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# Correlation of Sexual Maturity Stage with Testicular Echotexture in Young Nellore Bulls

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**ABSTRACT**

***Background***: Ultrasound images depend on the relative density of the tissues to be examined. During the period of sexual maturity, the cellular content and changes in the secretion of liquids from the genital organs assist in the identification of development changes. However, the establishment of normal ultrasound parameters for testicular dimensions and the characterization of normal testicular images are necessary to permit more detailed studies related to degenerative and pathological conditions of bovine testes. The objective of this study was to evaluate the pattern of echogenicity of the testicular parenchyma in young Nellore bulls at different stages of sexual maturity.

***Materials, Methods & Results***: The sample consisted of 405 young Nellore bulls between 21 and 33 months of age. All of the animals were evaluated for breeding soundness examination and ultrasound examination of the testes. All of the images were transferred to the computer with the help of Image J software for further analysis. To correlate the several intensities of pixel resolutions with sexual maturity stage and to study the pattern of testicular parenchyma echogenicity of the animals, the bulls were subdivided in three age classes: 1) 21 to 25.9 months, 2) 26 to 29.9 months, and 3) 30 to 33 months. Additionally, according to the physical and morphological semen features, the animals were classified into the following five breeding soundness classes: 1= animals sound for breeding; 2= animals sound for breeding in a natural mating system; 3= animals temporarily unsound for breeding; 4= animals excluded due to imperfect spermiogenesis; 5= animals excluded on the basis of genital morphological abnormalities. SAEG software version 9.1 was used to perform the statistical analyses. There was no difference between the mean pixel intensity values (103.8, 105.1, 103.9 and 102.0 for classes 1, 2, 3 and 4, respectively) or in relation to the age within the same breeding soundness class ($P > 0.05$). There were no differences in the average pixel intensity values between the breeding soundness classes or age classes ($P > 0.05$). These results indicate that the breeding ability and age were not reflected by the testicular echotexture in the group of evaluated animals. There was a slight correlation between the PI of the testicular regions with the SC ($r = 0.11$, 0.12 and 0.13 for the LT, RT and LRT, respectively) and the TV ($r = 0.11$, 0.12 and 0.13 for the LT, RT and LRT, respectively). No other correlations were found between the studied characteristics and the PI values of the studied images ($P > 0.05$).

***Discussion***: In the current study, the animals were in the growth phase near or at the end phase of the establishment of sexual maturity, and the volume of the seminiferous tubules would have already increased. The structure of the seminiferous epithelium most likely did not differ between the breeding soundness classes. Instead, the different classes are due to the spermiogenesis process or spermatic cell metamorphosis. Therefore, the testicular echotexture would not be expected to show a difference among the breeding soundness classes. There was a low correlation between the scrotal circumference and testicular volume and the pixel intensity of the testicular images. Therefore, these results indicate that testicular echotexture evaluation does not allow the prediction of the sexual maturity stage in young bulls.

**Keywords:** breeding soundness evaluation, ultrasonography, zebu bulls.

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## INTRODUCTION

Ultrasound images depend on the relative density of the tissues to be examined. During the period of sexual maturity, the cellular content and changes in the secretion of liquids from the genital organs assist in the identification of development changes [1,2]. However, the establishment of normal ultrasound parameters for testicular dimensions and the characterization of normal testicular images are necessary to permit more detailed studies related to degenerative and pathological conditions of bovine testes [6,11].

Based on this knowledge it was 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 [8].

The purpose of this paper was to study the pattern of echogenicity of the testicular parenchyma in young bulls at different stages of sexual maturity and correlate this pattern with the physical and morphological characteristics of semen.

## MATERIALS AND METHODS

### *Local, animals and diets*

The animals used for this study were 405 young Nellore bulls between 21 and 33 months of age, from a herd raised in São Paulo State (latitude 20-21º South and longitude 50-51º West). These animals were pastured on grassland consisting primarily of *Brachiaria decumbens*, and after 18 months of age, they were confined and fed corn silage, mineral salt and water *ad libitum* until the breeding soundness examination.

To correlate the several intensities of pixel resolutions with sexual maturity stage and to study the pattern of testicular parenchyma echogenicity of the animals, the bulls were subdivided in three age classes: 1) 21 to 25.9 months, 2) 26 to 29.9 months, and 3) 30 to 33 months.

### *Seminal and testicular measurements*

Semen was collected using electroejaculation for physical and morphological evaluation according to the methods of the Brazilian College of Animal Reproduction [9]. Scrotal circumference (SC) was assessed with a tape measure, and testicular volume (TV) was calculated [3]. Additionally, according to semen features, the animals were classified into one of five groups described by Siqueira *et al*. [13] based on the spermatogenesis process, the pathophysiology of reproductive organs, and the physical and morphological sperm features: 1= animals sound for breeding according to the Brazilian College of Animal Reproduction standards; 2= animals with indices of sperm pathologies that do not harm the fecundation capacity of the ejaculate and are classified as sound for reproduction under natural mating conditions; 3= animals temporarily unsound for breeding; 4= animals culled because of ineffective spermatogenesis; and 5= animals culled because of morphological alterations of the reproductive organs, such as testicular asymmetry, papillomatosis, and seminal vesicle inflammation.

### *Ultrassonography*

The Mindray branded equipment[1] that was used for the ultrasound evaluation was coupled to a linear 7.5 MHz transducer to obtain longitudinal images from the testicular parenchyma of the caudal face of the left and right testes. All of the obtained images were transferred to a computer and analysed using the software "Image J" (National Institutes of Health, USA) which captured the average pixel intensity (PI) of each image of the testicular regions on a pixel scale varying from 0 (anechoic) to 255 (hyperechoic). To evaluate the homogeneity of the testicular echogenicity and the representative pixel area, each region of the selected images was divided into squares with areas of 20, 40, 80, 120, 160 and 200 $mm^2$.

### *Statistical analysis*

SAEG software version 9.1 [12] was used to perform the statistical analyses. Descriptive analyses (averages and standard deviations) were performed for all variables. An analysis of variance (ANOVA) was used to analyse the effect of groups on all studied features. Non-parametric tests (Kruskal-Wallis test, 5%) were used for all of the features that did not meet the requirements for ANOVA. In addition, Pearson's single correlations were performed for the testicular and semen features and the ultrasound evaluation.

## RESULTS

The distribution of the bulls into the breeding soundness classes were as follows: 56.1% (227/405) for class 1, 12.6% (51/405) for class 2, 22.2% (90/405) for class 3, 4.9% (20/405) for class 4, and 4.2% (17/405) for class 5. A total of 68.7% of the animals were classified as sound for breeding (classes 1 and 2), and 31.3% of the animals were classified as unsound for breeding.

Age class 1 (21 to 25.9 months of age) consisted of 59.7% (242/405) of the animals, and 67% of them were considered sound for breeding (breeding soundness classes 1 and 2). Age class 2 (26 to 29.9 months of age) consisted of 25.7% (104/405) of the animals, and 74% of these animals were considered sound for breeding. Age class 3 (30 to 33 months of age) consisted of 14.6% (59/405) of the animals, and 66.1% of these animals were classified as sound for breeding.

The testicular biometrics and body weight of the animals are described according to the breeding soundness classes and the age classes (Table 1). The animals of breeding soundness class 5 had higher average SC and TV values (36.4 cm and 1098.6 cm3) than the animals in class 1 (34.5 cm and 952.4 cm3) and class 3 (34.2 cm and 918.7 cm3). The SC and TV values did not differ among the other classes studied ($P > 0.05$).

**Table 1.** Body weight, scrotal circumference (SC), testicular volume (TV) and seminal features of young Nelore bulls, aged from 21 to 33 months, in accordance with the breeding soundness classes.

| Features | Breeding soundness classes | | | | | General average |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| Weight (Kg)* | 471.9 ±_72.3$^{a}$ | 487.0 ± 35.8$^{a}$ | 476.6 ± 34.3$^{a}$ | 486.1 ± 29.2$^{a}$ | 490.6 ± 39.4$^{a}$ | 476.3 ± 59.1 |
| SC (cm) | 34.5 ± 2.2$^{b}$ | 34.9 ± 2.2$^{ab}$ | 34.2 ± 2.7$^{b}$ | 35.7 ± 2.5$^{ab}$ | 36.4 ± 3.4$^{a}$ | 34.6 ± 2.4 |
| TV ($cm^3$) | 952.4 ± 190.9$^{b}$ | 958.5 ± 184.3$^{ab}$ | 918.7 ± 227.3$^{b}$ | 1037.4 ± 215.1$^{ab}$ | 1098.6 ± 303.0$^{a}$ | 955.9 ± 208.2 |
| MOT (%) | 71.5 ± 9.9$^{b}$ | 69.8 ± 9.8$^{ac}$ | 62.1 ± 17.9$^{a}$ | 48.0 ± 27.5$^{ad}$ | 62.3 ± 21.5$^{a}$ | 67.7 ± 15.0 |
| VIG (0-5) | 3.2 ± 0.5$^{b}$ | 3.1 ± 0.5$^{ac}$ | 2.8 ± 0.7$^{a}$ | 2.2 ± 1.1$^{ad}$ | 3.0 ± 0.8$^{a}$ | 3.1 ± 0.6 |
| MAJDEF (%) | 11.8 ± 4.3$^{bc}$ | 19.7 ± 4.9$^{b}$ | 37.6 ± 18.8$^{a}$ | 52.7 ± 16.4$^{a}$ | 23.1 ± 16.9$^{b}$ | 20.8 ± 16.4 |
| MINDEF (%) | 4.2 ± 2.3$^{b}$ | 6.5 ± 4.8$^{a}$ | 8.0 ± 6.8$^{a}$ | 7.4 ± 5.7$^{a}$ | 5.2 ± 2.9$^{a}$ | 5.5 ± 4.5 |
| TDEF (%) | 16.0 ± 5.3$^{b}$ | 26.2 ± 5.1$^{b}$ | 45.6 ± 19.6$^{a}$ | 60.2 ± 17.9$^{a}$ | 28.2 ± 18.5$^{b}$ | 26.3 ± 18.0 |

Breeding soundness classes: 1) sound for breeding; 2) sound for breeding under natural crossbred regime; 3) temporarily unsound for breeding; 4) discarded in view of the imperfect spermiogenisis; and 5) discarded in view of the morphological amendments of the sex organs. $^{a,b}$Average values followed-up by different small letters in the same line differ among themselves through Tukey test ($P < 0.05$); *Non-parametric analysis through Kruskal-Wallis test ($P > 0.05$); MOT= Rectilinear progressive spermatic motility; VIG= Spermatic vigor; MAJDEF= % of major defects; MINDEF= % of minor defects; TDEF= % of total defects.

The physical quality of the semen was higher in breeding soundness class 1 than in the other classes ($P < 0.05$). The physical quality of the semen from class 2 was significantly higher than that of class 4 ($P < 0.05$), while the physical quality of the semen was equal between classes 3 and 5. The quality of the semen from classes 3 and 5 did not differ significantly from that of classes 2 and 4 ($P > 0.05$). There were fewer MINDEF in class 1 than in the other classes ($P < 0.05$), while there were no significant differences among the rest of the classes ($P > 0.05$). Classes 1 and 2 (sound animals) had significantly lower rates of MAJDEF and TDEF than classes 3 and 4 ($P < 0.05$) and were not significantly different from class 5 ($P > 0.05$). In terms of the MAJDEF and TDEF, the unsound animals (class 3) did not differ from the animals of class 4 ($P > 0.05$), although they had lower values than those of class 5 ($P < 0.05$). The animals of class 4 had higher MAJDEF and TDEF values than those of class 5 ($P < 0.05$).

To perform computational analysis of the ultrasonographic images of the testicular parenchyma, it is necessary to define an area that represents the entire image. Thus, the results of the representative pixel areas in these defined regions of the ultrasonographic images are described in Table 2.

Differences in the representative pixel areas of the pre-determined regions were observed between different testes and different pre-determined regions ($P < 0.05$). However, the testicular sizes and defined regions did not appear to correlate with the physical or morphological semen aspects or, consequently, with the classes of breeding soundness. Thus, we chose to use the average PI of the left and right testis and an area of 200 $mm^2$ in the analysis shown in tables 3 and 4, as this was a highly sampled area of the testicular parenchyma.

The animals of breeding soundness class 5 were excluded due to morphological abnormalities of the sexual organs, such as testicular asymmetry, papillomatosis, and seminal vesicle inflammation. Therefore, these animals were not used for the PI evaluations.

There were no differences in the average PI values between the breeding soundness classes (Table 3) or age classes (Table 4; $P > 0.05$). These results indicate that the breeding ability and age were not reflected by the testicular echotexture in the group of evaluated animals.

Pearson single correlations were performed for all features and for the PI of the left and right testes and the average between them. There was a slight correlation between the PI of the testicular regions with the SC (r = 0.11, 0.12 and 0.13 for the LT, RT and LRT, respectively) and the TV (r = 0.11, 0.12 and 0.13 for the LT, RT and LRT, respectively). No other correlations were found between the studied characteristics and the PI values of the studied images ($P > 0.05$).

**Table 2.** Pixel intensities according to representative pixel areas of the pre-determined testicular regions.

| Pre-determined region ($mm^2$) | LT | RT | LRT |
|---|---|---|---|
| 20 | $116.4 \pm 14.5^{aA}$ | $114.0 \pm 15.0^{bA}$ | $115.2 \pm 14.8^{A}$ |
| 40 | $115.0 \pm 13.6^{aAB}$ | $114.4 \pm 15.9^{aA}$ | $114.7 \pm 14.6^{A}$ |
| 80 | $112.5 \pm 14.9^{aBC}$ | $111.5 \pm 15.5^{aAB}$ | $112.0 \pm 15.2^{B}$ |
| 120 | $110.4 \pm 14.7^{aCD}$ | $109.2 \pm 15.5^{aB}$ | $109.8 \pm 15.1^{C}$ |
| 160 | $107.6 \pm 14.5^{aDE}$ | $105.4 \pm 15.6^{bC}$ | $106.5 \pm 15.1^{D}$ |
| 200 | $105.3 \pm 14.3^{aE}$ | $102.6 \pm 15.3^{bC}$ | $103.9 \pm 14.9^{E}$ |

LT= Left testis; RT= Right testis; LRT= Average between left and right testes. [a,b]Average values followed-up by different small letters in the same line differ among themselves through Tukey test ($P < 0.05$); [A,B]Average values of the same parameter followed-up by capital letters in the same column differ themselves through Tukey test ($P < 0.05$).

**Table 3.** Testes pixel intensities of young Nelore bulls, aged from 21 to 33 months, in accordance with breeding soundness classes.

| Features | Breeding soundness classes* | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| LT | 105.0 ± 14.1 | 106.7 ± 16.0 | 105.3 ± 14.7 | 104.2 ± 10.7 |
| RT | 102.6 ± 15.2 | 103.6 ± 14.7 | 102.6 ± 14.3 | 100.0 ± 14.2 |
| LRT | 103.8 ± 13.1 | 105.1 ± 12.5 | 103.9 ± 13.8 | 102.0 ± 9.9 |

Breeding soundness classes: 1) sound for breeding; 2) sound for breeding under natural crossbred regime; 3) temporarily unsound for breeding; and 4) discarded in view of the severe imperfect spermiogenisis. *ANOVA ($P > 0.05$); LT= Left testis; RT= Right testis; LRT= Average between left and right testes.

**Table 4.** Testes pixel intensities of young Nelore bulls, in accordance with the age classes.

| Features | Age classes* | | |
|---|---|---|---|
| | 1 (21 to 25.9 months) | 2 (26 to 29.9 months) | 3 (30 to 33 months) |
| LT | 106.4 ± 13.8 | 103.0 ± 15.1 | 104.5 ± 14.5 |
| RT | 102.9 ± 15.6 | 101.0 ± 15.0 | 103.8 ± 14.8 |
| LRT | 104.7 ± 13.1 | 102.0 ± 12.9 | 104.2 ± 12.8 |

LT= Left testis; RT= Right testis; LRT= Average between left and right testes. *ANOVA ($P > 0.05$).

## DISCUSSION

As shown in Table 1, the animals of breeding soundness class 5 had higher average SC and TV values than those of class 1. This difference is probably due to the low number of animals in this class (n = 17) and the subsequently reduced variation, because higher numbers of animals result in higher variation. Higher-than-average SC measurements were related to higher testicular volumes and weights and higher daily spermatic production. Low SC measurements have frequently been associated with small testes and have been related to infertility. However, bulls of the same age with below-average SC values and with long testes have higher testicular weights and volumes than bulls with oval-shaped testes, and these animals demonstrate normal spermatic production [3].

The differences ($P < 0.05$) in the physical and morphological semen aspects noted here were expected, as the spermatic morphology was the main criteria used for the breeding soundness classification, where bulls were classified as unsound only by spermogram analysis. This finding explains the low values of spermatic abnormalities observed in class 5 (Table 1).

Previous studies did not provide information regarding the homogeneity of the testicular echotexture and representative pixel areas (Table 2). These results are important for the standardization of the normal ultrasonographic parameters of the testicular dimensions and for the characterization of normal testicular images. These parameters are necessary to permit more detailed studies related to the degenerative and pathological conditions of bull testes [11]. Other authors also did not verify PI differences between the left and right testes [2,6].

The testicular parenchyma of adult taurine bulls is homogeneous and moderately echogenic [1], while the testicular parenchyma of young Nellore bulls is homogeneous and has low echogenicity [5,6]. However, the average PI of the animals in the current study (Table 3) was moderately echogenic (103.8, 105.1, 103.9 and 102.0 for classes 1, 2, 3 and 4, respectively), according to the scale used in this study (0 – 255).

In a recent study, it was observed an average PI of 115.3 (adjusted average to the program used in the current study and equivalent to 45.2%) in 18-month-old Nellore bulls considered sexually mature [6]. In Guzerá bulls, it were observed PI values of 102.3 ± 46.9 in animals between 21 and 24 months of age, 112.0 ± 35.3 in animals between 24.1 and 27 months of age and 127.5 ± 46.2 in animals from 27.1 to 30 months of age [7], values which are higher than those found in the current study (Table 4).

There was no correlation between age and PI in this study. However, testicular echogenicity was different between pubertal and prepubertal 15-month-old animals, where the echogenicity of pubertal bulls was higher than that of prepubertal animals, suggesting that ultrasonography could be used as an indicator of sexual precocity in young bulls [8]. Other authors [2] came to the same conclusion when they used ultrasound technology to study the testicular development of 20 taurine bulls.

In previous studies, the testicular echotexture was measured in premature animals, a factor which probably led to the inclusion of animals in pubertal and prepubertal phases and resulted in lower pixel intensity values.

Higher levels of testicular echogenicity were reported in pubertal bulls compared to prepubertal bulls and concluded that SC, body weight and age are better indicators of sexual maturity [4].

The differences in PI reported by various authors could be related to the nutritional status of the animals studied, as Cardilli *et al*. [6] found that naturally pastured animals had lower testicular echogenicity than bulls fed diets designed for weight gain. This result could be explained by the fact that the breeding organs of sexually mature males are more resistant than those of immature males to the influence of nutrition, and they are the primary group studied by the author most often cited.

The animals of this study were fed a diet designed to cause weight gain (1.0 to 1.2 kg/day) for at least 60 days before the breeding examination. However, both sound and unsound animals were given the same nutritional treatment. The results indicated that the measurement of the PI could not effectively predict the spermatogenesis activity in the animal classes studied here. This finding is probably due to the fact that all of the animals were at a similar stage of maturity (end of adolescence and initial phase of sexual maturity). High spermatogenesis activity was observed even in unsound animals, and there were few differences in spermatic concentration between sound and unsound bull.

In contrast to this study, where the PI was found to have a low correlation with the parameters of the testicular biometric studied (r = 0.11 to 0.13), it was recorded high positive correlations between PI and SC (r = 0.83) and between PI and TV (r = 0.77) [6]. In Guzerat bulls, the correlation between the SC and PI was r = 0.94, due to the fact that the animals studied were prepubertal and pubertal [7] and the testicular echogenicity is expected to increase in direct proportion with the increases in SC and testicular volume [8]. In the current study, the animals were in the growth phase near or at the end phase of the establishment of sexual maturity, and the volume of the seminiferous tubules would have already increased [2].

No relationship was observed between the PI and spermatic pathologies in this study, as there was no difference in the PI between sound and unsound animals. In Nellore, Canchim and crossbred bulls and observed a relationship between the testicular echotexture and the percentage of spermatic changes. Additionally, the white intensity of the ultrasonographic images presented a negative regression curve with MAJDEF and TDEF, demonstrating an inverse correlation between the testicular echotexture and the percentage of spermatic pathology [10]. However, the PI was positively correlated with the MAJDEF in *Bos taurus* taurus bulls and the white intensity of the ultrasound image was positively associated with the semen quality [4].

The structure of the seminiferous epithelium most likely did not differ between the breeding soundness classes. Instead, the different classes are due to the spermiogenesis process or spermatic cell metamorphosis. Therefore, the testicular echotexture would not be expected to show a difference among the breeding soundness classes.

## CONCLUSIONS

The testicular parenchyma of bulls between 21 and 33 months of age was evaluated to allow the classification of the animals as sound or unsound for breeding. The testicular parenchyma exhibited a homogeneous, moderately echogenic pattern. However, the evaluation of the testicular echotexture alone did not permit the prediction of the sexual maturity stage of young bulls.

SOURCE AND MANUFACTURER

[1]Model DP - 2200 VET, São Paulo, SP, Brazil.

***Ethical approval*.** The procedure was approved (number 36/2009) by the Animal Welfare Committee of Federal University of Viçosa (UFV), MG, Brazil.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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