

Sergio Marcantonio 1, Giovanni Gnemmi 2, Arantxa Echegaray 3, Marcelo Miragaya 1

1. Universidad de Buenos Aires, Buenos Aires, Argentina
2. Bovinevet International, Huesca, Spain
3. HUMECO, Huesca, Spain

✉ araechegaray@humeco.net
 🌐 www.humeco.net
 ☎ +34 974 231 165

Objectives

In the last ten years, ultrasonography (US) has been taken into account as a non-invasive and atraumatic technique to evaluate the structure and functionality of the male testicular parenchyma. A computerized analysis of the ultrasound image allows revealing differences in the echotexture of the parenchyma, imperceptible to the human eye, enabling objective measurements. The work aimed to evaluate the potential of testicular US to predict the quality and freezability of bull semen.

Material and Methods

Semen samples were obtained from 57 bulls (Angus, Brangus, Bradford, Limangus, Polled Hereford, 1.5-10 years of age) which belonged to a bovine reproduction centre.

The ultrasound images were taken with an ultrasound machine (EXAGO® ECM, France) with a multi-frequency linear transducer (5 to 10 MHz), set at 7.5 MHz. A total of 3 transversal videos were taken per testicle, in 3 positions: dorsal, middle and ventral.

The echographic videos were analyzed by the algorithms, selecting a region of interest (ROI) and obtaining the average of the 3 videos for the following parameters: (EC1) black pixels, (EC2) white pixels, (EC3) mean gray level of pixels, (Density) density of hypoechogenic areas, (Diameter) mean diameter of hypoechogenic areas and (Area) percentage of hypoechogenic areas in the total area of the ROI.

Bulls ejaculates were obtained by the artificial vagina method. The volume was measured using the graduated scale of the collecting tube; sperm concentration by spectrophotometry. Microscopic mass motility was evaluated through a thick drop with a 100 x optical microscope with a thermostated stage. Progressive individual motility through a diluted sample in a mounted slide at 400X and a microscope with a thermostated stage. Sperm morphology with a sample diluted with formal saline solution in a mounted slide and in phase contrast microscope at 1000x, counting at least 100 cells per sample.

The criteria for acceptance of the ejaculates as suitable for freezing were: concentration \leq 500,000 sperm/ml; mass motility \leq 4 (scale from 0 to 5); progressive individual motility \leq 70%; vigour \leq 4 (scale from 0 to 5) and percentage of normal sperm \leq 70%.

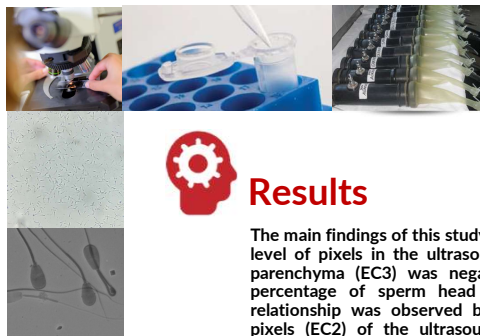
The frozen-thawed seminal quality assessment consisted of the analysis of the progressive and vigorous individual motility. The criteria for acceptance of straws as suitable were: progressive individual motility \leq 40% and vigour \leq 4.

A correlation and ANOVA analysis were performed between all the ECOTEXT variables and fresh and frozen-thawed semen quality, using SPSS statistical software. A p-value $<$ 0.05 was considered significant.



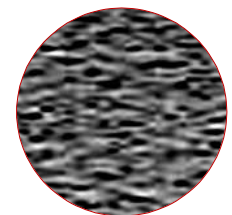
No freezable ejaculate

Freezable ejaculate



Results

The main findings of this study were that the average grey level of pixels in the ultrasound image of the testicular parenchyma (EC3) was negatively correlated with the percentage of sperm head abnormalities. A negative relationship was observed between the level of white pixels (EC2) of the ultrasound and the frozen-thawed sperm motility ($r = -0.316$, $p = 0.025$), and a positive relationship between EC2 and the percentage of proximal and distal cytoplasmic droplets ($r = 0.288$, $p = 0.034$; $r = 0.316$, $p = 0.020$, respectively). Besides, there were significant differences in the density of hypoechogenic areas between bulls that generated straws of good quality after freezing-thawing (approved) and those that generated straws of low quality (disapproved) at 0 days post-ultrasound ($p < 0.048$).



Discarded straws

Approved straws

Table 1. Echotexture values in the testis of bulls with approved or disapproved semen straws (Mean \pm SD).

| Group of bulls | EC1 | EC2 | EC3 | Area (micron ²) | Diameter (micron) | Density (n/cm ²) |
|---------------------------------|-----------------|------------------|-----------------|-----------------------------|-------------------|-------------------------------|
| Approved semen straws (n=30) | 14,2 \pm 9,5 | 29,5 \pm 36,3 | 86,4 \pm 9,0 | 11,5 \pm 2,7 | 122,7 \pm 17,2 | 141,8 \pm 15,4 ^a |
| Disapproved semen straws (n=24) | 16,0 \pm 12,4 | 76,2 \pm 150,9 | 85,7 \pm 14,8 | 12,3 \pm 4,2 | 130,4 \pm 29,9 | 132,3 \pm 19,2 ^b |

Values with different superscripts present significant differences ($p < 0.05$)

Conclusion

Some Ecotext parameters have different levels of correlation with sperm quality, especially with sperm morphology. The density of hypoechogenic areas into the testicular parenchymal ultrasonogram is a predictor of the freezability of the sperm.