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Relationship between echotextural and histomorphometric characteristics of stallion testes

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ABSTRACT

The goal of this study was to investigate correlations between objective measures of testicular echotexture and histomorphometric attributes related to the histological composition of stallion testes. Fifty-four scrotal testes were obtained from three groups of stallions during routine castrations: colts <1 yr old (n = 18), young stallions 1–5 yrs old (n = 27), mature stallions > 5 yrs old (n = 9). In addition, two scrotal testes with degeneration, 16 retained inguinal and 10 retained abdominal testes were surgically obtained. Cross-sectional and longitudinal ultrasonograms were obtained for each testis. Mean numerical pixel values (NPVs) as well as pixel standard deviations (PSDs) were determined for each image (ImageJ-1.5 software). Histomorphometric attributes of the seminiferous tubules (STs) were derived (three tissue samples per each testis) using image analysis software [relative STs area: RSTA = ST area/total cross-sectional area (TA) x 100%; relative STs lumen: RSTL = ST lumen area/TA x 100%; individual ST area; ISTA; individual ST lumen: ISTL; seminiferous epithelium height: SHE]. Degree of fibrosis was graded semi-quantitatively (0–3) in samples from 17 testes. All measures of testicular echotexture as well as all histomorphometric attributes of STs had highest values when obtained from the scrotal testes of young and mature stallions ($P < 0.05$). The NPVs and PSDs from the ultrasonographic images of the scrotal testes were significantly correlated with all histomorphometric attributes of STs ($P < 0.001$). However, there was no correlation between the majority of these measures and attributes if each group of the scrotal testes was analyzed separately. The NPVs from the ultrasonographic images of the retained inguinal testes were correlated with RSTA, RSTL, ISTA, and ISTL, while the NPVs from the retained abdominal testes were not correlated with any of the histomorphometric attributes of the STs. Testes with high degree of fibrosis had variable values of pixel intensity and pixel heterogeneity. Based on the results of this study, we concluded that the pixel intensity and pixel heterogeneity of stallion testes increase during the first year of life and remain stable in young and mature stallions. These changes occur in parallel to the development of the seminiferous tubules. Testicular echogenicity in stallions does not seem to reflect degree of testicular fibrosis. Retained abdominal testes have lower and less heterogeneous echogenicity than scrotal testes from stallions that are more than one year old. While pixel analysis cannot replace biopsy in assessing testicular histomorphology in young and mature stallions, testicular echogenicity is a good indicator of peri-pubertal growth and expansion of the seminiferous tubules.

1. Introduction

Testicular pathologies, such as testicular degeneration, are major causes of subfertility in stallions [1,2]. The efficiency of sperm production decreases in stallions impacted by such disorders, leading to low sperm numbers and poor semen quality [3]. Severe cases have irreversible changes to testicular parenchyma and cannot be successfully treated, while mild cases, which could benefit from various therapies, are difficult to diagnose [1]. In the advanced stages of testicular degeneration, there are characteristic changes that can be detected by scrotal palpation (soft, small testes)

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and by semen evaluation (small sperm numbers, large numbers of immature germ cells), while the early stages are often not associated with any obvious signs, making them difficult to diagnose and treat. The only accurate test of the current structure and function of testes is a histopathological examination of testicular tissue. In order to obtain a sufficient sample of testicular tissue, an invasive procedure - testicular biopsy has to be performed [4]. While this procedure can be done safely in stallions [5–7], it is rarely performed in the field conditions, due to a potential for detrimental complications [8,9]. A further challenge posed by biopsy is that the sample must be appropriately fixed, stained, and evaluated, which takes at least several days. Therefore, it is imperative to introduce non-invasive diagnostic methods that would be sensitive enough to assist in diagnosing various testicular problems, such as testicular degeneration.

Ultrasonography is a non-invasive technique that allows for the visualization of various organs in animals and humans. Recently, computer-based analysis of testicular echotexture was introduced as an alternative to invasive techniques of assessing male reproductive functions. This analytical method is based on noting changes in pixel intensity and pixel heterogeneity in the ultrasonograms of testicular parenchyma that are demonstrably related to microstructural attributes of the testes [10–12]. In prepubescent bull calves and ram lambs, there is an overall increase in testicular echogenicity with age [13–15]. The objective measures of testicular echogenicity remain consistent in post-pubertal bulls, but the correlation is inconsistent between these measures and sperm numbers and sperm morphology in semen collected at the same time [16]. In a scrotal insulation model of testicular degeneration, acute degenerative changes were reflected by pixel intensity, but not by semen parameters [10]. Interestingly, a strong correlation was seen in pixel intensity and semen parameters between two and four weeks later. This delay in changes of the parameters of ejaculated semen was most likely caused by the time required for the final stages of spermatogenesis, epididymal maturation, and transport. Furthermore, previous studies showed that differences in echotextural parameters of testicular parenchyma in rams are caused by variations in the histomorphology of testicular tissue [12,17]. Positive correlations were found between the size of the seminiferous tubules (outer and inner diameters, total and luminal areas) and pixel intensity of testicular tissue. All these data on the ruminant species strongly suggest that the computer-assisted pixel analysis of testicular ultrasonograms is a valid method for assessing testicular function and structure.

While ultrasonography is routinely used to evaluate the reproductive tract in stallions, a computer-based analysis of testicular echotexture has not yet been investigated in this species. Our long-term goal is to validate this technique for clinical application in horses. Veterinary practitioners would readily apply a quick and non-invasive assessment of testicular function and structure, which can be a non-invasive alternative to testicular biopsy. The aim of this study was to investigate correlations between objective measures of testicular echotexture and histomorphometric attributes related to the histological structure of stallion testes.

2. Materials and methods

Eighty two testes were obtained from stallions during castrations performed in the Large Animal Hospital (LAH) of the College of Veterinary Medicine, University of Florida, as well as in one local veterinary practice. Forty-nine testes came from Quarter horses, 25 from other light horse breeds, seven from draft horses, and one from an American Miniature Horse. The horses' ages ranged from seven months to 14 years. Fifty-four scrotal testes were obtained via routine castration procedures from stallions that had no history of any reproductive problems, with 18 testes from colts <1 yr old, 27 testes from young stallions 1–5 yrs old, and nine from mature stallions >5 yrs old. Two additional scrotal testes were obtained from nine years old Andalusian stallion, with a history of infertility, and typical signs of testicular degeneration (low sperm numbers, poor sperm motility, immature germ cells in ejaculates). Out of 56 scrotal testes, 44 were obtained from 22 stallions with two scrotal testes, one from a stallion with one scrotal testis, and 11 scrotal testes were obtained from 11 stallions with one scrotal and one retained testis. Furthermore, sixteen retained inguinal testes were obtained via inguinal surgical approach and 10 retained abdominal testes were obtained using abdominal laparoscopic surgery.

All testes obtained from castrations performed in the LAH UF were processed within 1 h after surgeries. All testes obtained from field castrations were placed in a cooler for a transport to the laboratory, and were processed within 4 h after surgeries. It has been previously shown that there is no difference between echotextural attributes of testicular tissue in rams obtained before castration with active blood flow, after castration with blood pooled, or after castration with blood drained [17]. Each equine specimen in our study was washed with tap water to remove blood, and the vaginal parietal tunic was removed to expose the testis. The deferent ducts and the spermatic cords were clamped with plastic tie straps, after which the specimens were carefully evaluated for any gross pathologies. For evaluation of testicular echotexture, each testis was placed in a custom-made, scrotal ultrasonographic training model of our own design (Fig. 1A). Briefly, the model consists of a stallion condom (Nasco, Fort Atkinson, WI) and a holding frame, made of 0.5″ PVC pipe and PVC connectors. A specimen (testis with epididymis and spermatic cord) was placed inside the condom, and space between the condom and testis was filled with a small amount of tap water to mimic the vaginal cavity. The condom with the specimen was then attached to the PVC frame using a plastic kitchen clamp and a 12″ reusable rubber twist tie. Inside the stallion condoms, the testes hung freely, similar to the scrotal testes. We did not observe increased attenuation of the ultrasound beam when the testes were placed in the stallion condoms as compared with the testes that were completely covered by the parietal vaginal tunic. Furthermore, a small amount of tap water between the testes and the condom appeared as a small, anechoic outline, which was useful for defining the testicular border.

Ultrasonographic evaluation of each specimen was performed using a B-mode ultrasound machine equipped with a 7.5 MHz linear transducer (Aloka SSD-900, Aloka Inc. Tokyo, Japan). The same image depth (7 cm) and standardized gain settings (Gain = 73) were used for all evaluations. Ultrasound gel was applied to the transducer for better coupling with the model. Two ultrasonographic images of each testis were captured for computer-assisted analysis: a cross-section and a longitudinal section [18]. For the cross-sections, the transducer was held on the lateral side of the mid-portion of each testis, perpendicular to its longitudinal axis. For the longitudinal sections, the transducer was held on the lateral side of the testis, parallel to its longitudinal axis (Fig. 1B). Computer-assisted analysis of the ultrasonographic images was performed using two techniques: the spot-meter technique and the region-area technique, both of which utilized the National Institutes of Health's (NIH) free research software (ImageJ-1.5, NIH, Bethesda, MD, USA). For the spot-meter technique (Fig. 1C and D), a total of four (cross-sections) or six (longitudinal sections) square-shaped spots (5 mm × 5 mm) were placed on each saved image of testicular parenchyma, excepting any image artifacts or hemorrhages resulting from local anesthesia [12]. For the region-area technique (Fig. 1E and G), an entire image of testicular parenchyma was outlined using a free-form tracing tool. Mean pixel intensity (numerical pixel values – NPVs) and pixel heterogeneity