Revista Brasileira de Zootecnia
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ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.41, n.8, p.1961-1965, 2012

Short Communication

Testicular echotexture and seminal quality of young Montana Tropical Compound bulls classified as sound and unsound for breeding

Rogério Oliveira Pinho[1], Deiler Sampaio Costa[2], Jeanne Broch Siqueira[3], Alberto Yukio Chaya[1], Tamires Miranda Neto[4], Leonardo Franco Martins[5], Simone Eliza Facioni Guimarães[1], José Domingos Guimarães[1]

[1] Universidade Federal de Viçosa, PH Rolfs Avenue, s/n, Viçosa - MG, Brazil.
[2] Universidade Federal de Mato Grosso do Sul, Campo Grande - MS, Brazil.
[3] Universidade Federal de Espírito Santo, Alegre - ES, Brazil.
[4] Agro-Pecuária CFM Ltda.
[5] Faculdade Anhanguera Educacional, Dourados - MS, Brazil.

ABSTRACT - The objective of this study was to investigate the relationship between testicular echotexture and seminal quality, in addition to evaluate the testicular parenchyma echogenicity pattern in young Montana bulls classified as sound and unsound for breeding. Fifty-two young Montana Tropical Compound bulls from 22 to 33 months of age were used. The animals were previously evaluated by breeding soundness evaluation and thereafter divided into two groups of breeding soundness classes: 1 = 16 animals sound for breeding; 2 = 36 animals unsound for breeding by means of physical and morphological analysis of semen. All animals underwent an ultrasound examination of the testes, and the images were analyzed with the software "Image J". ANOVA was used for statistical analysis, to determine the effect of groups in relation to testicular measurements, physical and morphological semen features and ultrasound pixel analysis. There was a difference between scrotal circumference between classes, with 39.7±2.1 cm and 37.3±3.1 cm for sound and unsound animals, respectively. Regarding testicular echotexture, mean values for the pixel intensity were 95.7 and 94.0 for sound and unsound animals for breeding, respectively, with no difference between the classes. None of the studied parameters were correlated with each other, indicating that the quantification of the pixel intensity in testicular echotexture was not effective in determining the degree of sexual maturity in Montana Tropical Compound bulls.

Key Words: breeding soundness evaluation, bulls, reproductive physiology, testicular ultrassonography

Introduction

Ultrassonography allows the assessment of reproductive tract in a noninvasive manner and can be used routinely (Abdel-Razek & Ali, 2005), and the ultrasound image depends on the relative density of tissues to be examined. So, during the period of sexual maturity, the cellular content and changes in the secretion of liquids on the genital organs aid the identification of development changes that could occur in these organs (Aravindakshan et al., 2000; Abdel-Razek & Ali, 2005).

However, the establishment of normal ultrasound parameters for testicular dimensions and characterization of normal testicular images become necessary to permit more detailed studies related to degenerative and pathological conditions of the testes of bulls, in addition to early detection of testicular changes and early disposal of these animals for breeding (Cardilli et al., 2010).

Based on this knowledge, Hamm & Fobbe (1994) and Chandolia et al. (1997) observed an increase in the gray scale of the testes in different stages of the sexual maturity, requiring the standardization of the gray scale at different ages, so that a precise diagnosis of puberty, sexual maturity or even of morphofunctional activity of the gonads can be possible.

Concerning the Montana Tropical Compound breed, it is comprised of 4 biological types (NABC), according to similarities of type, function, physiology, growth and breeding features. The N group is comprise of *Bos taurus indicus* (Nellore, Guzerat, Gir) animals; the A group, of animals from the *Bos taurus taurus* breed adapted to the tropics (Bonsmara, Caracu and Senepol); the B group is composed of *Bos taurus taurus* animals of British origin (Aberdeen Angus, Hereford and Red Angus), and the C group includes animals such as *Bos taurus taurus* from Continental Europe (Charolais, Limousin and Simental) (Ferraz et al., 1999a; Ferraz et al., 1999b).

Received March 1, 2011 and accepted May 4, 2012.
Corresponding author: rogerio_op@yahoo.com.br

Thus, the objective of this study was to investigate the relationship between testicular echotexture with seminal quality, in addition to evaluate the testicular parenchyma echogenicity pattern in young Montana Tropical Compound bulls classified as sound or unsound for breeding.

Material and Methods

Fifty two young Montana Tropical Compound bulls were used. Bulls had blood degree predominantly from group A, related to the biological types, aged between 22 and 33 months of age. The animals were evaluated at the end of the winter, raised in a herd in the state of São Paulo – Brazil, at latitude between 20º and 21º South and longitude between 55º and 51º West, with an average temperature of 24 ºC during the period of the experiment, and annual precipitation of 1,189 mm^3.

The animals were raised extensively, on predominant *Brachiaria decumbens* pasture, up to 14 months of age, and then confined and fed corn silage, mineral salt and water *ad libitum* until the time of breeding soundness evaluation.

After individual confinement of animals in appropriate cattle crushes, body weight measurement using electronic scales and determination of testis measurements was performed, composed of the scrotal circumference (SC), testicular length and width.

The ratio between width and length was calculated to determinate the testes shape, considering the average of the width and length values obtained from two measurements done in each animal. Five different types were defined, as in the study of Bailey et al. (1996), in which: ratio lower or equal to 0.5 = long testis; between 0.51 and 0.625 = long/moderate testis; between 0.626 and 0.75 = long/oval testis; between 0.751 and 0.875 = oval/spherical testis; higher than 0.875 = spherical testis.

The formula of the cylinder volume recommended by Fields et al. (1979) was adopted for the calculation of the total testicular volume (TV): $TV = 2 [(r^2) \times \pi \times h]$, in which r = radius calculated from the average of the testicular widths (L/2); $\pi = 3.141592654$; and h = average of length or testicular heights, for testes classified as long, long-moderate and long-oval, which was the case of the present study.

The semen was collected through the electroejaculation method and for the semen physical evaluation, mass motility (spermatic movement in mass in a scale from 0 to 5), rectilinear progressive spermatic motility (%) and spermatic vigor, 0 – 5 (CBRA, 1998) were measured.

The morphological analysis of the spermatozoa was made through the humid preparation in a buffered formalin-saline solution (Hancoch, 1957), with the aid of a microscope of phase contrast at 1000x magnitue. After, 400 cells were counted, determining the normal spermatozoa percentage and anomalies of acrossome, head, intermediary piece and tail, as mentioned by Colégio Brasileiro de Reprodução Animal (CBRA, 1998) and classified as major, minor and total spermatic defects, as the criteria described by Blom (1973).

The classification recommended by CBRA (1998) was used to interpret the breeding soundness evaluations, with the breeding potential predicted through recorded values for the physical and morphological semen characteristics, as follows: minimum of 70% of progressive sperm motility, major sperm defects lower than 20% and total sperm defects lower than 30%. Additionally, the animals were allocated into two groups: 1 = 16 animals sound for breeding; and 2 = 36 animals unsound for breeding.

A Mindray brand equipment, model DP – 2200 VET (Brazil), used for the ultrasound evaluation, was coupled to a linear transducer of 7.5 MHz to get images from testicular parenchyma. The ultrasound images were obtained under the same conditions of image adjustment controls of the device as well as the technical evaluation, providing that only the testicular parenchyma were assessed, forming images in longitudinal plans, on the caudal face of the left and right testes.

All images obtained were transferred to a computer, and with software Image J (version 1.46, National Institutes of Health, USA), the image analysis was performed, capturing the average pixel intensity (PI) of each image in a pixel scale varying from 0 (anechoic) to 255 (hyperechoic). To evaluate the homogeneity of the testicular echotexture and the pixel representative area, each region of selected images was divided in 200 mm^2 squares.

For the statistical analyses, software SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1) was used. Descriptive analyses for means and standard deviation were performed for all variables under study. The Lilliefors test was used to verify the data normality and the variation homogeneity between the treatment groups was evaluated using the Cochran-Bartlett test.

ANOVA was used to analyze the effect of constituted groups related to testicular biometry, physical and morphological semen features, and intensity of the ultrasound resolution. The non-parametric analysis was used with Wilcoxon test for all characteristics that did not meet the ANOVA assumptions. Pearson Single Correlations were performed for testicular and seminal characteristics and ultrasound examination. For all statistical procedures, $\alpha = 0.05$.

Results

After semen analysis, bulls were classified as: 30.8% (16/52) sound for breeding and 69.2% (36/52) unsound for breeding.

There was no difference in body weight between sound and unsound animals (P>0.05). However, standard error and testicular volume were higher (P<0.05) in sound animals (39.7 cm and 1315.0 cm^3, respectively), compared with unsound cattle (37.2 cm and 990.5 cm^3, respectively) (Table 1).

There was no difference in pixel intensity (PI) in the studied testicular images (P>0.05) between sound and unsound animals (Table 2), but there was a difference between left and right testes within the group of unsound animals (P<0.05).

With respect to correlations performed, there was no correlation of PI with the studied characteristics (P>0.05).

Discussion

The average scrotal circumference (SC) of sound and unsound animals was 39.7 and 37.2 cm, respectively (Table 1). In the study of Miranda Neto (2001), animals with a mean age of 17.5 months showed no tendency to stabilize SC, which was confirmed in routine breeding soundness evaluation of bulls that at 24 months had an average SC of 37.5 cm. Fernandes Junior & Franceschini (2007) obtained a lower SC mean, with 35.4 cm, but with animals at 22 months of age.

In relation to testicular volume, Fernandes Junior & Franceschini (2007) recorded an average of 780.95 cm^3 in Montana bulls at 22 months of age. The present study presented higher averages, but in older animals (between 22 and 33 months of age), with 1,315.0 and 990.5 cm^3 for sound and unsound animals, respectively.

The differences observed (P<0.05) in the physical and morphological semen characteristics (Table 1) were already expected, since sperm analysis was the criterion used for the breeding soundness classification. Nonetheless, Pinho et al. (2009), using the same criteria in this study, did not observe any differences between physical semen features for sound and unsound animals for breeding, observing difference only in the percentage of major defects (16.7 and 45.7%, respectively) and total defects (20.2 and 51.9%, respectively). Fernandes Junior & Franceschini (2007) observed percentages of major defects, minor and total defects of 14.5, 5.2 and 19.7%, for sound bulls, and 43.4, 7.6 and 51.0% for unsound bulls, respectively.

Regarding testicular echotexture (Table 2), there is no further literature referring to Montana Tropical Compound animals. There was no difference in PI between the testes, or between classes of bulls suitable for breeding (P>0.05), corroborating the studies of Aravindakshan et al. (2000) and Cardilli et al. (2009b), who did not report any difference in PI for the left and right testes. The difference observed between testis echotexture in unsound bulls is probably due to testicular asymmetry cases, which may have interfered with the pixel intensity.

According to Abdel-Razek & Ali (2005), the testicular parenchyma of adult Taurus animals is homogeneous and with moderate echogenicity, while testicular parenchyma in young Nelore bulls, according to Cardilli et al. (2009a; 2010), is homogeneous and with low echogenicity. Corroborating the results of Abdel-Razek & Ali (2005), the average PI of the animals used in this study presented moderate echogenicity (95.7 and 94.0 for sound and unsound animals, respectively) in relation to the scale used (0 – 255).

Similar PI results were observed by Cardilli et al. (2009b), who recorded 45.2% (equivalent to a 115.3 PI scale used in this study) of PI in young 18-month-old Nellore bulls, considered sexual mature. Carmo (2008), in Guzerat bulls, observed PI of 102.3±46.9 (animals aged from 21 to 24 months), 112.0±35.3 (animals aged from 24.1 to 27 months) and 127.5±46.2 (animals aged from 27.1 to 30 months), results similar to this study.

Table 1 - Body weight, scrotal circumference (SC), testicular volume (TV) and physical and morphological semen features of young Montana Tropical Compound bulls, classified as sound and unsound for breeding

Characteristics	Sound	SE	Unsound	SE	P value
Body weight (kg)	643.5	14.4	620.3	9.6	0.153
SC (cm)	39.7	0.6	37.2	0.4	0.089
TV (cm3)	1315.0	56.6	990.5	37.8	0.002
MOT (%)	73.1	9.5	53.0	6.3	0.000
VIG (0-5)	3.3	0.1	2.5	0.1	0.000
MAJDEF (%)	13.9	9.6	61.7	6.4	0.000
MINDEF (%)	4.8	9.6	11.1	6.4	0.001
TDEF (%)	18.7	9.6	72.8	6.4	0.000

SE - standard error; MOT - rectilinear progressive sperm motility; VIG - sperm strength; MAJDEF - % of major defects; MINDEF - % of minor defects; TDEF - % of total defects.

Table 2 - Mean value of pixel intensity of the testes of young Montana Tropical Compound bulls, classified as sound and unsound for breeding

Characteristics	Sound	SE	Unsound	SE	P value
LT	97.0	3.5	97.6	2.3	****
RT	94.3	4.2	90.3	2.8	****
LRT	95.7	3.2	94.0	2.2	0.016

SE - standard error; LT - left testis; RT - right testis; LRT - average between left and right testes.

Higher PI results were reported by Silva et al. (1997), who recorded a PI average of 163.7 in pubertal Nellore bulls at 15.6 months of age. Brito et al. (2002) studied animals with ages varying from 18 to 184 months, recording average PI of 196.1 for crossbred bulls, 192.7 for *Bos taurus indicus*, and 190.7 for *Bos taurus taurus*, and the other bulls with less than 36 months of age presented PI average of 188.6.

Contrarily to this study, in which no significant correlations of PI with the testicular biometry features were observed, Cartee et al. (1989) recorded correlation of PI with testicular volume. Cardilli et al. (2009b), also recorded high and positive correlations of PI with testicular volume (r = 0.77) and SC (r = 0.83), in which it is expected that the testicular echogenicity increases in direct proportion with the increase of SC and also with the testicular volume (Hamm & Fobbe, 1994; Chandolia et al., 1997).

This fact was confirmed by Carmo (2008), in which correlation between SC and Pi was r = 0.94. However, the animals of this study, according to sperm morphology evaluation, were in the final puberty phase and initial phase of sexual maturity, not showing high echogenicity in relation to biometric parameters, because, according to Aravindakshan et al. (1999), they do not possess a so evident increase of the volume of the seminiferous tubules.

There was no relationship between PI with sperm abnormalities in this study, since there was no difference between sound and unsound animals regarding PI. Kastelic et al. (1997), studying Nellore, Canchim and crossbred bulls observed the relationship of the testicular echotexture with the percentage of sperm abnormalities, and the white intensity of the ultrasonographic image presented a negative regression curve with the percentage of major defects and total defects, showing that the higher the testicular echotexture, the lower the percentage of sperm abnormalities.

Gabor et al. (1998) determined PI in Holstein bulls and observed average and negative correlation (r = -0.48) with the proportion of live spermatozoa. However, Brito et al. (2003), working with animals evaluated in the sperm recovery course after scrotal insulation, found positive correlation of PI with the percentage of major defects in *Bos taurus taurus*, and the white intensity of the ultrasound image presented association with the semen quality (r = -0.32) in relation to normal spermatozoa.

Thus, according to the results of this study, testicular echotexture was not capable of predicting the sperm quality, given the lack of correlation with PI and biometric parameters of the testis and physical and morphological semen evaluation, unlike the other authors mentioned (Cartee et al., 1988; Gabor et al., 1998; Brito et al. 2003; Cardilli et al., 2009b; Carmo, 2008).

Conclusions

The quantification of the pixel intensity only through ultrasound evaluation of testes is not effective at the determination of the degree of sexual maturity of Montana Tropical Compound bulls.

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