Relationships between pixel intensity of testicular ultrasonograms and sperm morphology in Nelore and Canchim bulls

(Relações entre a intensidade da ultra-sonografia testicular e morfologia espermática em touros das raças Nelore e Canchim)

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RESUMO - Touros adultos de duas fazendas (Fazenda A: 52 da raça Nelore e Fazenda B: 53 da raça Canchim) foram submetidos ao exame andrológico completo. A fim de complementar a avaliação andrológica foi realizado a ultrassonografia testicular, analisando através de computador, a imagem das áreas de intensidade (Pixel intensity), determinando a consistência testicular. Foi realizado a análise regressão linear para os defeitos maiores, menores e totais como variáveis dependentes não havendo diferenças na fazenda A. A variável independente idade foi significante (P<0,05) para a consistência testicular (Plm). Na fazenda B, os defeitos maiores e totais como variável dependente foram similares. Para os defeitos maiores e totais e a média da consistência testicular (Plm) e circunferência escrotal, como variáveis independentes, foram significantes (P<0,05). Por isso o aumento na média da consistência, intensidade de Plm dos testículos, foi um indicativo de alta porcentagem de espermatozóides anormais. Em conclusão, a ultrassonografia, bem como a determinação da consistência (intensidade de Plm) testicular, pode ser considerada potencialmente promissora, no auxílio da avaliação da capacidade reprodutiva de touros zebuínos.

Palavras chave: Ultrassonografia, testículos, exame andrológico, touros zebuinos.

SUMMARY: Breeding soundness evaluations were conducted on bulls at two fazendas (Fazenda A; 52 Nelore bulls; Fazenda B, 53 crossbred Zebu bulls). In addition, testicular ultrasonography and computerized image analysis of pixel intensity (echotexture) was also done. For Fazenda A, linear regression analyses for major sperm defects and for total (major and minor combined) sperm defects (as dependent variables) had similar results; the significant independent variables were age (with a positive slope) and the standard deviation of pixel intensity (positive slope). For Fazenda B, major and total sperm defects (as dependent variables) were similar; for both, mean testicular pixel intensity (positive slope) and scrotal circumference (negative slope) were significant independent variables. Therefore, increases in the mean or standard deviation of pixel intensity were predictive of a higher percentage of morphologically abnormal spermatozoa. Testicular ultrasonography and determination of pixel intensity have considerable potential in the breeding soundness evaluation of Zebu bulls.

Key words: Ultrasonography, testes, breeding soundness evaluation, Zebu bulls

INTRODUCTION - Ultrasonography is a noninvasive method for evaluating bull testes (Pechman and Eilts, 1987). In one study (Eilts and Pechman, 1988), visual assessment of the ultrasonographic examination of bull testes did not appear to enhance a standard breeding soundness evaluation. However, computerized image analysis to determine pixel intensity (ultrasonic echotexture) provides much more detailed information (Pierson et al., 1995). The principal objective of the present study was to determine the relationships between semen quality and pixel intensity of testicular ultrasonograms.

MATERIALS AND METHODS - Standard breeding soundness evaluations were conducted on bulls at two fazendas, including measurement of scrotal circumference (SC) and collection of semen by electroejaculation. Fazenda A had 52 Nelore bulls and Fazenda B had 34 Canchim and 19 MA (approximately 5/8 Charolais and 3/8 Zebu) bulls. A semen sample was preserved in formol-saline, a wet-mount was prepared and 100 spermatozoa were assessed with phase-contrast microscopy (1200x). Sperm abnormalities were recorded as major or minor, as previously described (Fonseca et al., 1992). For bulls on Fazenda A only, libido testing was done as described (Pineda et al., 1997).

A B-mode diagnostic ultrasound scanner with a 7.5 MHz transrectal transducer was used for ultrasonographic examination of the testes. Each testis was examined separately, with the transducer oriented vertically (parallel to the long axis of the testis). An ultrasound image (ultrasonogram) of each testis was frozen and subsequently recorded on a VHS recorder. The ultrasonograms were subsequently digitized and pixel intensity (scale of 0 to 255) was determined with image analysis software (National Institutes of Health, U.S.A.). For each bull, the average and the standard deviation of pixel intensity was determined separately for the two testes; the means for the average (MPI) and for the standard deviation (SDPI) were calculated and used for statistical analyses.

Statistical analyses were done separately for each of the two fazendas. Initially, correlations between minor, major and total sperm abnormalities, age (in months), SC, MPI and SDPI were determined. In addition, the correlations between libido score and major, minor and total defects, MPI, SDPI and SC were also determined. Multiple linear regressions were done for major, minor and total defects as dependent variables. The parameters eligible for independent variables were age, SC, MPI, SDPI and libido score; only independent variables that were correlated (P<0.10) with the dependent variable were used. Once the independent variables were chosen, the correlation between all possible pairs of independent variables was determined; if they were significantly correlated, the independent variable most highly correlated with the dependent variable was used.

RESULTS AND DISCUSSION - Data for age, SC, MPI, SDPI and percentage of defective spermatozoa are shown in Table 1. Although identical procedures were used for collection and evaluation of the ultrasound images from the two fazendas, the MPI for Fazenda A (Nelore bulls) was higher than for Fazenda B (crossbred bulls; 185 versus 153, respectively). Consistent with this difference, the minimum and maximum MPI for Fazenda A were each 30 units higher than for Fazenda B. This difference was unexpected and the reason is unknown. Overall, the proportion of defective spermatozoa seemed considerably lower in the Nelore bulls compared to the crossbred bulls, despite similarities in age and SC.

Table 1. Age (months), scrotal circumference (SC, cm), mean and standard deviation of pixel intensity (MMPI and SDPI), and percentage of major, minor and total sperm defects.

	Fazenda A			Fazenda B		
Parameter	Mean	Sd	Range	Mean	Sd	Range
Age	28.8	6.2	23-51	24.8	7.8	19-53
SC	31.0	2.5	27-38	32.5	2.5	28-40
MPI	185	12.5	151-220	153	14.4	121-190
SDPI	25.8	2.2	21-34	28.8	2.3	24-34
Major	6.2	2.9	1-13	16.5	13.0	3-68
Minor	1.9	1.8	0-9	10.2	4.3	3-20
Total	8.0	4.0	2-16	26.7	12.4	9-76

Correlation coefficients are shown in Table 2. There were many significant correlations for major and total sperm defects, but only one significant correlation for minor sperm defects. The average libido score was 8.2±2.0. Correlations between libido score and sperm defects, MPI, SDPI and SC were low (P>0.15), consistent with a previous study (Pineda and Lemos, 1994).

Table 2. Correlation coefficients for age, scrotal circumference (SC) mean and standard deviation of pixel intensity (MPI e SDPI) with the percentage of major, minor and total sperm defects.

	Fazenda A			Fazenda B		
Parameter	Major	Minor	Total	Major	Minor	Total
Age	.37[c]	.10	.32[b]	-.04	-.30[b]	-.14
SC	.27[b]	-.04	.18	-.21	-.15	-.27[b]
MPI	.03	-.04	-.01	.33[c]	-.15	.30[b]
SDPI	.23[a]	.12	.22[a]	.28[b]	-.02	.28[b]

[a] p<0.1; [b] p<0.05; [c] p<0.01

The results of linear regression analyses are summarized in Table 3. For Fazenda A, regressions for major and total defects were similar; both age and SDPI were significant independent variables and the slopes of each were positive. The positive slope for age was unexpected; in general, the number of abnormal spermatozoa declines with age (concurrent with an increase in SC), as previously described (Smith et al., 1981). However, in the present study, the percentage of abnormal spermatozoa was relatively low and some older bulls with a slightly higher percentage of defective spermatozoa were responsible for this outcome.

Table 3. Regression models for porcentage of major, minor and total sperm defects as dependent variables with age (months) mean and stadard deviation of pixel intensity (MPI and SDPI) and scrotal circumference (SC) as independent variables.

Dependent / Independent variables	Slope	Prob.
Fazenda A		
Major defects Model R^2 = 0.19; y-int.= -1.9)		
Age	3.3	.002
SDPI	2.3	.03
Total defects (Model R^2 = 0.14; y-int. = -1.7)		
Age	2.7	.01
SDPI	2.1	.04
Fazenda B		
Major defects (Model R^2 = 0.13; y-int. = 0.2		
MPI	0.3	.01
SC	-1.1	.11
Minor defects (Models R^2 = 0.08; y-int. = 14.3)		
Age	-0.2	.03
Total defects (Model R^2 = 0.13; y-int.= 29.9)		
MPI	0.3	0.03
SC	-1.3	0.04

Prob.= probability; y-int.= y intercept.

There were significant linear regressions for all three classes of defective spermatozoa for Fazenda B. Regressions for major and for total defects were similar; MPI was significant (positive slope), with some contribution from SC (negative slope). Therefore, as MPI increased, the percentage of major and total defects decreased. In addition, as SC increased, the percentage of major and total defects decreased. In the linear regression with minor defects as a dependent variable, only age was significant (with a negative slope). Therefore, as the bulls increased in age, the percentage of spermatozoa with a minor defect decreased, consistent with previous observations (Smith et al., 1981)

This is apparently the first report of pixel analysis of testicular ultrasonograms of Zebu bulls. In this study, increases in the mean or standard deviation of pixel intensity were predictive of a higher percentage of morphologically defective spermatozoa. These results indicated that testicular ultrasonography and determination of pixel intensity have considerable potential in the breeding soundness evaluation of Zebu bulls.

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