30.28±17.37^{a}
14	15.98±0.35^{b}	75.22±5.80^{a}
16	16.55±0.28^{bc}	83.21±5.27^{b}
18	17.34±0.27^{bc}	90.12±5.98^{b}
20	18.83±0.37^{bc}	134.81±11.01^{b}
22	23.5±2.02^{a}	240.40±44.04^{a}
24	24.11±0.36^{d}	282.89±11.31^{d}
30	26.16±0.53^{e}	359.93±23.91^{d}
36	27.38±0.58^{e}	410.80±26.28^{d}
42	31.18±0.75^{f}	611.96±38.69^{e}
48	28.31±0.49^{e}	471.92±22.69^{d}
60	28.38±0.38^{e}	534.25±25.36^{f}

^{a,b}Means followed by the same letter in the column do not differ statistically, according to the Duncan test (P<0.05).

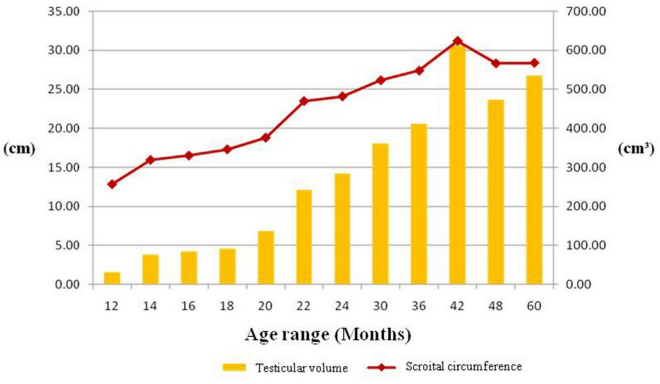

Figure 1. Testicular development, scrotal circumference (cm) and testicular volume (cm^3) of crossed buffaloes (Murrah x Mediterranean) according to age range (n=19).

tively. In turn, the testicular volume varied from 30.28±17.37cm³ to 611.96±38.69cm³, in both cases with statistical difference (P<0.05) between the different ages.

The scrotal circumference values found in the present study were smaller than those reported by Villares et al. (1979) in purebred Jafarabadi and Murrah animals, and also smaller than those found by Ohashi et al. (1988) and Ohashi et al. (2007) in Mediterranean x Jafarabadi crossbreeds, according to the authors kept in excellent extensive grazing conditions. The average found in our study for buffaloes with 60 months of age or more, were also smaller than that found by Bhosrekar (1993) for Indian water buffaloes which reported a scrotal circumference that ranged from 30 to 34 cm. This fact seems to be directly connected to the management and feeding, however, the results here observed were bigger than those obtained by Bongso et al. (1984) and McCool and Entwistle (1989) for swamp buffaloes.

The mean scrotal circumference values found in this study were smaller than those in the majority of studies of buffaloes in Brazil, as well as in India. This fact can be related to the type of herd management and genetic standard of the experimental animals. According to Vale et al. (2004), buffaloes that are bred in extensive grazing systems lose body weight, hence testicular mass and scrotal circumference in dry periods when there is insufficient forage, but recover themselves from these effects in the rainy season when forage grasses are plentiful again.

Testicular echogenicity

The mean pixel intensity levels varied from 78.67±6.36 to 114.05±2.42 in the animals with 12 to 60 monthsof age (Table 2).

The ultrasound appearance of the testis in this work was similar to the descriptions of Pechman & Eilts (1987), Cartee et al. (1989) and Gábor et al. (1998) in for cattle. Therefore, the results are in line with those of the mentioned authors, all of them agreed due to be noninvasivetechnique it is practical and easy to be carry out. In addition, the same conclusion was reached by Brass et al. (1989), studying sheep and the testicular parenchyma in that study revealed normal result tests, without palpable and non-palpable lesions. Chapwanya et al. (2008), studying in bovine, and Jucá (2005), who analyzed goats with ages of 18, 30 and 48 months, reported variations in the echotexture of the testicular parenchyma at all ages, with hypoechoic images of low and high intensity.

However, although it was observed through the development of this study a complex development pattern of increase and decrease of the numerical pixel values in the different age ranges, similar observations were also reported by Chandolia et al. (1997a, 1997b) in goats and cattle, and by Aravindakshan et al. (2000) studying males up to two years of age.

The interpretation for the echogenicity results found in the respective age ranges is in line with the results of Pechman and Eilts (1987), Cartee et al. (1989) and Cardilli et al. (2009a, 2009b, 2010), all of whom affirm that the testicular parenchyma of young animals is homogeneous with low echogenicity, but that this increases in direct proportion with the animals' ages.

In this physiological context, Evans et al. (1995, 1996) demonstrated in cattle that during the sexual maturation period, the cell content undergoes changes in the lumen of the seminiferous tubules, which according to the authors is triggered by the start of secretion of fluids, leading to diminished average pixel values. These same changes were noted in the animals between the ages of 36 and 60 months in the present study.

The changes in the echotexture of the testicular parenchyma found in the present study are indicators that can reflect important details of the testicular development stages. Aravindakshan et al. (2000) and Abdel-Razek & Ali (2005) evaluated such changes of the internal structure of the scrotum and testes of cattle during their development and attributed these changes to cell proliferation and the production of fluids, since ultrasound images depend on the relative density of the tissues to be examined and during the period of sexual maturity, the cellular content and changes in the secre-

Table 2. Testicular echographic intensity in pixels (mean ± standard deviation) of male crossed buffaloes (Murrah x Mediterranean) according to age range, evaluated during six consecutive months, raised in extensive management (n=19).

Age range	ECHOt (pixels)	Minimum	Maximum
12	78.67±6.36^{d}	68	90
14	94.22±3.40^{bc}	71	113
16	88.16±3.95^{c}	53	131
18	96.09±3.40^{bc}	61	132
20	103.12±3.86^{abc} ^{bc}	67	127
22	98.4±5.87	77	110
24	114.05±2.42^{a}	92	132
30	109.24±3.13^{ab}	81	137
36	98.67±3.05^{bc} ^{abc}	74	111
42	99.33±2.01	83	108
48	96.17±1.90^{bc}	85	105
60	90.13±1.77^{dc}	76	103

^{a,b}Means followed by the same letter in the column do not differ statistically, according to the Duncan test (P< 0.05).

tion of liquids from the genital organs assist in the identification of development changes.

Therefore, based on this knowledge, Hamm & Fobbe (1994) observed an increase in the gray scale of the testes at different stages of sexual maturity, coinciding with puberty, and they standardized the gray scale at different ages to allow a precise diagnosis of puberty. In the same way, Chandolia et al. (1997b) observed differences in the testicular echogenicity between pubertal and prepubertal 15-month-old animals, where the echogenicity of pubertal bulls was higher than that of prepuberty animals, suggesting that ultrasonography could be used as an indicator of sexual precocity in young bulls. However, Pinho et al. (2012) didn't found correlation with sexual maturity and testicular echotexture, indicating that the quantification of the pixel intensity in testicular echotexture was not effective in determining the degree of sexual maturity in Montana breed bulls.

In another study correlating the increase in pixel intensity between the scrotal circumference of animals of different ages, Brito et al. (2004) proposed that this is due to the testicular maturation and multiplication of germinative cells. On the other hand, Carmo et al., (2012) reported that this increase in the echotexture of the testicular parenchyma can be associated with increasing age, attributed to the increasing cell proliferation and reduced production of fluids in the organ with the approach of puberty. The author deduced that the increase in the echotexture probably occurred due to the increase in the diameter of the seminiferous tubules in the initial phase of puberty.

In a male ovine study, Chandolia et al. (1997a) reported that to a great extent the increase in the numerical pixel values occurred in a context of rising serum concentrations of testosterone. The authors concluded that the increase in the numerical pixel values can be due to the gradual formation of more mature cell types required for spermatogenesis. Thus, our findings support this assumption, as shown in the Figure 2.

Testosterone concentrations

The mean testosterone level in the animals aged 12 and 13 months old was 0.070±0.026 ng/ml and it remained at relatively low levels in the animals from 12 to 19 months. But starting at 20 to 21 months of age, the levels rose progressively, peaking at 0.700±0.155 ng/ml in the range from 30 to 35 months, after which it remained at high levels (36 to 60 months), as shown in Table 3.

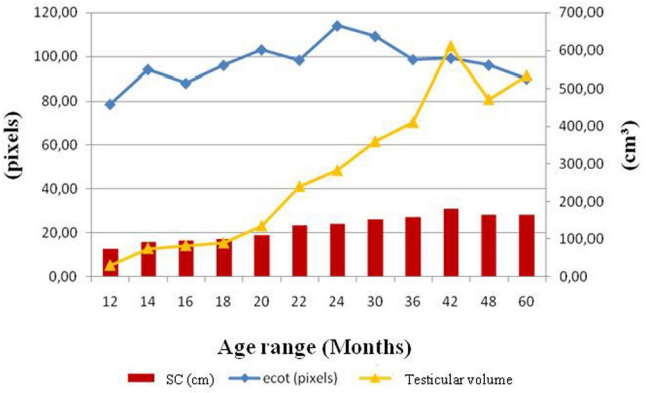

Figure 2. Testicular development, scrotal circumference (cm), testicular volume (cm^3) and testicular echotexture of male crossed buffaloes (Murrah x Mediterranean) according to age range (n=19).

Table 3. Average and standard deviations, frequency, minimum and maximum values of testosterone levels in crossed buffaloes (Murrah x Mediterranean) according to age range, in the period from May to November 2010. (n=19).

Age range	Ages (months)	Frequency	Testosterone ±SD ng/ml	Minimum	Maximum
12	12 and 13	6	0.070±0.026[c]	0.020	0.173
14	14 and 15	23	0.111±0.021[c]	0.020	0.347
16	16 and 17	31	0.073±0.013[c]	0.020	0.312
18	18 and 19	34	0.079±0.013[c]	0.020	0.356
20	20 and 21	18	0.125±0.024[c]	0.020	0.383
22	22 and 23	5	0.124±0.049[c]	0.020	0.277
24	24 to 29	22	0.155±0.025[c]	0.020	0.509
30	30 to 35	33	0.700±0.155[b]	0.020	3.330
36	36 to 41	12	0.466±0.175[a]	0.020	2.120
42	42 to 47	14	0.477±0.134[c]	0.020	1.660
48	48 to 59	12	0.542±0.215[c]	0.092	2.440
60	≥ 60	17	2.762±0.457[b]	0.058	5.730

[a,b]Average data followed by the same letter in the column do not differ statistically, according to the Duncan test (P<0.05).

The data demonstrate a positive association between testosterone concentration and age, confirming that as the scrotal circumference increases, the secretion of this hormone rises, a finding also reported by Ohashi et al. (2007).

In addition, the highest testosterone level found in this study was 2.762±0.457 ng/ml. According to Chacur (1999), this level can particularly influence the reproductive performance of buffaloes in the tropics, since the testosterone concentration of buffaloes is lower than in other domesticated animals.

The minimum serum testosterone considered for the onset of puberty in *Bos indicus* is 1 ng / ml testosterone (Evans et al. 1996).

CONCLUSIONS

There is an increase in scrotal circumference, testicular volume, pixel intensity and testosterone concentration with increasing age of the animals.

The analysis of testosterone levels associated with observation of changes in the testicular echotexture of buffaloes is effective in determining the pre-puberty, puberty and sexual maturity.

Ultrasonography showed to be an important tool to predict changes in the testicular parenchy- ma and whenever possible, should be used along with breeding soundness examination(BSE).

It is possible thattheextensive management conditions and feedinghaveinfluenced someparametersrelated toscrotal circumferenceand circulating-testosterone levelsin this study.

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