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ENDOCRINE AND THERMAL RESPONSES TO GnRH TREATMENT AND PREDICTION OF SPERM OUTPUT AND VIABILITY IN HOLSTEIN-FRIESIAN BREEDING BULLS

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ABSTRACT

A study was conducted to determine changes in serum LH and testosterone concentrations and in scrotal surface temperature (SST; measured with infrared thermography) following GnRH treatment and to predict the number of spermatozoa collected and the proportion that were viable. Holstein-Friesian breeding bulls (n=22, average age, 24.3 mo; range, 15 to 41 mo) were examined twice 30 d apart. Concurrently, semen was collected twice weekly with an artificial vagina. Treatment with GnRH (100 μg, im) increased ($P<0.0001$) serum LH and testosterone concentrations and increased ($P<0.0001$) SST (range 0.6 to 1.1°C; $P<0.05$) at the top and bottom of the scrotum. In regression models to predict the total number of spermatozoa, significant independent variables included ultrasonic echotexture of the testes (negative slope), scrotal width (positive slope) and SST at the bottom of the scrotum 45 min after GnRH treatment (positive slope). In regression models to predict the percentage of live spermatozoa, ultrasonic echotexture was a significant independent variable (negative slope). Measurement of testicular ultrasonic echotexture and SST after GnRH treatment augmented measurement of testicular size for predicting the number and percentage of live spermatozoa.

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Key words: bulls, testes, thermography, echotexture, semen

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INTRODUCTION

Although scrotal circumference is the most common method for predicting spermatozoa production in bulls (3) other technologies may also have merit. Ultrasonography is a noninvasive tool for assessing testes and detecting testicular pathology (2, 10, 12). Infrared thermography has been used to evaluate scrotal surface temperatures (SST; 1) and as an adjunct to a standard breeding soundness examination (11). Post (13) and Wekerle (16) suggested that blood testosterone concentrations following GnRH treatment could be useful in predicting reproductive potential in bulls.

The objectives of the current study were to 1) determine the change in SST and serum concentrations of LH and testosterone after GnRH treatment and 2) predict the number of spermatozoa collected and the percentage of live spermatozoa.

MATERIALS AND METHODS

Twenty-two Holstein-Friesian breeding bulls (average age, 24.3 mo; range, 15 to 41 mo) at the National AI Centre in Debrecen, Hungary, were examined on 2 occasions, designated as Examinations A and B, respectively, 30 d apart (October and November, 1995). During the examinations, ambient temperature in the barn ranged from 6 to 8°C. At each examination, the bulls were in the same sequence and the same procedures were conducted. Scrotal circumference (SC) was measured with a cloth scrotal tape (Examination 1) or with a Coulter Scrotal Tape (Examination 2; Trueman Manufacturing Ltd., Edmonton, AB). An image of the posterior aspect of the scrotum was recorded from a consistent distance with a video camera (JVC GR-AX200, Victor Co., Yokohama, Japan). The image was subsequently digitized (Super Match VideoSpigot Capture Card, VidCap 1.00 Microsoft software) and stored on the hard disk of an IBM Thinkpad (360 CSE; IBM U.K. Ltd., Greenock, UK). Customized image analysis software (Testigabsas Analysis) was used to determine scrotal width. Each testis was examined ultrasonically, using a B-mode diagnostic ultrasound scanner with a 7.5 MHZ linear transducer (Scanner 450 VET Echograph, Pie Medical, Maastricht, The Netherlands). The transducer was applied against the posterior surface of the scrotum (perpendicular to the vertical axis) and was aligned at the center of the testis. A custom electromechanical device for holding the transducer was used (7). When the transducer was applied with a force of approximately 0.76 kg/m^2, a small lamp lighted and the image was frozen and subsequently captured on a computer and analyzed with custom software, as described above. The mean grey level, designated as echotexture (range, 0 to 63 levels), was determined in an area 1.0 x 6.5 cm just below the tunica albuginea.

An intramuscular injection of 100 µg GnRH was administered. For Examination A, Ovurelin (Reanal, Hungary) was used, while Gonavet 50 (Veyx-Pharma, Bernburg, Germany) was used for Examination B. Scrotal surface temperature was measured before treatment and 45 min after (6). An AGEMA 880 LWB (AGEMA, Danderyd, Sweden) infrared thermography camera was used. Thermograms were stored on a computer and subsequently analyzed with Irwin2 image analysis software (AGEMA, Danderyd, Sweden). The SST at the top of the scrotum (SSTTOP) and the SST at the bottom of the scrotum (SSTBOTTOM) were measured using a line one pixel high and the width of the scrotum. The difference between these 2 measurements was calculated and denoted as

the SST gradient (SSTGRADIENT). Blood samples were collected by jugular venipuncture, on 2 occasions: just prior to GnRH treatment and 90 min after treatment. Blood was allowed to clot, after which it was centrifuged and stored at -20°C until analysis. Serum LH concentrations were determined (in the laboratory of Albert Szentgyörgyi, Medical University, Szeged, Hungary) with an in vitro, Leydig cell bioassay as previously described (15). For this assay, the amount of testosterone produced in response to serum LH was determined. The sensitivity of the assay was 0.2 U/L, and the intra- and inter-assay coefficients of variation were 7.6 and 12.5%, respectively. Serum testosterone concentrations were measured by radioimmunoassay using tritium, as previously described (4). The sensitivity of the assay was 0.3 nmol, and the intra- and inter-assay coefficients of variation were 7.1 and 11.7%, respectively.

Semen was collected with an artificial vagina twice-weekly from all bulls (1 ejaculation on each collection day), starting 2 wk before Examination A and ending 2 wk after Examination B. Sperm density and the percentage of live (motile) spermatozoa were determined by photometry and by visual microscopic evaluation, respectively. Data were used only if ejaculates met minimum standards, including a minimum of 80% morphologically normal spermatozoa and less than 10% with acrosomal defects and a minimum of 35% with linear motility post-thawing.

All statistical analyses were conducted with the Statistical Analysis System (14). The 2 examinations were analyzed separately. Pearson correlation coefficients were calculated between the number of spermatozoa, the percentage of live spermatozoa, and all other end points. Stepwise linear regression analyses were performed for number of spermatozoa and for percentage of live spermatozoa as the dependent variables. For each analysis, all other end points that were correlated ($P<0.15$) with the dependent variable were considered as potential independent variables. First, Pearson correlation coefficients were calculated between all possible independent variables. If 2 possible independent variables were correlated ($P<0.1$), then only the one correlated with the dependent variable was included in the regression analysis. Paired Student's t-tests were used to determine the effect of GnRH treatment on serum concentrations of LH and testosterone and on SSTTOP, SSTBOTTOM and SSTGRADIENT.

RESULTS

Means (± SE) for all end points and Pearson correlation coefficients between the number of spermatozoa and the percentage of live spermatozoa and each other end point are shown (Table 1). The SSTTOP, SSTBOTTOM and serum concentrations of LH and testosterone consistently and significantly increased following GnRH treatment. There were several significant correlations between the number of spermatozoa and the percentage of live spermatozoa and the other end points. Regression models (model r^2, 0.14 to 0.69) for the number of spermatozoa and for the percentage of live spermatozoa as dependent variables are also shown (Table 2).

DISCUSSION

Serum concentrations of both LH and testosterone increased dramatically following GnRH treatment, as previously reported (13, 16). The SSTTOP and SSTBOTTOM measures increased significantly in response to GnRH treatment, with the increase at the bottom exceeding the increase

Table 1. Means (± SE) for all end points and Pearson Correlation coefficients between the number of spermatozoa and percentage of live spermatozoa and all other end points for bulls (n=22) examined on 2 occasions (A and B). For LH, testosterone and scrotal surface temperature, 1 and 2 refer to before and after GnRH treatment, respectively, and Δ refers to the difference between these 2 measurements.

	Mean + SE		Correlation coefficients			
			No. of sperm cells		Live sperm cells (%)	
End points	Examination A	Examination B	A	B	A	B
Morphologic						
Scrotal circumference (cm)	37.1 ± 0.8	36.2 ± 0.6	.58[c]	.32	-.03	.25
Scrotal width (cm)	13.9 ± 0.3	14.9 ± 0.4	.22	.55[c]	.10	.17
Echotexture (units, 0-63)	22.8 ± 1.0	22.2 ± 0.9	-.70[d]	-.29	-.07	-.48[b]
Serum LH (U/l)						
LH1	3.7 ± 0.8	7.6 ± 2.1	.39[a]	.02	-.26	.05
LH2	13.0 ± 1.3	16.7 ± 1.8	.01	-.03	.03	.01
LHΔ	9.3 ± 1.6[d]	9.1 ± 2.3[d]	-.20	-.04	.16	.04
Serum testosterone (nmol/l)						
Testosterone1	6.8 ± 1.7	10.7 ± 2.5	.15	-.03	-.30	.03
Testosterone2	20.6 ± 1.3	24.6 ± 1.7	.20	-.05	-.38[a]	.17
TestosteroneΔ	13.8 ± 1.3[d]	13.9 ± 2.3[d]	.01	-.01	.02	.09
Scrotal surface temperature (^{o}C)						
TOP1	34.2 ± 0.2	36.0 ± 0.4	-.30	-.02	-.11	-.33
TOP2	34.9 ± 0.2	36.6 ± 0.3	.19	.02	-.09	-.28
TOPΔ	0.7 ± 0.2[c]	0.6 ± 0.3[b]	.58[b]	.05	.04	.16
BOTTOM1	29.1 ± 0.2	30.3 ± 0.3	-.08	.13	.07	-.39[a]
BOTTOM2	29.9 ± 0.2	31.4 ± 0.3	.43[b]	.42[b]	-.09	-.06
BOTTOMΔ	0.8 ± 0.2[d]	1.1 ± 0.3[d]	.47[b]	.31	-.15	.44[b]
GRADIENT1	5.1 ± 0.2	5.6 ± 0.2	-.28	-.21	-.20	.03
GRADIENT2	5.0 ± 0.2	5.2 ± 0.3	-.18	-.40[a]	-.03	-.22
GRADIENTΔ	-0.1 ± 0.2	-0.4 ± 0.2[b]	.16	-.31	-.22	.33
Spermatozoa						
No. per ejaculate ($x10^{9}$)	6.3 ± 0.4	6.3 ± 0.3	--	--	--	--
Live (%)	63.8 ± 0.4	63.9 ± 0.4	--	--	--	--

[a-d]Means with a superscript indicate a difference between measurements made before and after GnRH treatment; correlation coefficients with a superscript indicate a correlation with the endpoint listed in the same row: [a]P<0.1; [b]P<0.05; [c]P<0.01; [d]P<0.001.

Table 2. Regression models for total number of spermatozoa and percentage live spermatozoa in bulls (n=22) examined on 2 occasions (A and B). For testosterone and scrotal surface temperature (BOTTOM), 2 refers to measurements after GnRH treatment, respectively, and GRADIENTΔ refers to the difference between top and bottom measurements for scrotal surface temperature made before and after GnRH treatment.

	Slope	R^2	Prob.
Number of spermatozoa			
Examination A (y intercept = -20.64, R^2 = 0.69)[a]			
Echotexture	-0.30	0.49	0.0003
BOTTOM2	1.13	0.20	0.002
Examination B (y intercept = -14.15, R^2 = 0.44)[a]			
Scrotal width	0.47	0.30	0.008
BOTTOM2	0.43	0.14	0.042
Percentage live spermatozoa			
Examination A (y intercept = 66.1, R^2 = 0.14)[a]			
Testosterone2	-0.11	0.14	0.080
Examination B (y intercept = 68.3, R^2 = 0.36)[a]			
Echotexture	-0.21	0.24	0.022
GRADIENTΔ	-0.61	0.12	0.073

[a]R^2 = total R^2 for the model.

at the top. In a previous study (8), when bulls were exposed to different ambient temperatures, changes in bottom SST were larger than in top SST. Therefore, bottom SST seems to be more labile. Increased serum testosterone concentrations may have been associated with the increased SST (e.g. due to increased blood flow). Further study is needed to confirm the increase in SST and to determine the mechanism responsible.

Scrotal circumference was highly correlated ($r=.58$, $P<0.01$) with the number of spermatozoa collected in Examination A, consistent with previous reports (3). Although this correlation was lower in Examination B ($r=.32$, $P<0.15$), the correlation between the number of spermatozoa and scrotal width (another measure of testicular size) was large and significant ($r=.55$, $P<0.01$). The correlation of echotexture with number of spermatozoa in Examination A was likewise noteworthy ($r=-.70$, $P<0.001$). In Examination B, this correlation was negative but much smaller ($r=-.29$, $P<0.2$). In

previous investigations (5) using the same equipment and software as that used in the present study, we found a negative correlation between the testicular echotexture and the cross sectional area of the seminiferous tubules ($r=-.50$, $P<.001$). Lenz et al. (10) reported that the ultrasonic texture score was lower in human testes with active seminiferous tubules than in those that were inactive. Therefore, echotexture appears to be related to the sperm-producing capabilities of the testis. There were also significant correlations of SST with number of spermatozoa, including SSTBOTTOM2, which was significant in both Examinations A and B.

Using step-wise regression equations for the number of spermatozoa collected, we were able to account for much of the variation (model r^2 of 0.69 and 0.44 in Examinations A and B, respectively). In Examination A, echotexture accounted for nearly half of the variation ($r^2=.49$) in the number of spermatozoa, while in Examination B scrotal width accounted for 30% of the variation. In both examinations, SSTBOTTOM2 was a significant independent variable. The slope for SSTBOTTOM2 was positive; therefore, the warmer the bottom of the scrotum was (following GnRH treatment), the higher the number of spermatozoa collected. Perhaps the greater increase in SSTBOTTOM2 indicates increased thermoregulatory ability.

The percentage of live spermatozoa was negatively correlated to testosterone2 ($r=-.38$, $P<0.1$; Table 1) in Examination A. Consequently, the step-wise regression model did not account for much of the variation (model $r^2=.14$, $P<0.08$; Table 2). In Examination B, the percentage of live spermatozoa was negatively correlated with echotexture ($r=-.48$, $P<0.05$; Table 1), SSTBOTTOM1 and SSTBOTTOMΔ. The latter 2 independent variables could not be included in the step-wise regression analysis because they were significantly correlated with echotexture. The SSTGRADIENTΔ measurement in Examination B had a relatively low correlation with the percentage of live spermatozoa ($r=0.33$, $P<0.13$; Table 1), but it did contribute to the regression model (Table 2).

In conclusion, SST and serum concentrations of LH and testosterone increased following GnRH treatment. Measurement of testicular echotexture and SST after GnRH treatment will augment the use of measurement of testicular size for predicting the number and percentage of live spermatozoa.

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