Relationships among scrotal and testicular characteristics, sperm production, and seminal quality in 129 beef bulls

J.P. Kastelic, R.B. Cook, R.A. Pierson, G.H. Coulter

Abstract

Standard breeding soundness examinations plus measurement of scrotal surface temperature (SST), internal/scrotal testicular temperatures, testicular ultrasonographic echotexture, daily sperm production, and epididymal sperm reserves were conducted on 129, 16-month-old crossbred beef bulls. There were significant positive linear correlations between SST and internal scrotal/testicular temperatures, a positive linear regression ($P < 0.06$) of bottom SST with the incidence of secondary sperm defects, but a negative linear regression ($P < 0.01$) with the incidence of primary sperm defects. Testicular echotexture had a positive linear regression with daily sperm production ($P < 0.002$) and testicular tone had a negative linear regression ($P < 0.008$) with epididymal sperm reserves. Scrotal circumference had a positive linear regression ($P < 0.04$) with the percentage of progressively motile sperm, a negative linear regression ($P < 0.1$) with the incidence of primary sperm defects, and a positive linear regression ($P < 0.0001$) with epididymal sperm reserves. In addition to seminal quality and scrotal circumference, testicular ultrasonographic echotexture has considerable promise for augmenting breeding soundness examinations of bulls.

Résumé

Un examen régulier du potentiel reproducteur ainsi qu'une mesure de la température scrotale superficielle (TSS), des températures scrotale interne et testiculaire, un examen par ultrasonographie de la texture testiculaire, une évaluation de la production quotidienne de sperme et des réserves épididymales de sperme furent obtenues de 129 taureaux croisés âgés de 16 mois. Une corrélation linéaire positive significative entre la TSS et les températures scrotale interne et testiculaire, une régression linéaire positive (P < 0,06) entre la TSS inférieure et la fréquence de défauts spermatiques secondaires, ainsi qu'une régression linéaire négative (P < 0,01) avec la fréquence de défauts spermatiques primaires furent observées. Une régression linéaire positive (P < 0,002) entre la texture testiculaire et la production quotidienne de sperme fut notée, alors qu'une régression linéaire négative (P < 0,008) était notée entre le « tonus » testiculaire et les réserves épididymales de sperme. La circonférence scrotale présentait une régression linéaire positive (P < 0,04) avec le pourcentage de mobilité progressive du sperme, une régression linéaire négative (P < 0,1) avec la fréquence de défauts spermatiques primaires, et une régression linéaire positive (P < 0,0001) avec les réserves épididymales de sperme. En complément à un examen de la qualité de la semence et de la mesure de la circonférence scrotale, l'examen ultrasonographique de la texture testiculaire semble prometteur pour l'évaluation du potentiel reproducteur des taureaux.

(Traduit par docteur Serge Messier)

Introduction

In many mammals, the testes must be a few degrees cooler than body temperature for viable spermatogenesis (1,2). Infrared thermography is a non-invasive method of measuring scrotal surface temperature (SST). Scrotal thermograms from bulls with normal testicular thermoregulation had horizontal temperature bands across the scrotum (warm near the body and progressively cooler distally); more random temperature patterns were associated with poor seminal quality (3).

Diagnostic ultrasonography of the testes has no effect on seminal quality or on sperm production (4). Although the ultrasonographic anatomy of the bull testis has been reported (5), ultrasonography has not been widely used as an adjunct to the standard breeding soundness examination. We postulated that there may be an association between ultrasonographic testicular echotexture and seminal quality and sperm production.

We have demonstrated the thermoregulatory function of the scrotum, testes, and testicular vascular cone in bulls and rams (2,6–9). However, regression analyses have not been conducted to explore relationships among independent variables such as testicular temperatures, size, tone, and echotexture, and dependent variables, such as seminal quality and sperm production. The objectives of the current study were to determine: 1) relationships among scrotal circumference, testicular tone, SST, internal scrotal and testicular temperatures, and testicular echotexture; and 2) relationships

Research Centre, Agriculture and Agri-Food Canada, Box 3000, Lethbridge, Alberta T1J 4B1 (Kastelic, Cook and Coulter); Department of Obstetrics and Gynecology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0X0 (Pierson).

Address correspondence and reprint requests to Dr. John P. Kastelic, telephone: 403-317-2236, fax: 403-382-3156, e-mail: kastelic@em.agr.ca.

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Table I. Mean (± SE) and range for scrotal/testicular characteristics, seminal quality and sperm production in 129 beef bulls

Endpoint	Mean ± SE	Minimum	Maximum
Scrotum and testes			
Scrotal circumference (cm)	33.7 ± 0.3	27.0	41.0
Testicular tone (mm displacement)	20.1 ± 0.1	18.0	22.0
Testicular echotexture (pixel intensity)	158.5 ± 2.1	107.5	211.3
Spermatozoa (%)			
Progressively motile sperm cells	47.7 ± 1.4	10.0	80.0
Normal sperm cells	62.1 ± 1.5	13.0	97.0
Primary sperm defects	7.3 ± 0.6	0.0	45.0
Secondary sperm defects	30.6 ± 1.4	2.0	77.0
Scrotal surface temperature (°C)			
Top	31.5 ± 0.2	24.7	34.8
Bottom	27.6 ± 0.3	16.4	32.1
Average	29.7 ± 0.2	21.6	33.2
SST gradient	3.9 ± 0.2	−1.5	8.9
Subcutaneous temperature (°C)			
Top	32.8 ± 0.1	27.6	37.4
Middle	32.4 ± 0.1	25.2	36.0
Bottom	32.2 ± 0.1	24.1	35.8
Intratesticular temperature (°C)			
Top	34.0 ± 0.1	27.0	36.2
Middle	34.1 ± 0.1	26.8	36.4
Bottom	34.1 ± 0.1	26.8	36.4
Epididymal temperature (°C)			
Caput	35.5 ± 0.1	30.0	38.0
Corpus	34.2 ± 0.1	28.8	37.3
Cauda	32.6 ± 0.1	27.0	35.8
Sperm production and reserves			
Daily sperm production (spermatozoa × 10^6/g)	14.0 ± 0.6	4.8	48.8
Epididymal sperm reserves (spermatozoa × 10^9)	16.9 ± 1.0	1.4	65.1

between those variables listed in Objective 1 and seminal quality, sperm production, and epididymal sperm reserves. The experiment was conducted in accordance with the guidelines of the Canadian Council on Animal Care (10) and with the approval of the Animal Care Committee at the Lethbridge Research Centre.

Materials and methods

Breeding soundness examinations were performed on 129 cross-bred (Angus cross) beef bulls approximately 16 mo of age. Scrotal circumference (Coulter Scrotal Tape; Trueman Manufacturing, Edmonton, Alberta) and testicular tone (Foote and Hahn Tonometer; Lane Manufacturing, Denver, Colorado, USA) were determined; the majority of these measurements were done by a single, experienced operator. Scrotal surface temperature (SST) of the caudal aspect of the scrotum was assessed with infrared thermography (Thermovision 782; AGA Infrared Systems AB, Danderyd, Sweden). Thermographic images were saved in a digital format and analyzed using dedicated computer software (Viewsoft Version 1.2; Viewscan Ltd., Concord, Ontario). The SST of the entire scrotum and SST at the top, middle, and bottom of the testes was determined as described (6). Testicular ultrasonography was performed (Tokyo-Keiki Model 3000 scanner, 7.5 MHz transducer; Tokyo-Keiki, Tokyo, Japan). Scanner settings that affect image attributes (i.e., time-gain compensation, gain) were standardized to predetermined values. Ultrasound examinations were recorded on an 8-mm camcorder (Sony CCD-V5000; Sony Corporation, Tokyo, Japan) using a composite video signal. Ultrasonographic images (frames of video) of the testis were recorded at different levels of the testis in transverse and longitudinal planes. The ultrasound image was frozen on the screen and the videotape allowed to run freely; this allowed for a stable section of tape for digital image acquisition (Panasonic AG-7355 S-VHS video recorder; Matsushita Electric, Tokyo, Japan) equipped with digital frame memory. Still images were captured by computer for analysis using a digital image acquisition system (PCVISION +, Imaging Technology, Woburn, Massachusetts, USA) with a resolution of 640 × 480 pixels and 256 shades of gray. Images from the ultrasound scanner, which had a gray-scale resolution of 256 levels, were maintained at 256 levels during image acquisition.

The echotexture of the testes was analyzed using a customized image analysis program optimized for ultrasonographic images (Synergyne, R.A. Pierson, Saskatoon, Saskatchewan) running on a high-level graphics workstation (SparcStation20; Sun Microsystems, Mount View, California, USA). The program was designed to

Table II. Pearson correlations between internal scrotal/testicular temperatures and scrotal surface temperature (SST) scrotal circumference (SC), testicular tone, and testicular echotexture in 129 beef bulls

	SST				Testicular		
	Top	Bottom	Average	Gradient	SC	Tone	Echotexture
Subcutaneous temperature							
Top	0.3808[a]	0.3915[a]	0.4496[a]	−0.2470	0.1150	0.2325[b]	0.2636[b]
Middle	0.3862[a]	0.4129[a]	0.4701[a]	−0.2624[b]	0.0820	0.2074[c]	0.2697[b]
Bottom	0.3926[a]	0.4483[a]	0.4862[a]	−0.2662[b]	−0.0069	0.1090	0.2161[c]
Intratesticular temperature							
Top	0.2672[b]	0.2507[b]	0.3004[a]	−0.1844[c]	0.1783[c]	0.1141	0.1820[c]
Middle	0.2567[b]	0.3099[a]	0.3323[a]	−0.2332[b]	0.1491[d]	0.0250	0.1016
Bottom	0.2676[b]	0.3116[a]	0.3390[a]	−0.2412[b]	0.1662[d]	0.0040	0.1358
Epididymal temperature							
Caput	0.1304[e]	0.0507	0.1126	0.0909	0.0762	0.0976	−0.0489
Corpus	0.2019[c]	0.1786[c]	0.2275[c]	−0.0954	0.0659	0.1964[c]	0.1614[d]
Cauda	0.1879[c]	0.2112[c]	0.2708[b]	−0.1323[e]	0.0355	0.0347	0.2066[c]

[a–e] Probabilities under the null hypothesis: [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$, [d]$P < 0.1$, [e]$P < 0.15$

quantify characteristics for user-selected regions of an image. Echotexture was defined in terms of mean pixel value, quantified using values from 0 (black) to 255 (white). Mean pixel values were evaluated by selecting 6 randomly selected computer-generated circles containing 20 pixels (approximately 2 mm in diameter) within the central portion of the image of the testis, taking care to avoid the mediastinum testis and the tunica albuginia. To avoid artifacts induced by enhanced through transmission and/or shadowing, the areas of the testis were confined to the 10 o'clock and 2 o'clock positions of the testis. Calculations were done on the mean values from 120 pixels. Image analysis was done without knowledge of any other data collected for each bull.

Semen was collected by electroejaculation; 0.2 mL was diluted with 2.7% sodium citrate for determination of progressive motility and 0.2 mL was diluted with 0.2% glutaraldehyde fixative for morphological examination. The percentage of morphologically normal and abnormal spermatozoa (primary defects were those affecting the head; secondary defects were those affecting the midpiece or tail) and the percentage of progressively motile spermatozoa were evaluated with phase-contrast microscopy at 1000X.

Assessment of SST was conducted before and approximately 20 min after administration of caudal epidural analgesia using xylazine HCl (Rompun; Chemagro Ltd., Etobicoke, Ontario), 0.07 mg/kg, as described (6). Following post-epidural assessment of SST, scrotal subcutaneous (SQT), intratesticular (ITT), and intraepididymal temperatures (IET) were measured (with needle thermocouples) at 3 locations in each testis (top, middle, and bottom) as previously described (6). Following temperature measurements, testes were either collected immediately (via surgical castration, $n = 91$) or at slaughter (approximately 5 d later, $n = 38$). Daily sperm production and epididymal sperm reserves were determined as described (11).

A paired student's t-test was used to compare SST before versus after analgesia. Stepwise regressions were performed (12), with dependent variables including progressive sperm motility, sperm morphology, daily sperm production per gram of testicular parenchyma and epididymal sperm reserves. Independent variables included scrotal circumference, testicular tone, testicular echotexture, and top, middle, and bottom measurements for SST, SQT, ITT, and IET. Correlations were determined between dependent and independent variables and only those that approached significance ($P < 0.15$) were considered for inclusion in the regression analyses. Furthermore, collinearity tests (12) were performed to remove independent variables that were similar, leaving only those with the highest correlations to be included in regression analyses. Scatter plots were done to insure that a small number of outliers did not unduly affect the analyses.

Results and discussion

There was no difference ($P > 0.2$) in SST before versus after analgesia. Mean values and ranges for breeding soundness examination variables, SST and internal scrotal/testicular temperatures, daily sperm production, and epididymal sperm reserves are shown in Table I. Correlation coefficients (and their probabilities) are shown in Table II (SST and testicular echotexture) and Table III (seminal quality). There were consistent positive correlations between SST and internal scrotal/testicular temperatures, indicating that SST was related to underlying temperatures. Coulter et al (13) reported positive correlations ($P < 0.0001$) between SST and both subcutaneous temperature and temperature of a surrogate testis, $r = 0.95$ and 0.91, respectively. In a previous study (6), correlation coefficients between SST and SQT were moderate to high, those between SQT and ITT were low to moderate, and those between SST and ITT were low. Furthermore, we have shown that SST decreases from the top of the scrotum to the bottom, while ITT is significantly cooler at the top than the bottom (6–8). Therefore, caution should be exercised in the interpretation of scrotal thermograms as a method to predict testicular temperature, as previously indicated (6). Negative correlations between SQT, ITT, IET, and testicular echotexture in conjunction with positive correlations between the percentage of morphologically normal sperm, daily sperm production, and testicular echotexture were consistent with the principle that increased testicular temperature is deleterious to spermatogenesis.

Table III. Pearson correlations between dependent and independent variables in 129 beef bulls

	Spermatozoa (%)					
	Motile	Normal	Primary defects	Secondary defects	DSP[a] ($\times 10^7$/g)	ESR[b] ($\times 10^9$)
Scrotal circ.	0.1993[f]	0.0636	−0.1795[f]	0.0195	−0.2232[f]	0.3464
Testic. tone	0.0830	0.0496	−0.1380[g]	0.0254	0.0408	−0.2544[e]
Echotexture	0.0773	0.0646	−0.0052	−0.0664	0.3046	0.2258
Top SST[c]	0.1888[f]	−0.1161	−0.0112	0.1206	−0.0583	0.0795
Bottom SST	0.2044[d]	−0.0799	−0.2241	0.1917[d]	−0.1159	0.0381
Average SST	0.2682	−0.0677	−0.1340[g]	0.1352[g]	−0.0394	0.0662
SST gradient	−0.0548	0.0862	0.1807[f]	−0.1866[f]	−0.0761	−0.1425

[a] Daily sperm production

[b] Epididymal sperm reserves

[c] Scrotal surface temperature

[d–g] Probabilities under the null hypothesis: [d]$P < 0.001$; [e]$P < 0.01$; [f]$P < 0.05$; [g]$P < 0.15$

Table IV. Regression models for sperm motility, seminal quality, daily sperm production, and epididymal sperm reserves for 129 beef bulls

Dependent/Independent variables	Slope	R^2	Probability
Motile sperm (%; y-intercept = −39.05)			
Average SST	1.74	0.07	0.003
Scrotal circumference	1.03	0.03	0.037
Primary sperm defects (%; y-intercept = 34.34)			
Bottom scrotal surface temperature	−0.51	0.05	0.012
Scrotal circumference	−0.39	0.02	0.094
Secondary sperm defects (%; y-intercept = 6.73)			
Bottom SST	0.84	0.03	0.060
Daily sperm production (cells $\times 10^6$/g; y-intercept = 10.89)			
Testicular echotexture	0.07	0.09	0.002
Scrotal circumference	−0.43	0.04	0.024
Epididymal sperm reserves (cells $\times 10^9$/g; y-intercept = 48.41)			
Scrotal circumference	1.22	0.14	0.0001
Testicular tone	−2.68	0.05	0.008

Regression models for sperm motility and morphological characteristics, daily sperm production and epididymal sperm reserves as dependent variables are shown in Table IV. There was a positive linear regression ($P < 0.06$) of bottom SST with secondary sperm defects, consistent with the expectation that higher SST will impair seminal quality. However, there was a negative linear regression ($P < 0.012$) of bottom SST with primary sperm defects and a positive linear regression ($P < 0.003$) of average SST with the percentage of progressively motile sperm. These findings were inconsistent with expectations and previous reports where a decrease in average SST was associated with a decrease in the incidence of sperm defects (2) and an increase in SST gradient was associated with increased sperm motility (3). Although the testes must be maintained at a temperature cooler than that of the body cavity (1), it appears that the relationship between SST and seminal quality is complex.

Testicular echotexture had a positive linear regression with daily sperm production ($P < 0.002$); as the image became whiter (and, therefore, more dense) sperm production increased. Therefore, determination of testicular echotexture may be a valuable additional tool for breeding soundness evaluation in bulls. Previously, daily sperm production in the live bull could only be determined through biopsy of the testicular parenchyma (14). In a previous study with a small number of bulls (15), there was no apparent change in the ultrasonographic appearance of the testes in bulls with induced testicular degeneration. However, in that study, evaluations were only done visually; perhaps computerized image analysis would have detected subtle changes that were not apparent visually.

Testicular tone had a negative linear regression ($P < 0.006$) with epididymal sperm reserves. These results contradict previous findings that indicated that bulls with higher testicular tone have better seminal quality and sperm production (16). Unfortunately, with the current tonometers, measurements are typically in a narrow range (18 to 23 mm displacement). A more accurate, less subjective tonometer would improve the usefulness of this measurement. Perhaps ultrasonography could be used in lieu of a tonometer as they are both measuring the density of the testicular parenchyma.

Scrotal circumference had a positive linear regression ($P < 0.037$) with sperm motility, a negative linear regression ($P < 0.094$) with primary sperm defects, and a positive linear regression ($P < 0.0001$) with epididymal sperm reserves. These results were in agreement with

previous findings (2,17,18) where scrotal circumference was positively associated with sperm motility, seminal quality, and sperm production. The negative regression between scrotal circumference and daily sperm production observed in the present study contradicted the relationships with motility, defects and sperm reserves and were perhaps associated with some other variable not identified in the present study. Bulls with large testes generally produce large numbers of motile, morphologically normal spermatozoa (19).

Reproductive potential varies considerably among beef bulls (19). However, selection criteria to identify bulls with superior reproductive capacity remain poorly defined. Unfortunately, no single test or measurement has great predictive value; even a large number of measurements using very different technologies usually account for only a small proportion of the variation in fertility. For example, a linear regression model for predicting bull fertility involving scrotal circumference, seminal quality, backfat thickness, and libido accounted for only 29% of the total variation in fertility of 277 bulls used under multiple-sire, range breeding conditions (20). In the present study, although the regression models were significant, they accounted for less than 20% of the variation. Perhaps neural network modelling would result in better predictive models than those derived with multiple linear regression, as has been done with prediction of beef tenderness (21). Furthermore, although there was a considerable range in the independent variables measured in the present study, the bulls were of a similar genetic background and age, which may limit the extent to which these findings can be extrapolated. Notwithstanding, it appears that scrotal circumference is still the best indicator of reproductive potential; bulls with large testes usually produce large numbers of normal spermatozoa. In addition to seminal quality and scrotal circumference, testicular ultrasonographic echotexture has considerable promise for augmenting breeding soundness examinations of bulls.

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