

# COMPUTER ANALYSIS OF VIDEO AND ULTRASONOGRAPHIC IMAGES FOR EVALUATION OF BULL TESTES

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### ABSTRACT

The objectives of this study were to determine relationships between scrotal size (SC; estimated from a video image) and testicular size, and between ultrasonographic echotexture of the testis and seminiferous tubule area in bulls. Video images of the scrotum of 49 Holstein-Friesian (H-F) bulls were recorded and digitized. Scrotal width and length were measured with custom software. After slaughter, scrotums (containing testes) were excised, SC and testicular height, width and volume were measured, and the testes were examined ultrasonographically. Correlations between SC and testicular width or volume (r=0.86, P<0.001 and r=0.84, P<0.001, respectively) were much higher than those between scrotal width and testicular width or volume (r=0.23, P<0.11 and r=0.28, P<0.06). Histological examination of the testes was performed in 31 of the bulls. Ultrasonographic echotexture of the testes (determined with custom software) was highly correlated (r=-0.5, P<0.005) with seminiferous tubule area. Although SC was superior to video imaging for estimating testicular size, ultrasonographic imaging of the testes has considerable potential for the evaluation of testicular function in bulls.

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#### INTRODUCTION

Testicular volume, as estimated by measuring SC or testicular size, is often used to predict sperm production (3, 5, 10). Recent advances in video imaging and image analysis provide new opportunities for assessing testicular size. Ultrasonography can also be used to predict testicular volume and sperm production in bulls. In one study (8), ultrasonographic assessment of testicular diameter was more highly correlated with testicular volume than was scrotal circumference. Lenz et al. (4) reported that ultrasonographic measurements of testicular volume in men were correlated (P<0.001) with sperm count. Computerized analysis of ultrasonographic images can provide substantial information regarding the structure and function of tissues (7). Bull testes are homogeneous and moderately echogenic (6), with an increase in echodensity as bulls reach puberty (1).

The objectives of the present study were to determine if 1) measurement of video images of the scrotum accurately predict testicular size and 2) ultrasonographic echotexture of the testis is related to seminiferous tubule area.

#### MATERIALS AND METHODS

Forty-nine Holstein-Friesian bulls (age, 16 to 19 mo; weight, 501±23 kg) were examined just prior to slaughter. Video-images of the posterior scrotal surface were recorded with a Camcorder (JVC GR-AX200, Victor Co., Yokohama, Japan) held a fixed distance (approximately 60 cm) behind the bull. Images were digitized (Super Match VideoSpigot Capture Card, VidCap 1.00 Microsoft software) to the hard disk of an IBM Thinkpad (360 CSE; IBM UK Ltd., Greenock, UK). A calibrated, distance-measuring cursor was used to estimate scrotal width and length, as described in Figure 1. Bull temperament and facilities for restraint precluded other in vivo measurements.

Immediately after slaughter, the scrotum (containing the testes) was excised and the SC was measured with a cloth tape. Ultrasonographic examinations of each testis (within the scrotum) was done with a B-mode diagnostic scanner (Pie Medical, Model 450; Maastricht, The Netherlands) with a 7.5 MHz linear-array transducer. Gain settings were held constant for all examinations. Coupling gel was applied to the transducer, which was aligned at the center of each testis, perpendicular to the vertical axis. A customized, electromechanical device was used to insure that consistent pressure was applied. At approximately 0.76 kg/cm<sup>2</sup> pressure, an indicator lamp lighted, and the ultrasound image was manually frozen. The frozen image was subsequently captured and stored (as described for the video image) and then analyzed as described (Figure 2).

Testes were removed from the scrotum, and the testicular vascular cones and epididymides were excised. The length and width of each testis was measured with calipers, and volume was determined by water displacement. Parenchymal tissue samples were taken from both testes of 31 bulls and fixed in 10% phosphate-buffered formalin (pH 7.0). Tissues were routinely processed for histology, including imbedding in paraffin, sectioning (7-µm thick), mounting on glass slides, and staining with hematoxylin and eosin. Tissue sections were examined (magnification, x 100) with a Leitz-Diaplan microscope (Leitz Wetzlar GmbH, Wetzlar, Germany) with an integral color



Figure 1. The video camera was held a fixed distance from the scrotum and a grid of known size projected onto the scrotum. The height and width of the scrotal margins were measured with the software.

videocamera and frame grabber. Image-analysis software (CYTOSOFT, Pictron Ltd., Budapest, Hungary) was used to measure cross-sectional area of symmetrical seminiferous tubules. For each testis, images were made of 10 microscopic fields, and approximately 10 to15 tubules were measured in each field.

Pearson correlation coefficients (9) were calculated between scrotal length, width and circumference and testicular length, width and volume, and between seminiferous tubule area and testicular echotexture.

### **RESULTS AND DISCUSSION**

Mean ( $\pm$ SE) values for all end points are shown (Table 1). Correlations (Table 2) between scrotal circumference and testicular length, width and volume were all high (correlation coefficients, 0.63, 0.86 and 0.84, respectively; P<0.001 for each), consistent with previous reports that SC is an accurate predictor of testicular size (3, 5, 10). In contrast, correlations between scrotal length or width (derived from the video image) and testicular length, width and volume were much lower



Figure 2. Ultrasonograms of the left and right testes are recorded on the left panel. Pixel analysis was performed in an area 1 cm high x 6.5 cm wide (just under the tunica albuginea). The average echotexture of these ultrasonograms is indicated by arrowheads on the grey scale at the bottom (0=black, 63=white) and the numeral 27 to the left of the grey scale. The frequency distribution of grey scales is shown in the histogram in the right panel.

(correlation coefficients, 0.23 to 0.33), with the exception of scrotal width and testicular length (correlation coefficient, 0.61). Therefore, video imaging of the scrotum was clearly inferior to SC for measurement of testicular size. Perhaps the video imaging technique can be refined and improved. For example, markers at the sides and top of the scrotum would more clearly delineate the borders.

Ultrasonic echotexture of the testis was highly correlated (r = -0.5, P<0.005) with seminiferous tubule area. In other studies, ultrasonography has been shown to be a noninvasive tool for assessing testes and detecting testicular pathology (2, 4, 6). Visual analysis of testicular ultrasonograms is of limited value; in the absence of pathology, the parenchyma appears homogenous, with an echodense mediastinum testis (6). However, computerized image analysis provides detailed information regarding tissue structure and function (7). For example, there was a profound increase in testicular echodensity as bulls achieved puberty (1). Furthermore, Lenz et al. (4) reported that the ultrasonic texture score was lower in human testes with active seminiferous tubules compared with those that

were inactive. Therefore, ultrasonographic evaluation of the testis and determination of testicular echotexture is another method for assessing spermatogenic capacity of the testis.

In conclusion, video imaging of the scrotum was inferior to measurement of scrotal circumference for prediction of testicular size. Ultrasonic echotexture of the testis was highly correlated with seminiferous tubule area, indicating that ultrasound imaging of the testis has considerable potential for the evaluation of testicular function in the bull.

Table 1. Mean ( $\pm$  SE) measurements of the scrotum and testes in 49 bulls.

Mean ± SE
$13.4 \pm 0.2$
$13.3 \pm 0.2$
$33.7 \pm 0.4$
$32.6 \pm 0.2$
$5.4 \pm 0.1$

<sup>a</sup>Only 31 or the 49 bulls were examined.

 Table 2.
 Pearson correlation coefficients between several scrotal and testicular measurements (n=49 bulls).

Testicular measurement		
Length	Width	Volume
0.63 <sup>c</sup>	0.86 <sup>c</sup>	0.84 <sup>c</sup>
0.25 <sup>a</sup>	0.33 <sup>b</sup>	0.32 <sup>b</sup>
0.61 <sup>c</sup>	0.23 <sup>a</sup>	0.28 <sup>b</sup>
	Testic Length 0.63 <sup>c</sup> 0.25 <sup>a</sup> 0.61 <sup>c</sup>	Testicular measurementLengthWidth0.63°0.86°0.25°0.33°0.61°0.23°

<sup>a-c</sup>Probability that the correlation coefficient is significant: <sup>a</sup>P<0.1; <sup>b</sup>P<0.05; <sup>c</sup>P<0.001.

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