# Semen parameters and computer-assisted quantitative analysis of testicular parenchyma in rams with presence and lack of fibrotic lesions

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**PURPOSE:** The aim of this study was to compare different semen parameters and testicular parenchyma intensity in rams with presence and lack of fibrotic lesions.

**MATERIAL AND METHODS:** The experiment was carried out with twenty six mature rams (Assaf and their cross-breeds with Synthetic Bulgarian Milk population) separated in two groups according to presence or not of testicular fibrotic lesions. Group I (with on testicular fibrotic lesions) included 14 rams and group II (presence of testicular fibrotic lesions in one or both testes) 12 rams. Initially, the animals were weighed and scrotal circumference was measured by pulling the testes firmly down into the lower part of the scrotum and placing a measuring tape around the widest point. Semen was collected by electro-ejaculator method (Fig. 1-A), then all ejaculates were placed on a water bath at 35°C and assessed within 5 min after collection. Volume of ejaculates (VE) was measured in graduated collecting tube. Mass motility (MM) was evaluated under light microscope on the base of sperm wave motion (scale 0-5, Evans and Maxwell, 1987). Concentration of the spermatozoa (CS) (x$10^9$/$cm^3$) was determined by a Photometer SpermaCue (Minitube, Germany), calibrated for small ruminant semen. Abnormal spermatozoa were determined after staining of the smears by Diff-Quik method and counting of 200 cells under microscope at magnification 200-400×. A trans-scrotal ultrasonography of both testes using of ultrasound scanner SonoScape A5 Vet (SonoScape, China) and multifrequency (7-12 MHz) linear transducer was performed by the same operator. Before examination the settings for focus, gain, brightness, and contrast were standardized and were the same for each animal. Before testicular scanning an ultrasound gel was used as a coupling material between the scrotum and transducer (Fig. 1-B). The gray-scale images were obtained in a longitudinal plane and frozen when visualization of the mediastinum of the testes was clear and apparent. A computer-assisted quantitative analysis based of pixel intensity (PI) measurement of each ultrasonogram was made by image analysis software (Adobe Photoshop CS, Version 8, Adobe Inc. Corporation, USA). After conversion of the image in a gray scale (0-255 pixels) a pixel intensity of 3 equal outlines of non-fibrotic testicular parenchyma (PINTP) for each testis, located below testicular mediastinum was determined. Additionally, a pixel intensity of testicular fibrotic lesions (PITFL) was recorded. The mean values of PI and the standard deviations for left and right testis of each ram were measured and their means were given as final values for the individual ram (Fig. 2).The results were processed by computer program Statistica version 7.0 (Stat-Soft., 1984-2000 Inc., Tulsa, OK, USA). The parameters for each group were presented as mean and standard deviation. The mean values between both groups were compared by t - test for comparison of two means and percentages. Statistical significance was accepted at P level < 0.05.


*Fig. 1. Semen collection by electroejaculation method (A) and ultrasound examination of testis (B)*

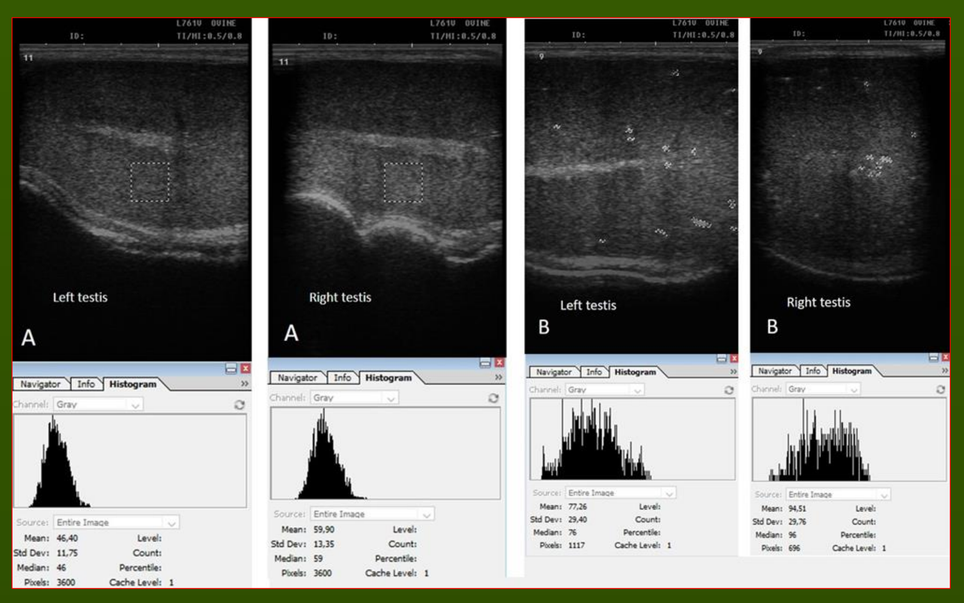


*Fig. 2. Ultrasonogram of testes and pixel intensity histogram in ram with no fibrosis (A) and testicular fibrotic lesions (B)*

**RESULTS AND DISCUSSION:** The present study showed no significant differences (P < 0.05) between mean values for age, body weight, scrotal circumference, volume of ejaculate and concentration of spermatozoa (Table 1). This result was no in correspondence with a similarity of the pixel intensity for non-fibrotic testicular parenchyma in both groups and can be an evidence for preserved sperm production capacity in rams, in spite of fibrotic lesions presence in their testes. In contrast, the mass motility and the abnormal spermatozoa were decreased in group II (P < 0.05). Probably, the testicular fibrosis can be attributed more to negative changes of sperm quality than sperm production. The additional investigation showed significantly higher (P < 0.05) pixel intensity of fibrotic lesions compared to the obtained values for non-fibrotic testicular parenchyma in the groups. This data indicated, measuring of pixel intensity of testicular parenchyma as reliable method for distinguishing between normal and affected parenchymal tissue. It can be used for diagnosis of previous and current pathologic processes into the testes. An ultrasound exam of testes immediately before the breeding season is recommended for a selection of the best rams for semen collection or mating.

*Table 1. Values of different parameters in rams with no testicular fibrosis (group I) and presence of testicular fibrotic lesions (group II) (Mean±SD)*

| Parameters | Group I (n=14) | Group II (n=12) |
|---|---|---|
| Age (months) | 29.3±9.1 | 23.3±10.4 |
| BW (kg) | 75.7±19 | 72.9±15 |
| SC (cm) | 34.4±3.1 | 34.9±3.5 |
| VE ($cm^3$) | 1.2±0.4 | 1.0±0.3 |
| CS (x $10^9$/ml) | 1.4±0.5 | 1.6±0.9 |
| MM (score 1-5) | 4.3±0.6$^a$ | 3.2±0.5$^b$ |
| AS (%) | 15.6±2.9 $^a$ | 24.9±5.8 $^b$ |
| PINTP (pixels) | 61.5±11 | 69.2±21$^1$ |
| PIFTL (pixels) | - | 102.6±14$^2$ |

BW - body weigh; SC - scrotal circumference; VE - volume of ejaculate; CS - concentration of spermatozoa; MM - mass motility; AS - Abnormal spermatozoa, PINTP - pixel intensity of non-fibrotic testicular parenchyma, PIFTP - pixel intensity of fibrotic testicular lesions
Different superscripts in a row indicate differences between the values at P<0.05
Different numbers in a column indicate differences between the values at P<0.05

**CONCLUSION**

The testicular parenchyma lesions can affect negatively the sperm mass motility and led to higher percent of abnormal spermatozoa into the ejaculate. The computer-assisted quantitative analysis of testicular parenchyma is a reliable method for assessment of testicular status in rams.