Echotexture of testicular parenchyma as a predictor of the seminal quality in AI beef bulls

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SUMMARY

Fertility is a key variable in bovine production. Although both sexes take part in the pregnancy results, the fertility of bulls plays a fundamental role. In the last twenty years, ultrasonography (US) has begun to be considered a non-invasive tool for evaluating the structure and function of the male reproductive tract. The objective of this study was to evaluate the potential of testicular ultrasonography to predict the semen quality produced by bulls belonging to Semen Collection and Processing Centers (SCPC). A total of 57 semen-donor beef bulls were included in the study. Bulls were between 1.5 and 10 years of age and comprised the following beef-producing breeds: Angus, Brangus, Braford, Limangus and Polled Hereford. Ultrasound images were taken using an EXAGO® ultrasound (ECM Angulemme, France) with a multifrequency linear transducer configured at 7.5 MHz. The images were analyzed using the ECOTEXT® software. A correlation analysis was carried out between all the Ecotext variables (EC1= black pixels, EC2= white pixels, EC3= mean gray level of pixels, Density= density of hypoechoic areas (HA), Diameter= mean diameter of HA and Area percentage of HA in the total area of the region of interest) and semen quality. A highly significant negative relationship was observed between the percentage of abnormal heads and EC2, EC3 and tubule diameter (r= -0.37; -0.52; -0.37 respectively). Diameter was also negatively correlated with the percentage of other sperm morphological abnormalities (r= -0.30). EC1 was positively correlated with the area, density and diameter of the seminiferous tubules (r=0.69; 0.46 and 0.53, respectively). All bulls with up to 115 HA/cm^2 produced semen that was not apt for freezing. In conclusion, despite having shown different levels of association between the Ecotext parameters and semen quality, it remains necessary to continue the studies to be able to confirm the use of this tool to predict potential fertility of semen-donor bulls from SCPC.

KEY WORDS

Echotexture; testicles; bovine; semen; quality.

INTRODUCTION

Reproductive efficiency determines the productivity and profitability in beef and dairy herds[2, 19]. In cow-calf operations, it has been classically accepted that reproductive traits have twice the relative economic importance of production traits and ten times the importance of carcass attributes[30]. Twenty years later, using a bioeconomic model to compare the relative economic importance of the traits for a commercial cow-calf producer, it was established that although there is no consistent relationship with profitability, fertility traits have the greatest economic impact[3].

Although both genders intervene in the pregnancy outcome, the quantitative male: female ratio makes the fertility of the bulls play a fundamental role. For each bull there are 20 to 40 females, which means that the importance of the individual fertility of the males is greater than that of the females[25]. In the case of semen-donor bulls from Semen Collection and Processing Centers (SCPC), the enormous capacity to multiply the reproductive potential provided by artificial insemination (AI) makes the importance of male fertility much greater.

Breeding soundness evaluation (BSE) is a powerful tool to estimate the potential fertility of bulls. The examination process has several components: a) a general physical examination, b) a detailed examination of the genital tract, c) an evaluation of the health status, d) a functional evaluation of the reproductive system and e) an evaluation of seminal quality. Approximately twenty years ago, ultrasonography (US) began to be considered a non-invasive and atraumatic technique to evaluate the structure and functionality of the male reproductive system[15, 16, 24]. However, US has not developed in the bull to the same level as in the cow[22, 27]. US is not only used to assess animal fertility, but it has multiple applications. In addition, its use in bulls is beginning to increase[13]. The objective of this study was to evaluate the potential and feasibility of using testicular US as a fertility prediction tool in SCPC bulls.

MATERIALS AND METHODS

The experimental protocol was evaluated and approved by the Institutional Committee for Animal Experimentation in Argentina (CICUAL), University of Buenos Aires.

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Location and animals

The research was carried out at the facilities of the bovine Reproduction Center (CRB), a SCPC located near the town of San Antonio de Areco, province of Buenos Aires, Argentina. Sperm morphology evaluations were performed in the sperm lab of the Department of Animal Reproduction, INITRA, FCV-UBA.

A total of 57 semen-donor bulls, between 1.5 and 10 years of age, belonging to the Angus (n= 30), Brangus (n= 12), Braford (n= 10), Limangus (n= 3) and Polled Hereford (n= 2) breeds were enrolled in this study.

Semen collection and processing

The semen was collected using an artificial vagina, following the SCPC routine. The semen-donor bulls were in a "2 x 2" collection regime, that is, obtaining 2 ejaculates per day, twice a week.

A seminal quality control was performed, including the following determinations: volume (ml), concentration (sperm/mm^3), microscopic mass motility (scale from 0 to 5), progressive individual motility (percentage of sperm with progressive individual motility), vigor (scale from 0 to 5) and sperm morphology (percentage of normal spermatozoa). The volume was measured using the graduated scale on the collecting tube; sperm concentration was estimated by spectrophotometry (SDM Photometer 1, Minitube®); all motility evaluations were carried out using a warm stage and phase contrast microscopy: microscopic mass sperm motility was evaluated in a large drop of undiluted semen (100x), progressive individual motility was evaluated in a diluted sample (400x) and morphology in a sample diluted in formalin solution (1000x) counting at least 100 cells per sample. Ejaculates with a concentration ≥500,000 sperm/mm^3; microscopic mass motility ≥4 (scale from 0 to 5); progressive individual motility ≥ 70%; vigor ≥4 (scale from 0 to 5) and percentage of normal spermatozoa ≥70% were considered approved.

The post-thaw semen quality evaluation consisted of the analysis of progressive individual motility and vigor, carried out between the slide and cover slip at 400 x on a warm stage. Approved straws were those with post-thaw progressive individual motility ≥40% and vigor ≥4.

Ultrasonographic Imaging Method

Images were taken with an EXAGO® model ultrasound (ECM Angulemme, France) with a multifrequency linear transducer (5 to 10 MHz), set at 7.5 MHz. Three images were taken per testicle, with the transducer placed transversely in 3 positions: dorsal, middle and ventral (6 images per bull).

The ultrasound images were objectively analyzed with the ECOTEXT® software (computerized image analysis)[17]. The program uses several algorithms developed for the analysis of testicular ultrasonograms based on the histogram of pixels and on the size and density of hypoechoic areas within a parenchyma area of 2.64 cm^2. It measures a total of six parameters: three at normal resolution (Ecotext1, Ecotext 2 and Ecotext 3) and three at high-resolution[15]. Ecotext 1 (EC1) refers to the number of black pixels (a value 0 or close to 0); Ecotext 2 (EC2) corresponds to the highest number of pixels (a value of 256 or close to 256); and Ecotext 3 (EC3) to the number of intermediate or gray pixels. The software evaluates the density (hypoechoic areas (HA)/cm^2), total area (% of hypoechoic areas; HA) and mean diameter (μm) of hypoechoic areas. The high-resolution images provide data related to the seminiferous tubules: a) density of tubules / cm^2; b) percentage of area occupied by the lumen of the tubules in the parenchyma and c) mean diameter of the lumen of the seminiferous tubules in microns.

Statistical analysis

A statistical analysis was performed on the values of the six Ecotext ultrasound parameters to determine if there were differences between testicles (left or right) or scan position (dorsal, middle or ventral). It was first checked if the variables follow a normal distribution (Kolmogorov Smirnoff test).

A correlation analysis was performed between all Ecotext variables and semen quality. Pearson correlation would use if the distribution of the variables was normal or Spearman correlation if the distribution of the variables were not normal (only Ecotext 1 and Ecotext 2 had a non-normal distribution).

A bivariate analysis and multivariate were performed to predict the freezability of ejaculates based on breed, age or tubule density (density of hypoechoic areas) determined by ultrasound. If the variables follow a normal distribution, we do

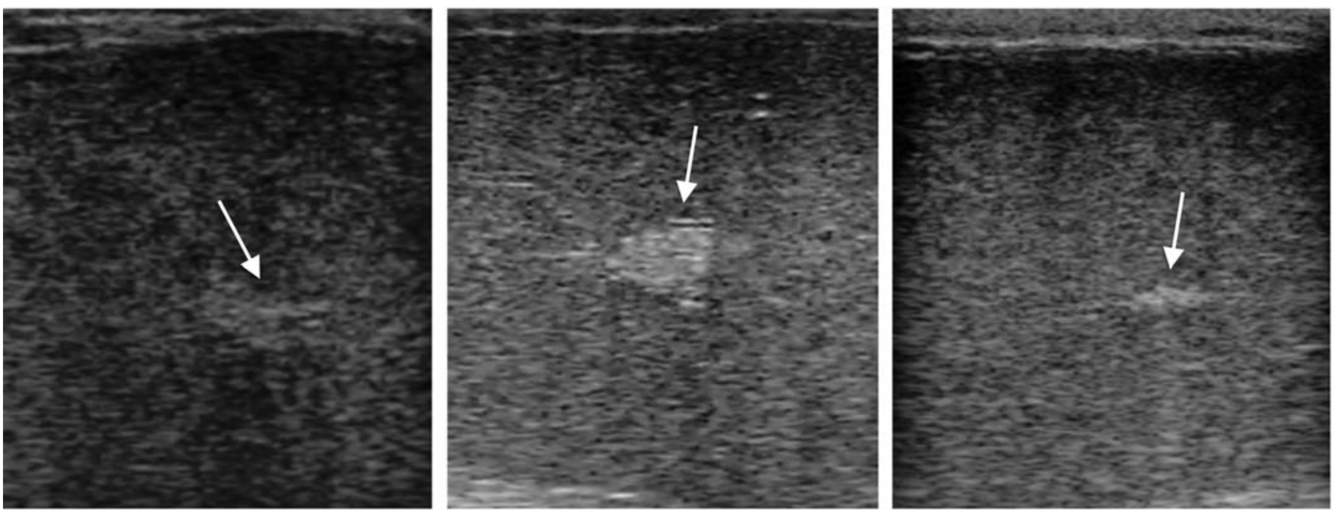

Figure 1 - Ultrasonograms of testicular parenchyma from different bulls included in the study. The testicular echotexture of the three bulls correspond to images with different homogeneity and echogenicity. The transducer is placed transversally and the hyperechoic areas correspond to the *rete testis* of the parenchyma (white arrow).

Student's t or one-way ANOVA test with Duncan multiple range tests for multiple comparisons if the variables are normal and Kruskal-Wallis otherwise. Prior to one-way ANOVA the Levene's Test for Equality of Variances was also performed. Wald's test was used to test the true value of the parameters (breed, age, tubule density) as model significant variables.
Logistic regression analysis (univariate and multivariate) was used to correlate the dichotomous result (freezable/non freezable ejaculate) data to the ECOTEXT parameters. A ROC was also used to calculate the elective breaking point (cut-off value) for each ECOTEXT variable in relation to the freezability of the ejaculate. The breaking point was in relation with the point that maximized specificity and sensitivity.
The results were analyzed using a statistical program SPSS/PC+, Version 15.0.1[28]. A p value <0.05 was considered significant.

RESULTS

A negative and highly significant (p<0,01) relationship between EC2, EC3 and density of HA was obtained with the percentage of abnormal heads (r = -0.37, -0.52 and -0.37, respectively). The diameter of the tubules was negatively related to the percentage of other anomalies (r= -0.30) (Table 1).
Because an important number of ultrasound images with EC3 showed values below 80 (darker and underexposed images), correlation analyses were carried out not only on all bulls but also on two subgroups: animals with EC3>80 and EC3>88 (to discard EC3 <80 and <88 values due to possible artefacts in the technique). In bulls with mean gray level values (EC3) >88, a significant (p<0.05) positive relationship was observed between % HA and diameter with ejaculate volume (r= 0.45 and 0.43, respectively). Likewise, there was a significant (p<0.05) positive correlation between density and ejaculates with normal sperm (r= 0.54). Ejaculates produced by bulls with a density of HA/cm^2 ≤150 had a lower percentage of normal sperm and a higher percentage of head abnormalities (p <0.05) (Table 2). The mean level of gray was related to the head anomalies (Table 3).
A multinomial logistic regression model was used to verify which of the Ecotext data could have a predictive value with respect to the result of freezing. The result expresses that the HA density and the age of the bull are the parameters included in the model that participate significantly (p <0.05). Breed was not a significant variable in the model (p= 0.12). However,

Table 1 - Correlation between the parameters of echotexture and testicular microstructure and sperm morphology.

Sperm morphology		EC1	EC2	EC3	Area (% HA)	Diameter (µm)	Density (HA/cm^2)
Normal	n	52	54	54	53	54	54
	r	-0.06	0.02	0.02	-0.06	-0.11	0.22
Head abnormalities	n	52	54	54	53	54	54
	r	0.26	**-0.37****	**-0.52****	**0.46****	**0.50****	**-0.37****
Tail abnormalities	n	52	54	54	53	54	54
	r	-0.01	0.06	0.06	0.12	0.10	-0.18
Proximal cytoplasmic droplet	n	52	54	54	53	54	54
	r	0.03	0.18	0.08	0.11	0.09	-0.02
Distal cytoplasmic droplet	n	52	54	54	53	54	54
	r	0.09	0.15	0.02	0.16	0.07	-0.03
Other abnormalities	n	51	53	53	52	53	53
	r	-0.15	**0.29***	**0.37****	-0.23	**-0.30***	**0.38****
	r	0.06	-0.26	**-0.36****	**0.29***	**0.34***	-0.23

** p< 0.01; * p< 0.05.

Table 2 - Seminal quality from bulls grouped according to ultrasonographic hypoechoic areas into ≤150 or >150 HA/cm^2.

Density (HA/cm^2)		Vol. (ml)	Conc. (sp/mm^3)	MM (0-5)	IM (%)	Norm sperm (%)	Head (%)	Tail (%)	PCD (%)	DCD (%)	Others (%)
≤150	n	38	38	38	38	35	35	35	35	35	34
	Mean ±S.D.	5,12 ±2,11	936.84 ±418.60	4.13 ±0.91	82.24 ±14.6	**68.40 ±18.85** ^{a}	**6,43 ±8,39** ^{a}	10.8 ±9.20	10.11 ±13.81	1.74 ±3.48	4.44 ±7.90
>150	n	15	16	15	15	16	16	16	16	16	16
	Mean ±S.D.	6,17 ±2,04	875.00 ±382.97	4.00 ±0.53	85,00 ±8,24	**77.44 ±22.32** ^{b}	**2.31 ±4.44** ^{b}	5.06 ±4.17	3.94 ±3.47	1.00 ±2.85	4.87 ±5.82

^{a,b} Different letters in the same column indicate statistical differences (p <0.05) between different testicular parenchyma densities (hypoechoic areas / cm^2).
Vol.= volume; Conc.= Concentration; MM= mass motility; IM= individual motility; Norm= morphologically normal sperm; Head= head morphology abnormalities; Tail= tail abnormalities; PCD= proximal cytoplasmic droplet; DCD= distal cytoplasmic droplet; Others = other morphological abnormalities.

Table 3 - Seminal quality from bulls grouped according to mean gray level values (EC3) into ≤80, 80-100 or >100.

EC3		Vol. (ml)	Conc. (sp/mm^3)	MM (0-5)	IM (%)	Norm (%)	Head (%)	Tail (%)	PCD (%)	DCD (%)	Others (%)
< 80	n	20	21	20	20	20	20	20	20	20	20
	Mean	**4,67**	971.43	4.15	78.25	72.75	**9.35**	9.40	5.65	2.15	3.10
	±S.D.	**±1,61** [a]	±482.33	±0.99	±18.30	±14.66	**±8.62** [a]	±11.24	±6.51	±4.17	±7.74
80-100	n	27	27	27	27	25	25	25	25	25	24
	Mean	**6,30**	864.81	4.11	86.85	71.72	**2.08**	8.36	8.56	0.76	5.75
	±S.D.	**±2,26** [b]	±351.32	±0.58	±6.67	±24.12	**±4.21** [b]	±6.22	±14.11	±1.42	±7.20
>100	n	6	6	6	6	6	6	6	6	6	6
	Mean	**3,92**	975.00	3.83	81,67	64.17	**3.83**	9.67	15.00	2.50	4.83
	±S.D.	**±1,20** [a]	±376.50	±1.17	±13.66	±20.22	**±9.39** [b]	±5.35	±14.80	±5.17	±5.71

[a,b] Different letters in the same column indicate statistical differences ($p < 0.05$) between the categories/groups of gray pixels.
EC3: number of intermediate or gray pixels; Vol.= volume; Conc.= Concentration; MM= mass motility; IM= individual motility; Norm= normal sperm; Norm; Head= head abnormalities; Tail= tail abnormalities; PCD= proximal cytoplasmic droplet; DCD= distal cytoplasmic droplet; Others= other abnormalities.

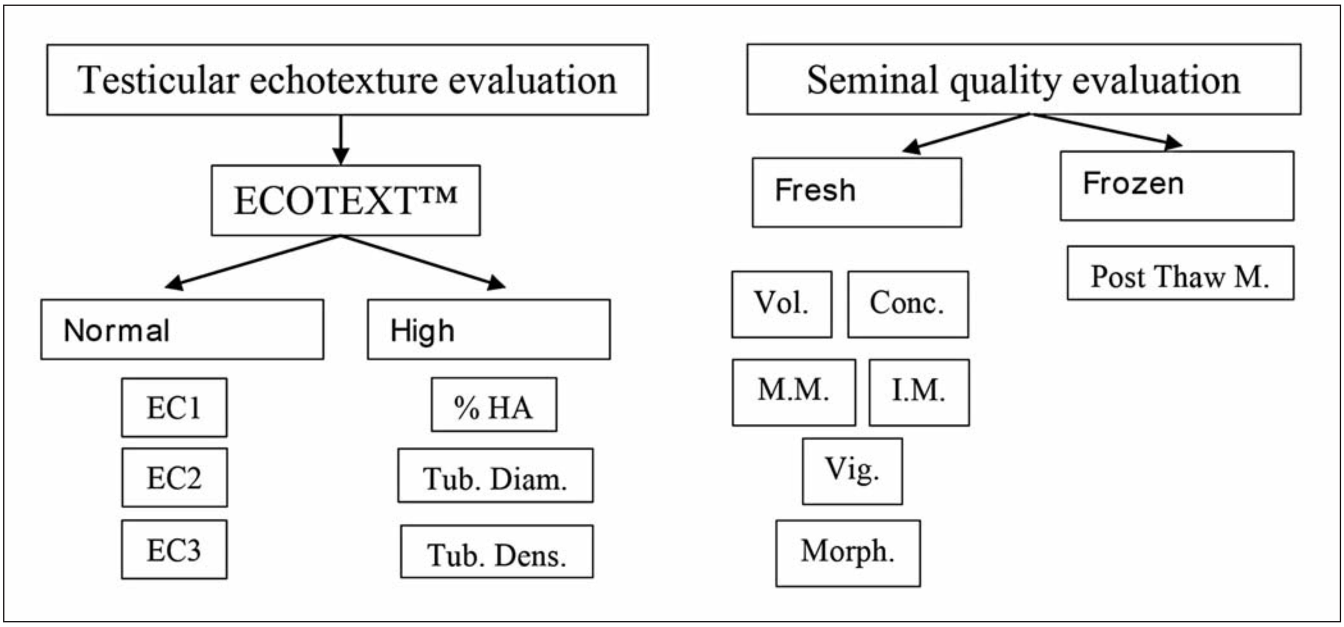

Figure 2 - Variables included in the testicular echotexture and seminal quality evaluations.
EC1 = black pixels, EC2 = white pixels, EC3 = mean gray level of pixels; % HA = % hypoechoic areas; Tub. Diam. = tubular diameter; Tub. Dens. = tubular density; Vol. = volume; Conc. = Concentration; M.M. = mass motility; I.M. = individual motility; Vig. = vigor; Morph. = morphology; Post Thaw M. = post thaw motility.

with the Wald test, the null hypothesis regarding age (age does not influence) cannot be rejected, so the density of HA remains the only significant variable ($p< 0.01$). Regression analysis indicates a strong relationship between density and freezability (exp $B=5{,}6 \times 10^{-9}$). For the prediction of non-freezable ejaculate producing animals, using a cut-off value ≤115 HA/cm^2, a sensitivity of 33.3% (6/18) and a specificity of 100% (25/25) were observed. All bulls with a density value ≤115 HA/cm^2 had non-freezable semen.

DISCUSSION

Reproductive US in the bull represents a tool with great potential[16,23]. Its application could be particularly useful in the evaluation of semen-donor bulls in SCPC, as a component for the prediction of fertility and efficiency in the production of straws. The novelty of the study resides in the incorporation of new echotexture parameters such as those that estimate the percentage, area and diameter of hypoechoic areas representing the seminiferous tubules.

The testicular echotexture values expressed in mean gray levels (EC3) obtained in the present study correspond to homogeneous images and a moderate hypoechogenicity, coinciding with the results of previous studies carried out in *Bos taurus* bulls belonging to beef cattle breeds[23,24]. Echegaray et al.[8] described similar EC3 values in dairy bulls. While Silva et al.[27] and Carmo et al.[6] reported a higher average pixel intensity (more echogenic images) in *Bos indicus* bulls under 2 years of age (163.7 and 115.3 respectively). This variation between one work

group and another can be attributed to differences in the age and biotype of the animals included in the respective studies, as well as possible discrepancies in the calibration and type of equipment and in the methodology used[23].
Regarding age, it has been described that echogenicity increases as the animal matures sexually[5,7] and then does not change significantly beyond puberty[5, 18]. One of the reasons for the lower echogenicity of testicular images in prepubertal animals is the presence of cross-sections of seminiferous tubules without germ cells or with immature cells[12]. The use of testicular echotexture as an indicator of sexual precocity has even been suggested[5, 7]. In the present study, no differences were found in the testicular echotexture of bulls as a function of age, within the range of 1.5 to 10 years, coinciding with Brito et al.[5] and Hamm and Fobbe[18]. There were also no differences in echogenicity between breeds in our study, however, the number of animals included in some of the breeds or age ranges was low. All the individuals evaluated were over 15 months of age, that could explain the high homogeneity of this parameter, in addition to the fact that it was a population of active semen-donor bulls, without pathologies.
Among the factors that define the echotexture of the testicular parenchyma, the density and diameter of the seminiferous tubules and the characteristics of their content stand out[12]. In recent histological studies of the testicular parenchyma in pigs, a high correlation was reported between the density of HA estimated by ultrasound and the presence of large seminiferous tubules (r = 0.71)[21]. However, the density of HA identified by Ecotext is lower than the total density of tubules measured by histology, where around 1000 tubule sections/cm^2 appear compared to the 150 to 200 HA/cm^2 visualized by ultrasound[10]. Nevertheless, when sections of tubular structures greater than 300 µm in diameter are counted by histology, their density is similar to the HA seen by ultrasound[10]. The above suggests that HA could be the ultrasound reflection of those areas in which the ultrasound beams cut the tubules of greater diameter. In beef bulls, tubular diameter has a range of 63 to 351 µm[5]. For this reason, three microstructure parameters, that estimate the area, diameter and density of the seminiferous tubules, were incorporated into the software.
The negative correlation between the mean gray level (EC3) and the diameter of the tubules and the percentage of HA (r= -0.87 and r= -0.84, respectively) is consistent with previous results[5, 14]. In contrast, EC3 had a moderate positive correlation with ultrasound-estimated tubule density (r= 0.54).
Analyzing the distribution of the density values as a function of EC3, it was observed that the percentile of EC3 <88 belonged to the dark or underexposed images, which could correspond to images taken with less contact between the transducer and the testicular parenchyma. When this subpopulation (EC3 <88) was excluded from the analysis, a negative correlation (r= -0.61) was observed between EC3 and tubule density. Thus, the data subpopulation with EC3 >88 excludes the data that generated the positive correlation of EC3 with density.
The inclusion of the EC1 and EC2 parameters in the software made it possible to evaluate new associations between the echotexture and the structure of the testicular parenchyma. Taking into account the total population of bulls in the study, a moderate positive correlation was found between EC1 and the diameter of the seminiferous tubules (r= 0.56) and the percentage of HA (r= 0.65). When the percentile of EC3 data >80 was analyzed, EC1 still showed a positive correlation with the diameter of the seminiferous tubules (r= 0.47) and the percentage of HA (r= 0.71). These results would indicate an association between the number of black pixels measured in the ultrasonogram and the tubular diameter and density. The relationship is lost if "noise" appears due to the presence of other factors that increase the number of black pixels, such as poor contact, edema, or decreased cell density in the parenchyma[10].
The correlations of EC2 in the total population of bulls were negative with the percentage of HA (r= -0.68) and with the diameter of the tubules (r= -0.74). However, a moderate positive correlation was observed between EC2 and the density of HA (r= 0.53). This positive correlation of EC2 with HA density does not coincide with the results of previous work from the same group[9]. Nevertheless, in the data percentile of EC3 >88, the correlations between EC2 and percentage of HA, diameter and density of the tubules were all negative (r= -0.62, r= -0.54 and r= -0.47, respectively).
An association of Ecotext parameters with sperm morphology was observed, but not with motility parameters. The main sperm abnormalities found in the analyzed ejaculates were head morpho-anomalies (vacuoles and craters, pyriform, tapered, macro and microcephaly), followed by tail abnormalities, proximal cytoplasmic droplet and distal cytoplasmic droplet. A moderate negative correlation was obtained between EC3 and the percentage of abnormal sperm heads (r= -0.52). Bulls with a more echogenic testicular echotexture produced ejaculates with greater head abnormalities. Brito et al.[5] also found a negative correlation between pixel intensity and normal sperm morphology. In studies carried out with the same software, a positive correlation of EC3 was found with the percentage of sperm abnormalities in pigs[20] and with the percentage of major abnormalities (head, midpiece and proximal cytoplasmic drop) in sheep[11]. In contrast, Kastelic et al.[20] found a negative correlation between echotexture and the percentage of sperm abnormalities after evaluating 16-month-old Holstein bulls. Brito et al.[4] found no association between both variables in *Bos indicus* bulls and *Bos indicus x Bos taurus* crossbreeds.
The correlation values of testicular function and echotexture, although significant, are lower than those that associate echotexture with the structure of the testicular parenchyma. Regarding the relationship between the ultrasound microstructure parameters (high-resolution) and the functionality of the testicular parenchyma, the HA density was negatively associated with the percentage of head abnormalities (r= -0.37). A study in which the same software was used in Holstein bulls also showed a low negative correlation between density and the percentage of major abnormalities (r= -0.34) and a positive correlation between density and the number of motile spermatozoa (r= 0.65) and the number of normal sperm (r= 0.63)[17]. Both these studies agree that the bulls whose testicular images had higher density values produced ejaculates with a higher proportion of normal sperm. Similarly, low negative correlations of HA density with the percentages of total abnormalities (r = -0.38), of intermediate piece abnormalities (r= -0.39), of distal cytoplasmic drop (r= -0,35) and tail (r= -0.32) were reported in rams[22].
The percentage of HA and the tubular diameter were positively correlated with the percentage of head abnormalities (r= 0.46 and r= 0.50, respectively). In contrast, in rams the percentage of HA and tubular diameter were negatively associated with the percentage of intermediate piece abnormalities (r= -0.40 and -0.34) and with the percentage of major abnormalities (r= -

0.39 and -0.35)[22]. However, it should be noted that in the present study these high-resolution parameters were associated with only one major morphological abnormality (abnormal heads) whereas in the study in rams all major abnormalities were considered together.

The correlation values between the microstructure and functionality parameters of the parenchyma are moderate to low (they do not exceed 50%), which would reduce its potential as a predictor of fertility.

Another aspect considered in this study was the effect of the conditions of the testicular parenchyma of the bulls evaluated by US and the post-thaw seminal quality of the straws obtained from their ejaculates. In this regard, post-thaw semen quality was not associated with the echotexture of the testicular parenchyma. No associations were found between the Ecotext parameters and post-thaw motility. In the percentile of more echogenic ultrasound scans (EC3 >80), although not significant, EC3 tended to be different between those bulls that produced good quality straws that were approved after thawing and those bulls that produced poor quality straws that were rejected (p=0,07). There were significant differences in tubular density between bulls that generated good quality post-thaw straws (approved) and those that generated low quality straws (rejected), which establishes a predictive value for this parameter.

The multinomial logistic regression model expressed that the density of HA on US was the only parameter included in the model that had a predictive value regarding the fate of the straws. With a cut-off value of ≤115 HA/cm^2, it showed a sensitivity of 33.3% and a specificity of 100% to determine that the semen would not be successfully frozen. All bulls with values ≤115 HA/cm^2 had non-freezable semen. A previous study carried out with Ecotext evaluating postmortem testes established a sensitivity of 100% and a specificity of 42.9% for HA density as a predictor of the quality of the sample obtained from the epididymis[9]. Although the same equipment and software were used, the origin of the samples was different (ejaculate vs. epididymis). In pigs, using the appropriate cut-off value for the species (80 HA/cm^2), specificity was higher (100% sensitivity and 83.5% specificity)[22]. Arteaga et al.[1], analyzing the predictive capacity of echotexture with regard to ejaculates with a high percentage of abnormalities in bulls (> 30%), they found a sensitivity of 60% and specificity of 33%. The predictive capacity of the echotexture variable used depends on the cutoff value chosen and the criteria used to define the dependent variable. In the case of the present study, the approval or rejection of the straws was defined using motility attributes, in addition to sperm morphology. This may be the reason for the lower sensitivity observed. In other studies from the same group[9, 22], only the percentage of sperm abnormalities was used. It is interesting to note that in rams, the most predictive parameter of semen quality was not density of HA, but instead the percentage of HA, with cut-off values lower than 6.5% and higher than 13%, obtaining a sensitivity of 100% and a specificity of 77.4%[12]. Perhaps increasing the number of bulls evaluated, the percentage of HA could also become predictive in this species.

CONCLUSIONS

Ecotext parameters show different levels of correlation with the structure and functionality of the testicular parenchyma, especially with abnormal sperm heads. The density of HA on US was the only parameter that had a predictive value regarding the fate of the straws. Despite having demonstrated different levels of association between the Ecotext parameters and semen quality, it is necessary to continue with the studies to verify the usefulness of this tool for predicting the potential fertility of SCPC semen-donor bulls.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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