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Morphologic, endocrine and thermographic measurements of testicles in comparison with semen characteristics in mature Holstein–Friesian breeding bulls

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Abstract

Twenty Holstein–Friesian breeding bulls (62–79 months of age) were examined 3 times, at 30-day intervals. Scrotal thermograms for assessment of scrotal surface temperature (SST) and blood samples for plasma testosterone concentrations were taken just before and then 45 and 90 min, respectively, after treatment with GnRH (50 μg, Gonavet, i.m. per bull). Following GnRH treatment, there generally were significant increases in mean values of both top SST (range, -0.1 to 1.4°C) and bottom SST (range, 0.3 to 1.8°C). Scrotal circumference was highly repeatable but SST and video-measurements of scrotal dimensions were less repeatable, because apparently they were affected by ambient temperature. Plasma testosterone concentrations before GnRH treatment were more repeatable than those after GnRH treatment. Correlations between examinations of 0.67 to 0.81 and -0.14 to 0.47, respectively, but the converse was true for SST measurements. Semen was collected with an artificial vagina 3 times per week for 12 weeks starting 2 weeks before the first examination. The total number of spermatozoa per ejaculate was highly repeatable and the percentage of motile and live spermatozoa were relatively consistent. Separate regressions for each variable and for each examination were conducted for these 3 semen characteristics as dependent

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variables. For the number of spermatozoa per ejaculate and for the percentage of motile spermatozoa, significant independent variables were plasma testosterone concentrations and difference between top and bottom SST, respectively. The slopes of these equations were nearly all negative and the R^2 was from 0.15 to 0.42. For prediction of the percentage of live spermatozoa, both SST gradient and plasma testosterone concentrations were significant independent variables. For these regressions, the slopes were negative and the regression coefficients were generally lower than for the other 2 dependent variables (range, 0.16 to 0.25). Treatment with GnRH and assessment of SST and plasma testosterone concentrations have some correlation with the semen production in the mature bull. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Infrared thermography; Echotexture; Testosterone; Cattle—male reproduction; Testis characteristics; Semen characteristics

1. Introduction

Many approaches have been utilized to predict semen characteristics in bulls. The most common method for predicting spermatozoa production is scrotal circumference (Coulter and Foote, 1976). Post (1978, 1987) reported that serum testosterone concentrations following GnRH treatment were closely correlated with the 24-h testosterone profile. Braun et al. (1988) showed the potential value of measuring testosterone and LH secretion (after administration of GnRH) as an indicator of semen quality in adult bulls. Lunstra and Coulter (1997) reported that infrared thermography of the scrotum showed promise for predicting semen quality and fertility in the bull. Ultrasonography, a noninvasive diagnostic tool (Pechman and Eilts, 1987; Coulter and Bailey, 1988), can be used to detect testicular pathology. Furthermore, changes in echotexture in peripubertal bulls (Evans et al., 1996) suggested that ultrasonography may be useful for prediction of sperm production.

The objectives were to: (1) determine the effect of GnRH treatment on plasma testosterone concentrations and Scrotal Surface Temperature (SST); (2) determine the repeatability of different morphologic, thermal and endocrine measures before and after GnRH treatment; and (3) examine the correlation among the total number of spermatozoa and the proportion of live and motile spermatozoa with the different morphologic, thermal and endocrine measures before and after GnRH challenge.

2. Material and methods

Twenty Holstein–Friesian breeding bulls (71.3 ± 4.4 months of age), at the National AI Centre, Gödöllö, Hungary, were used in this experiment. Bulls were examined 3 times, at 30-day intervals (starting in mid-April). Scrotal circumference (SC) was measured with a Coulter Scrotal Tape (Trueman Manufacturing, Edmonton, AB). Scrotal width (SCW) and length (SCL) was determined with our computer assisted video-digitizing method (Gábor et al., 1996). A Scanner 450 VET echograph with a 7.5 MHz linear-array transducer (Pie Medical, Netherlands) was used for ultrasonic examinations of the testes. A custom electro-mechanical device was used to insure a consistent

pressure (approximately 0.76 kg) between the transducer and the scrotal surface (Gábor et al., 1996). The ultrasound image was frozen, recorded on the hard disk drive of an IBM Thinkpad 360 CSE (IBM, Greenock, UK) and testicular echotexture determined with custom software (Testigabsas; Gábor et al., 1996).

The bulls were not in service prior to the start of the experiment. Therefore, semen was collected (with an artificial vagina) 3 times prior to Examination 1 and then 3 times each week for 14 consecutive weeks. For all ejaculates, the percentage of live and the percentage of motile spermatozoa was determined with a HTM 2000 motility analyzer (IMV, L'Aigle, France). The total number of spermatozoa was determined only for ejaculates that were of sufficient quality for cryopreservation.

An AGEMA 880 LWB (AGEMA, Danderyd, Sweden) infrared thermography camera was used to measure SST. Just before and 45 min after a single i.m. injection of 50 μg

Table 1
Morphologic, thermal, sperm, and endocrine data and correlation analyses for 20 bulls examined on 3 occasions

Measures	Examination (Mean ± SE)			Correlation coefficients		
	1	2	3	1 and 2	2 and 3	1 and 3
Morphologic						
Scrotal circumference, (cm)	39.9 ± 0.6	40.1 ± 0.5	39.3 ± 0.5	0.58[b]	0.77[c]	0.69[c]
Scrotal width, (cm)	15.0 ± 0.3	15.1 ± 0.3	17.8 ± 0.2	0.59[b]	0.53[b]	0.17
Scrotal length, (cm)	16.3 ± 0.3	16.9 ± 0.3	19.2 ± 0.2	0.32	0.27	−0.01
Echotexture (64 gray scale)	14.8 ± .5	19.4 ± 0.6	21.1 ± 0.5	0.65[b]	0.73[c]	0.58[b]
Scrotal Surface Temperature, °C						
TOP1 (before GnRH)	33.7 ± 0.1	34.8 ± 0.2	36.4 ± 0.1	0.28	0.49[b]	0.21
TOP2 (after GnRH)	35.1 ± 0.1	35.1 ± 0.2	36.3 ± 0.2	0.39	0.59[b]	0.35
TOP Δ	1.44 ± 0.1	0.40 ± 0.1	−0.10 ± 0.2	−0.09	0.49[b]	0.08
BOT1 (before GnRH)	30.4 ± 0.2	30.8 ± 0.2	33.1 ± 0.2	0.31	0.56[b]	0.35
BOT2 (after GnRH)	32.2 ± 0.2	31.5 ± 0.2	33.4 ± 0.2	0.44[a]	0.67[c]	0.29
BOT Δ	1.80 ± 0.2	0.70 ± 0.2	0.30 ± 0.2	0.30	0.43[a]	0.38
GRAD1	3.3 ± 0.2	3.9 ± 0.3	3.3 ± 0.3	0.38	0.56[b]	0.32
GRAD2	2.9 ± 0.2	3.6 ± 0.2	2.9 ± 0.2	0.43[a]	0.66[b]	0.40
GRAD Δ	−0.37 ± 0.1	−0.3 ± 0.2	−0.36 ± 0.2	0.42[a]	0.53[b]	0.29
Spermatozoa						
Total number ($\times 10^9$)	6.5 ± 0.6	6.2 ± 0.8	6.7 ± 0.7	0.49[b]	0.77[c]	0.74[c]
Live (%)	67.6 ± 0.6	66.6 ± 0.7	67.6 ± 1.0	0.21	0.15	0.65[b]
Motile (%)	86.5 ± 1.2	87.3 ± 3.5	89.0 ± 1.1	0.61[b]	0.25	0.15
Endocrine						
Serum testosterone (nmol/l)						
Initial	28.5 ± 3.9	20.6 ± 3.9	29.7 ± 2.2	0.67[c]	0.81[c]	0.75[c]
GnRH induced	39.3 ± 3.4	39.8 ± 4.9	49.3 ± 5.9	−0.14	0.47[a]	0.04
Δ	10.8 ± 5.1	19.2 ± 3.5	19.5 ± 5.0	−0.39[a]	0.13	−0.51[b]

[a,b,c] Correlations significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.
TOP, BOT = Measurement at the top and bottom of the scrotum, respectively.
GRAD = Gradient from top to bottom of the scrotum, TOP minus BOT.
Δ = Differences in measures before and after GnRH.

GnRH (Gonavet, VetPharma, Germany), thermograms of the posterior surface of the scrotum were obtained, stored in the computer, and subsequently evaluated with Irwin2 software (AGEMA). The temperature of a line one pixel high and the width of the scrotum were determined at the top and bottom of the scrotum (SST TOP and SST BOT, respectively). Blood samples were collected just before and 90 min after GnRH treatment. Samples were centrifuged and plasma frozen pending determination of plasma testosterone concentration by ^{3}H radioimmunoassay (Gábor et al., 1992). 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.

All statistical analyses were conducted with the Statistical Analysis System (SAS, 1990, Cary, NC). First, Pearson correlation coefficients were calculated between all end points. Then, regression analyses were conducted for sperm end points as dependent variables. Independent variables were morphologic, SST or plasma testosterone concentrations that were significantly correlated with sperm end points. Initially, single independent variables were used. Thereafter, several independent variables were subjected to Maximum R-square Improvement Analysis (multiple regression). A one-sample Student's *t*-test was used to determine the effect of GnRH treatment on plasma testosterone concentrations and SST.

3. Results

Morphologic, thermal, sperm, and endocrine data are in Table 1 and measurements before and after GnRH treatment are in Table 2. Scrotal circumference was repeatable among all 3 examinations. The bulls were mature (no substantial age-related changes in SC were expected over a 3-month period) and a consistent tension measuring tape was used. The SCW and SCL were consistent between the first 2 examinations, but were substantially greater in Examination 3. There were highly significant correlations among examinations for testicular echotexture. However, the mean echotexture was relatively larger with each successive examination.

Table 2
A one-sample *t*-test for the determination of the effect of the GnRH treatment on the change (Δ, Mean $\pm$ SE) in scrotal surface temperature and plasma testosterone concentrations in 20 bulls examined on 3 occasions

Variable	Examination 1	*P*	Examination 2	*P*	Examination 3	*P*
Thermal						
TOP Δ	1.44 ± 0.13	< 0.00001	0.40 ± 0.13	< 0.03	-0.1 ± 0.15	< 0.63
BOT Δ	1.80 ± 0.15	< 0.00001	0.70 ± 0.15	< 0.001	0.30 ± 0.16	< 0.07
GRAD Δ	-0.37 ± 0.13	< 0.01	-0.30 ± 0.22	< 0.08	-0.36 ± 0.16	< 0.12
Endocrine						
Testosterone Δ	10.8 ± 5.10	< 0.04	19.2 ± 3.49	< 0.01	19.5 ± 4.98	< 0.0001

TOP, BOT = Measurement at the top and bottom of the scrotum, respectively.
GRAD = Gradient from top to bottom of the scrotum, TOP minus BOT.
Δ = Differences in measures before and after GnRH.

Furthermore, there was an apparent increase in Leydig cell response, as the increase in plasma testosterone concentrations following GnRH was greater at Examinations 2 and 3 compared to Examination 1 (Tables 1 and 2). As expected (Byerley et al., 1990; Thompson et al., 1992, 1994), plasma testosterone concentrations increased substantially after GnRH treatment (Tables 1 and 2). However, our basal concentrations of plasma testosterone were more repeatable than those following GnRH treatment. Consequently, the testosterone had inconsistent repeatability (Table 2).

Top and bottom SST and the SST gradient (Table 1) were generally similar to previous reports (Kastelic et al., 1997). The ambient temperature in the barn was moderate during Examinations 1 and 2 but high during Examination 3 (approximately 10, 20 and 26°C, respectively). Remarkably, the average SST gradient for Examination 3

Table 3
Correlation coefficients and probability values (in parentheses) for endocrine and semen variables for 20 bulls examined on 3 occasions

Independent variables	Dependent variables		
	Examination 1	Examination 2	Examination 3
	Total sperm number per ejaculate		
Endocrine			
Testosterone			
Initial	−0.47(0.04)	−0.62(0.004)	−0.35(0.13)
GnRH induced	0.31(0.19)	−0.47(0.04)	−0.59(0.006)
Δ	0.58(0.008)	−0.27(0.24)	−0.44(0.05)
Thermal			
	Motile sperm %		
SST			
TOP1	0.11(0.62)	−0.41(0.08)	−0.48(0.03)
TOP2	−0.36(0.11)	−0.39(0.09)	−0.36(0.12)
TOP Δ	−0.27(0.25)	−0.10(0.66)	0.06(0.79)
BOT1	−0.15(0.53)	0.33(0.15)	0.63(0.003)
BOT 2	0.02(0.93)	0.15(0.52)	0.18(0.044)
BOT Δ	0.26(0.26)	−0.17(0.48)	−0.49(0.03)
RAD1	0.28(0.24)	−0.43(0.06)	−0.65(0.002)
GRAD2	−0.14(0.56)	−0.41(0.08)	−0.40(0.08)
GRAD Δ	−0.55(0.01)	0.07(0.76)	−0.38(0.10)
	Live sperm %		
SST			
TOP1	0.37(0.10)		
TOP Δ	−0.37(0.10)		
BOT2	0.46(0.04)		
GRAD2	−0.49(0.03)		
GRAD Δ	−0.39(0.09)		
Testosterone			
Initial		−0.50(0.03)	

TOP and BOT = Measurement at the top and bottom of the scrotum.
GRAD = Gradient from to to bottom of the scrotum.
1, 2, Δ = Measurement 1 min before (1) and 45 min after (2) GnRH and change (1 minus 2) due to GnRH.

was 3.3°C. The SST increased after GnRH treatment (Table 2; with the exception of top SST for Examination 3). This increase was greater at the bottom than at the top (difference not always significant), resulting in a decreased gradient of approximately 0.3°C. In general, SST after GnRH treatment was more repeatable (higher correlations) than SST before GnRH treatment.

The total number of spermatozoa (Table 1) was very repeatable among examinations (similar means and highly significant correlation coefficients). The percentages of live and motile spermatozoa did not have consistently high correlations. However, the means were similar and the standard errors were relatively small, indicating that overall these measures were relatively consistent.

The results of correlation analyses are shown in Table 3. This table includes only independent variables with at least one significant correlation with semen variables. There were more significant correlations for the percentage of motile sperm than for the total number of sperm and percentage of live sperm. Plasma testosterone concentrations were significantly correlated with the total number of spermatozoa. For the percentages of motile and live spermatozoa, significant correlations were generally not consistent among the 3 examinations.

Some additional correlation data are not shown in the tables. Bull age was positively correlated with pre-treatment plasma testosterone concentrations ($r = 0.19$ to 0.30, $P < 0.05$) and echotexture (ET) ($r = 0.11$ to 0.28, $P < 0.05$) and slightly correlated with the SST BOT 2 ($r = 0.06$ to 0.32, $P > 0.05$) and SC ($r = 0.06$ to 0.35, $P > 0.05$). The

Table 4
Prediction of total sperm number as dependent variable using endocrine end points as independent variables for 20 bulls examined on 3 occasions

Simple regression					Maximum R^2 improvement analysis		
Independent variables tested	R^2	Intercept	Slope	P	Independent variables selected	R^2	Equations
Examination 1							
GnRH Induc. Test.	0.22	8.75	−0.077	0.04			
Test. Δ	0.33	5.72	0.076	0.008	Test. Δ	0.33	$Y = 5.72 + 0.076$ Test Δ
Examination 2							
Initial Test.	0.39	10.37	−0.201	0.004	Initial Test.	0.39	$Y = 10.37 - 0.201$ Initial T.
GnRH Induc. Test.	0.22	8.5	−0.058	0.03			
Examination 3							
GnRH Induc. Test.	0.35	11.30	−0.094	0.006	GnRH Induc. Test.	0.35	$Y = 11.30 - 0.094$ GnRH Induc. Test.
Test. Δ	0.19	8.5	−0.098	0.05			

GnRH Induc. Test. = Testosterone induced after GnRH.
Test. Δ = Change in testosterone from before to after GnRH.
Initial Test. = Testosterone before GnRH.

ET was slightly negatively correlated with pre-treatment testosterone concentrations ($r = -0.10$ to 0.46, $P < 0.05$).

The results of regression analyses for the total number of spermatozoa as a dependent variable are shown in Table 4. Plasma testosterone concentrations were significantly correlated with the total number of spermatozoa (Table 3) and were the important independent variables in the regression equations. However, most of the correlations (and therefore the slopes of the regression lines) were negative.

For the percentage of motile spermatozoa, most of the significant correlations were either bottom SST or gradient (Table 3). For the regression analyses, the SST gradient was the only significant independent variable (Table 5). The slope of the regression lines for all 3 examinations were negative. Therefore, motility was inversely related to the gradient.

There were few significant correlations or regression for the percentage of live spermatozoa (Tables 3 and 6, respectively). For the regression equations, the significant independent variables were plasma testosterone concentrations and SST gradient. In both cases, the slope was negative, consistent with the negative slopes for these 2 indepen-

Table 5
Prediction of motile sperm % as dependent variable using scrotal surface temperature values as independent variables for 20 bulls examined on 3 occasions

Simple regression					Maximum R^2 improvement analysis		
Independent variables tested	R^2	Intercept	Slope	P	Independent variables selected	R^2	Equations
Examination 1							
GRAD Δ	0.31	84.65	−4.94	0.011	GRAD Δ	0.31	$Y = 84.65 - 4.94$ GRAD Δ
Examination 2							
GRAD1	0.19	95.10	−1.98	0.056	GRAD1	0.19	$Y = 95.10 - 1.987$ GRAD 1
TOP1	0.16	206.19	−3.42	0.075			
TOP2	0.15	173.94	−2.46	0.087			
GRAD2	0.16	94.39	−1.95	0.076			
Examination 3							
GRAD1	0.42	98.77	−2.97	0.001	GRAD1	0.42	$Y = 98.77 - 2.97$ GRAD1
BOT1	0.40	−73.74	4.921	0.003			
TOP1	0.23	225.06	−3.74	0.03			
BOT Δ	0.24	90.13	−4.06	0.03			
GRAD2	0.23	95.07	−2.07	0.08			

GRAD = Gradient from top to bottom of the scrotum, TOP minus BOT.
GRAD1 = GRAD before GnRH.
GRAD2 = GRAD after GnRH.
GRAD Δ = Change from before to after GnRH.
TOP1 and TOP2 = Temperature before and after GnRH, respectively at top of scrotum.
BOT1 and BOT2 = Initial temperature at bottom of scrotum and change in bottom temperature from before to after GnRH.

Table 6
Prediction of live sperm % as dependent variable and testosterone and scrotal surface temperature end points as independent variables for 20 bulls examined on 3 occasions

Simple regression					Maximum R^2 improvement analysis		
Independent variables tested	R^2	Intercept	Slope	P	Independent variables selected	R^2	Equations
Examination 1							
Scrotal surface temperature							
GRAD2	0.24	72.93	−1.83	0.03	GRAD2	0.24	$Y = 72.93 - 1.83$ GRAD2
BOT2	0.21	26.97	1.55	0.04			
GRAD Δ	0.16	66.93	−1.82	0.08			
Examination 2							
Initial testosterone	0.25	70.95	−0.211	0.03	Initial testosterone	0.25	$Y = 70.95 - 0.211$ Initial Test.

SST = Scrotal surface temperature; number 1, 2 are 0 and 45 min after GnRH.
TOP = Measure at the top of scrotum; TOP Δ, TOP 2 − TOP 1.
BOT = Measure at the bottom of scrotum; BOT Δ, BOT 2 − BOT 1.
GRAD2 = Difference between measures at top and bottom of scrotum after GnRH.
GRAD Δ = Change in gradient from top to bottom of the scrotum from before to after GnRH.

dent variables as predictors of the total number of spermatozoa and the percentage of motile spermatozoa.

4. Discussion

The SCW and SCL were consistent between the first 2 examinations, but were substantially greater in Examination 3, probably as a result of higher ambient temperatures increasing testicular descent. Perhaps a marker showing the location of the testes or perhaps a correction factor (based on ambient temperature) would improve the accuracy and repeatability of these measurements.

The highly significant correlations among examinations for testicular echotexture satisfies the using of this measure on practical level. The reason for the apparent increase of relatively larger mean echotexture with each successive examination is not yet known. Perhaps an absence of semen collection for several months followed by continuous collection affects testicular echotexture. This hypothesis is supported by the results of testosterone measures. As we earlier found the results of 2nd and 3rd challenges resulted practically in the same differences (ΔT) between the initial and stimulated levels of testosterone in plasma (Gábor et al., 1995) which were probably influenced by the previous break of semen collection and/or service.

Post et al. (1987a,b) concluded that the testosterone response to GnRH was highly repeatable in 5 bulls given 6 different doses of GnRH on 6 occasions. However,

differences in GnRH dosage, age and breed of bulls, in his study could cause conflicting results with our findings.

The ambient temperature has a greater effect on basal (pre-treatment) SST than SST following GnRH. This result supports the combination of infrared thermography of SST and the GnRH challenge in order to decrease the effect of the ambient temperature.

In a previous study (Kastelic et al., 1996) the gradient was only 1.3°C at an ambient temperature of 25°C. Regardless, it is recommended that these examinations be conducted at moderate (10 to 20°C) ambient temperatures, and it was also shown that SST was more labile at the bottom of the scrotum than at the top (Kastelic et al., 1996).

Lunstra and Coulter (1997) demonstrated (used unselected yearling beef bulls) that the sperm concentration was best when the SST gradient was between 1.8 and 3°C, and was poorer when the gradient was smaller than 1.2°C. In our study only pre-selected AI bulls (which were earlier in service) were used, that is why the SST gradients were relatively high and changed between 2.9 and 3.9°C.

Although independent variables with at least one significant correlation with semen characteristics were only used in Table 3, there are several possible reasons why relatively few significant correlations were found with the dependent variables (percentage of motile sperm, the total number of sperm and percentage of live sperm). The most valuable correlations were found between plasma testosterone concentrations and the total number of spermatozoa. In Examination 1 this (positive) correlation is controversial with others because the previous break of semen collection and/or service. This statement is confirmed by the results of Examination 2 and 3 where the similar negative tendencies were found.

The inconsistency in correlations of the percentages of motile and live spermatozoa among the 3 examinations may also be caused by the different ambient temperature.

Although the results of regression analyses for the prediction of the semen characteristics (Tables 4–6) showed relatively low predictive value of the above examined parameters in 6–7 years old breeding bulls, in our opinion some of them (such as echotexture, serum testosterone concentration and SST after GnRH challenge) promise more opportunity in precise evaluation of the testicular function.

In conclusion, GnRH treatment significantly increased plasma testosterone concentrations and usually caused significant increases in SST. Scrotal circumference and the total number of spermatozoa per ejaculate were highly repeatable. Other measurements were less repeatable, with an apparent effect of ambient temperature on video-measurements of the scrotum and assessment of SST. Significant regression equations were derived for the total number of spermatozoa and the percentage of motile spermatozoa; plasma testosterone concentrations and SST gradients, respectively, were the significant independent variables.

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