



## Short communication

## Lack of relationship between testicular echotexture and breeding soundness evaluation in adult Nelore bulls



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## ABSTRACT

The objective of this study was to describe the pattern of testicular echogenicity and the degree of commitment caused by fibrotic lesions in semen quality in adult Nelore bulls. A total of 402 extensively raised adult Nelore bulls were evaluated for breeding soundness and submitted to ultrasound examinations of their testes. Additionally, a testicular fibrosis score ranging from 0 to 5 was assigned to each bull to quantify the patterns, sizes and frequencies of the fibrotic lesions. No correlation was detected among the studied semen features and the pixel intensity values of the studied images. The testicular fibrosis score was only slightly correlated with scrotal circumference, testicular volume, and animal age. The other examined features did not correlate with the studied ultrasound parameters ( $p > 0.05$ ). No correlation was detected between the fibrosis score and testicular sperm quality ( $p > 0.05$ ). Thus, the appearance of the testicular parenchyma of the adult bulls did not correlate with breeding ability. Moreover, the varying degrees of testicular fibrosis did not appear to affect sperm quality. As a result, ultrasonic evaluation of the testes is not a useful tool for evaluating breeding soundness in old Nelore bulls.

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### 1. Introduction

The evaluation of testicular echogenicity allows palpable and non-palpable injuries to be identified, thus facilitating a diagnosis of focal or diffuse injuries (Chapwanya et al., 2008; Gnemmi and Lefebvre, 2008). According to Barth et al. (2008), fibrotic lesions are commonly encountered in the testicular parenchyma; therefore, it is necessary to determine the prevalence of these injuries through the testicular ultrasonographic evaluation of bulls of different

ages to relate the ultrasound image information to semen quality.

To establish a reliable diagnosis and prognosis for testicular fibrosis, a detailed history, a general and breeding soundness clinical evaluation, an evaluation of the physical and morphological semen features, and an ultrasonographic evaluation are required. Furthermore, these evaluations should be performed over the course of several consecutive examinations to avoid culling bulls that may fail a single evaluation, as recovered bulls are able to regain their normal fertility (Van Camp, 1997).

The objective of this paper was to study the pattern of testicular echogenicity and the degree of commitment caused by fibrotic lesions in semen quality in extensively raised adult Nelore bulls.

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## 2. Material and methods

A total of 402 adult Nelore bulls aged between 2.8 and 11.1 years of age were used. The animals were selected from a herd raised in the state of Mato Grosso do Sul, Brazil, at latitudes between 20° and 21° south and longitudes between 55° and 56° west. The experimental site had an average temperature of 21 °C and annual precipitation between 1000 and 1400 mm<sup>3</sup>. The experimental animals were predominantly raised on *Brachiaria decumbens* pasture and provided with mineral salt and water ad libitum.

Semen was collected using electroejaculation for physical and morphological evaluation according to the methods of the Brazilian College of Animal Reproduction (CBRA, 1998). Scrotal circumference (SC) was assessed with a tape measure, and testicular volume (TV) was calculated according to the method described by Bailey et al. (1998). Additionally, according to semen features, the animals were classified into one of five groups described by Siqueira et al. (2012a,b) 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 CBRA standards (1998); 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.

The Mindray branded equipment (model DP-2200 VET, São Paulo, Brazil) 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 200-mm<sup>2</sup> squares.

A fibrosis evaluation, whereby the ultrasound probe was laterally positioned in the middle region of each testis, was performed, as was a complete 90° scan of the testicular parenchyma. Subsequently, a testicular fibrosis score ranging from 0 to 5 was assigned to quantify the patterns, dimensions, and frequencies of the fibrotic lesions, where 0=zero, 1=1–10, 2=11–30, 3=31–50, 4=51–100, and 5=> 100. To correlate the scores of testicular fibrosis points (STFP) with animal age, the animals were subdivided into three age classes: (1) aged 2.8–3.9 years, (2) aged 4.0–6.9 years, and (3) aged 7.0–11.1 years.

SAEG software version 9.1 (SAEG, 2007) 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 or Wilcoxon test, 5%) were used for all of the features that did not meet the requirements for ANOVA. Categorical data were analysed with the Chi-square test ( $\chi^2_{gl_1}=3.84$ ;  $p=0.05$ ). In addition, Pearson’s single correlations were performed for the testicular and semen features and the ultrasound evaluation.

## 3. Results

A total of 367 of the 402 evaluated animals (91.3%) were found to be sound for breeding, with 85.8% (345/402) of the evaluated animals assigned to class 1 and 5.5% (22/402) assigned to class 2. The remaining 8.7% of animals (35/402) were found to be unsound for breeding, with 4.8% (19/402) of animals assigned to class 3, 2.2% (9/402) to class 4, and 1.7% (7/402) to class 5.

No differences in the average values of SC ( $38.5 \pm 2.6$  cm) and TV ( $1321.2 \pm 245.8$  cm<sup>3</sup>,  $p > 0.05$ ) were observed among animals of the different classes. There were also no differences in physical semen features (rectilinear progressive sperm motility and sperm vigour) among animals of the different classes ( $p > 0.05$ ). Differences in the morphological features of semen were observed among animals of different classes ( $p < 0.05$ ). The animals in classes 1 and 5 had lower average numbers of major sperm defects ( $8.5 \pm 3.7$  and  $14.8 \pm 14.9\%$ , respectively) than the other classes. The animals from class 3 had more minor sperm defects ( $10.1 \pm 9.8\%$ ) than the other classes ( $p < 0.05$ ). The animals in classes 1 and 5 had had <20% of total sperm defects ( $12.1 \pm 4.8$  and  $17.6 \pm 16.8$ , respectively;  $p > 0.05$ ), while the animals from the other classes had higher values ( $21.8 \pm 2.9\%$ ,  $39.9 \pm 12.3\%$ , and  $54.7 \pm 17.8\%$  for classes 2, 3 and 4, respectively).

No differences in average PI values were observed between the left and right testes or between animals of different breeding soundness classes ( $p > 0.05$ ). No differences in PI values were observed among the breeding soundness classes ( $91.6 \pm 14.7$ ,  $90.6 \pm 20.0$ ,  $89.9 \pm 18.8$ , and  $95.8 \pm 18.2$  for the classes 1, 2, 3 and 4, respectively;  $p > 0.05$ ).

The number of animals in each category of testicular fibrosis point score was determined, and the age classes and the fibrosis score of both the left and right testis were considered. No differences in STFP were observed between the left and right testes within each age class ( $\chi^2_{gl_1}=3.84$ ,  $p=0.05$ ).

A greater number of animals with zero fibrosis points in the testicular parenchyma were observed in age class 1 compared to the other age classes (45.0 vs 34.2 and 13.8,  $p < 0.05$ ). More animals with a fibrosis score of 1 were in age class 2 compared to age class 1 (24.5 vs 15.4); similar numbers of animals with a fibrosis score of 1 were observed in age class 1 and age class 3 ( $p < 0.05$ ). Fewer animals with a fibrosis score of 4 were in age class 1 compared to other classes (2.2 vs. 9.8 and 17.2%,  $p < 0.05$ ); for animals with other fibrosis scores (2, 3 and 5), there were no differences among the age classes ( $p > 0.05$ ).

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