Effects of GnRH treatment on scrotal surface temperatures in bulls

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Abstract

Two experiments were conducted to characterize scrotal surface temperature (SST) in bulls treated with gonadotropin releasing hormone (GnRH). In Experiment 1, Angus bulls (n = 10, 18 mo, 597 kg) were given GnRH (400 ng/kg) or saline, IV. Bottom SST increased approximately 1.7°C ($P < 0.005$) over time (0 to 90 min) at an ambient temperature of 5°C. However, there was no significant effect of GnRH treatment and temperature increases were attributed to stress. When the experiment was repeated at an ambient temperature of 25°C, SST was elevated prior to treatment, with no subsequent significant increase. Experiment 2 was conducted with Charolais bulls (n = 6, 12–14 mo, 517 kg) with an emphasis on minimizing stress. Bottom SST increased approximately 2°C ($P < 0.05$) between 0 and 45 min after GnRH treatment, supporting the hypothesis that GnRH treatment increases SST in bulls. In conclusion, it was apparent that stress, high ambient temperatures, and GnRH treatment can all increase SST in bulls.

Résumé

Deux expérimentations furent entreprises afin de caractériser la température scrotale superficielle (TSS) de taureaux traités avec l'hormone relâchante de la gonadotropine (GnRH). Lors de la première expérimentation, des taureaux de race Angus (n = *10, 18 mois, 597 kg) reçurent de la GnRH (400 ng/kg) ou de la saline par voie intraveineuse. À une température ambiante de 5 °C, la TSS en partie inférieure augmenta d'environ 1,7 °C* (P < *0,005) durant la période d'observation (0 à 90 min). Toutefois, le traitement à la GnRH n'eut pas d'effet significatif et l'augmentation de température fut attribuée au stress. Lorsque l'expérimentation fut répétée à une température ambiante de 25 °C, la TSS était déjà élevée avant le traitement et aucune augmentation significative ne fut notée suite au traitement. Lors de la deuxième expérimentation, des taureaux de race Charolais furent utilisés* (n = *6, 12–14 mois, 517 kg) et des efforts furent faits pour minimiser le stress. La TSS en partie inférieure augmenta d'environ 2 °C* (P < *0,05), entre 0 et 45 min suivant l'administration de GnRH, ce qui tend à accréditer l'hypothèse qu'un traitement à la GnRH augmente la TSS chez les taureaux. Il apparaît évident que le stress, la température ambiante élevée et un traitement à la GnRH sont tous des facteurs pouvant faire augmenter la TSS des taureaux.*

(Traduit par docteur Serge Messier)

Treatment with gonadotropin releasing hormone (GnRH) increases plasma concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH); the increased LH acts on Leydig cells within the testis, stimulating them to release testosterone (1). Treatment with GnRH may be useful for predicting potential fertility in bulls. In 1 study (2), there was close agreement between rankings for reproductive performance (libido and pregnancies achieved) in bulls and plasma testosterone concentrations following GnRH treatment. In another study in bulls (3), it was suggested that blood testosterone concentrations following GnRH treatment could be useful for predicting reproductive potential. In a recent study (4), when young (average age, 24.3 mo) Holstein-Friesian breeding bulls were treated with GnRH, SST increased (range, 0.6 to 1.1°C; $P < 0.05$) at both the top and bottom of the scrotum. In that study, measurement of testicular ultrasonographic echotexture and SST after GnRH treatment augmented measurement of testicular size for predicting the number and percentage of live spermatozoa.

In a second study, using mature Holstein-Friesian bulls (average age, 71.3 mo), SST generally increased following GnRH treatment (5). In these 2 studies, all bulls were treated with GnRH; there was no concurrent control group that did not receive GnRH.

The objectives of the present study were to further characterize scrotal and testicular temperatures and testicular echotexture in bulls in response to GnRH treatment. Our hypothesis was that GnRH treatment increases SST in bulls. Furthermore, since ambient temperature has been shown to substantially affect SST (6), a secondary objective was to determine if the effects of GnRH treatment on SST were dependent upon ambient temperature. Two experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care (7) and with the approval of the local institutional Animal Care Committee.

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Table I. Mean and SEM for rectal temperature; top, average, and bottom scrotal surface temperatures, plasma concentrations (ng/mL) of LH and testosterone; and testicular echotexture in bulls (n = 10) treated with GnRH or saline at 2 ambient temperatures (experiment 1)

	Interval after treatment (min)							
	Ambient 5°C				Ambient 25°C			
Variable	0	45	90	SEM	0	45	90	SEM
Temperature (°C)								
Rectal	39.4[a]	39.8[b]	39.6[c]	0.2	39.7	39.8	39.8	0.3
Top	29.5	29.2	29.2	0.5	33.3	33.9	33.9	0.4
Average	25.7	26.3	26.1	0.3	31.4	31.5	31.5	0.4
Bottom	23.0[d]	24.8[e]	24.6[e]	0.5	29.9	30.0	30.0	0.6
Testicular echotexture								
	28.7[d]	28.8[d]	29.9[e]	0.8	28.6	29.3	29.5	0.7
Plasma LH								
GnRH	0.3[a]	7.2[b]	10.4[b]	0.9	0.2[d]	6.2[e]	5.0[e]	0.5
Saline	0.2[a]	0.2[a]	0.2[a]	0.1	0.1[d]	0.2[d]	0.3[d]	0.1
Plasma testosterone								
GnRH	1.8[a]	5.9[b]	9.6[c]	0.5	1.3[d]	4.6[e]	8.8[f]	0.6
Saline	1.7[a]	0.8[a]	1.2[a]	0.6	2.4[d]	1.3[d]	1.2[d]	0.7

Within a row (for each ambient temperature), means with unlike superscripts differ: [abc]($P < 0.05$), [def]($P < 0.01$)

At 5°C, there were main effects of time for rectal temperature ($P < 0.001$), bottom temperature ($P < 0.005$), and testicular echotexture ($P < 0.006$)

Treatment, time and their interaction were all significant ($P < 0.01$) for plasma concentrations of both LH and testosterone at both ambient temperatures

For both ambient temperatures, the main effect of treatment and the treatment × time interaction were not significant for testicular echotexture nor any temperature endpoint

Aberdeen Angus bulls (n = 10), approximately 18 mo of age (body weight, 597 ± 16 kg; mean ± SEM), were used in Experiment 1. This experiment was initially conducted at an ambient temperature of 5°C and was repeated 48 h later at 25°C (with several hours for equilibration at each ambient temperature). A standard breeding soundness evaluation (8) was conducted and all bulls were judged satisfactory potential breeders (scrotal circumference, 33.0 ± 0.8 cm; 79.6 ± 3.6% morphologically normal spermatozoa). Bulls were allocated to receive either GnRH (Fertagyl; Intervet Canada Ltd, Whitby, Ontario), 400 ng/kg, or saline, IV (n = 5 bulls per treatment). The dosage of GnRH was based on previous reports in the literature (2–5) and on preliminary trials to determine plasma luteinizing hormone (LH) concentrations in response to different dosages of GnRH. Two bulls (1 per group) were simultaneously restrained in adjacent cattle chutes in the same room. Rectal temperature was recorded with an electronic thermometer (Model 500; GLA Agricultural Electronics, San Luis Obispo, California, USA). An infrared thermography camera (Inframetrics Model 760; Inframetrics Ltd., North Billerica, Massachusetts, USA) was held approximately 1 m behind the bull and an image of the caudal aspect of the scrotum was captured and recorded on a diskette. Bulls were then given either GnRH or an equivalent volume of saline, IV. Temperature measurements were repeated on 2 occasions, 45 min and 90 min after treatment. Immediately after temperature measurements, blood samples were collected (by venipuncture of the caudal or jugular vein), centrifuged, and the plasma was frozen pending determination of LH and testosterone concentrations. Following blood sampling, each testis was examined ultrasonographically with 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 long axis of the testis) and aligned at the centre of the testis. A custom electromechanical device for holding the transducer was used (9). When the transducer was applied with a force of approximately 0.76 kg/cm^2, a small indicator lamp lighted and the image was frozen and subsequently captured on a computer (IBM Thinkpad, Model 360CSE; IBM UK, Ltd., Greenock, United Kingdom).

Infrared thermograms were transferred to a dedicated computer system (Image; National Institutes of Health, Bethesda, Maryland, USA) for image analysis. Scrotal surface temperature was determined for areas at the top and bottom of the testes (top SST and bottom SST, respectively) and for the entire scrotal surface area overlying the testes (average SST), as described (10). For ultrasonograms, the mean grey level (designated as 'echotexture') was determined with customized software (9) in an area 1.0 cm × 6.5 cm, just under the tunica albuginea. Echotexture ranged from 0 (black) to 63 (white).

Plasma concentrations of LH and testosterone were determined with validated radioimmunoassays (11). For each hormone, all samples were measured in a single assay. For LH, the intra-assay coefficients of variation were 3.9% and 5.8% for reference sera of 0.48 ng/mL and 1.05 ng/mL, respectively; for testosterone, they were 9.4% and 6.9% for reference sera of 0.80 ng/mL and 1.90 ng/mL.

Statistical analyses were conducted (separately for the 2 ambient temperatures) with the Statistical Analysis System (SAS Institute,

Table II. Mean top and bottom scrotal surface temperature (°C) and overall difference between 0 and 45 min after treatment in bulls (n = 6) given GnRH or saline at 2 ambient temperatures (Experiment 2)

	GnRH		Saline		Difference		
	0	45	0	45	Mean	SE	P value
Ambient 6°C							
Top	28.4	29.3	29.2	28.6	1.5	0.3	0.41
Bottom	24.1	25.3	24.9	24.2	1.9	0.4	0.05
Ambient 11°C							
Top	29.4	29.9	29.3	28.5	1.3	0.3	0.06
Bottom	25.7	26.8	25.7	24.7	2.1	0.2	0.02

Cary, North Carolina, USA). Repeated-measures analyses of variance (ANOVAs) were conducted to determine the effects of treatment, time, and the treatment × time interactions on rectal temperature; top, bottom, and average SST; testicular echotexture; and plasma concentrations of LH and testosterone. If there was a significant main effect or interaction, a split-plot ANOVA was conducted and differences among means were identified with a least significant difference test.

In Experiment 2, Charolais bulls (n = 6; 12 to 14 mo of age; weight, 517 ± 16 kg; scrotal circumference, 34.4 ± 0.3 cm) were used. The experiment was initially conducted at an ambient temperature of 6°C and was subsequently repeated at an ambient temperature of 11°C (with several hours of equilibration prior to the experiment). Bulls were restrained in a stanchion and considerable effort was made to minimize stress. The experiment was done in a switchback format. On the 1st day, 3 bulls received GnRH (Cystorelin; Sanofi Animal Health, Victoriaville, Quebec), 400 ng/kg, and 3 received saline; 2 d later, each bull received the opposite treatment. A hand-held infrared thermometer (Telerm-100; Hod Mg.RT, Hodmezovasarhely, Hungary) was used to measure SST at 4 sites, 2 at the top of the scrotum (overlying the right and left testis, respectively) and 2 at corresponding sites at the bottom. For top and bottom, the average (of left and right temperatures) were used in the statistical analyses. Each site was marked with an indelible ink marker (to ensure consistency) and the area assessed was approximately 3 cm in diameter. Measurements were done just prior to treatment (time = 0) and were repeated 45 min after treatment.

Separate statistical analyses were conducted for top and bottom SST at each ambient temperature. First, a paired student's t-test was conducted to ensure that there was no difference between GnRH and saline treatments at time = 0. To determine the effect of treatment, we calculated (for each bull) the change in SST (between 0 and 45 min) following treatment with GnRH and following treatment with saline; these 2 numbers were added together to determine the net change in temperature attributable to GnRH treatment. Then, a 1-sample student's t-test was used to test the null hypothesis that this difference was equal to 0.

In Experiment 1, there was an effect of time on rectal temperature ($P < 0.001$), bottom temperature ($P < 0.005$), and testicular echotexture ($P < 0.006$) at an ambient temperature of 5°C (Table I). However, there were no significant effects of time on rectal temperature, SST, or echotexture at an ambient temperature of 25°C. Treatment, time, and their interaction were all significant ($P < 0.01$) for plasma concentrations of both LH and testosterone at both ambient temperatures. However, there were no significant effects of treatment nor treatment by time interaction for testicular echotexture or any temperature endpoint at either ambient temperature.

In Experiment 2, there was no significant difference ($P > 0.2$) between GnRH and saline treatments at time = 0 for top or bottom SST at both ambient temperatures. The SST increased following GnRH treatment, but decreased slightly following saline treatment (Table II). Combined for both ambient temperatures, the average difference in SST attributable to GnRH treatment was 1.4°C and 2.0°C at the top and bottom of the scrotum, respectively.

The Angus bulls used in Experiment 1 were with their dams on extensive range conditions prior to weaning and thereafter were maintained in pens in groups. No specific efforts were made to habituate these bulls to handling or restraint. While restrained in the chute (for the experiment), their behavior (e.g., movement, vocalization) clearly indicated that the confinement, experimental procedures, and close proximity to people were very stressful. This is further corroborated by their elevated rectal temperature (39.8°C) in the absence of any clinical indications of disease. Although we did not objectively measure stress in these bulls, our interpretation was that stress-induced (due to handling and restraint) increases in body temperature were sufficient to increase SST, thereby masking any increase in SST attributable to GnRH treatment. It is well known that stress induces an increase in body temperature. In 1 study (12), when dairy cows were restrained in a chute, body temperature increased, peaking 1 h after the onset of the restraint.

Since stress-induced increases in body temperature in Experiment 1 precluded an effective test of our hypothesis, we made substantial efforts to minimize stress in Experiment 2. In this experiment, the bulls were very docile and habituated to handling and displayed minimal responses to the restraint or manipulations. Unfortunately, rectal temperature was not recorded in this experiment. However, it was noteworthy that the bulls remained very calm throughout the study and showed minimal aversion or reaction, even to venipuncture for blood sampling. In this experiment, SST consistently declined (between 0 and 45 min) in saline-treated bulls; perhaps the initial restraint was minimally stressful, with a small rise in body temperature that subsequently decreased. Furthermore, SST consistently increased in GnRH-treated bulls in this experiment, supporting the hypothesis that GnRH treatment increases SST in bulls. Similarly, in 2 recent studies utilizing Holstein-Friesian bulls

(4,5), SST generally increased following GnRH treatment. Perhaps GnRH treatment increased SST by increasing blood flow. In hypophysectomized rats given GnRH, testicular blood flow increased approximately 35% and 50% at 2 h and 4 h, respectively, after treatment (13). Furthermore, increased metabolic activity associated with the production and release of testosterone (in response to increased LH concentrations) may have contributed to the increase in testicular temperature. Further studies are needed to elucidate the physiological mechanisms associated with GnRH-induced increases in testicular temperature.

Ultrasonographic echotexture increased significantly over time at an ambient temperature of 5°C in Experiment 1. Since rectal temperature and SST also increased significantly over time in these bulls, there was a temporal association between increases in testicular echotexture and body temperature. Perhaps the increased echotexture was due to stress-related increases in blood flow. The association between ultrasonographic echotexture and body temperature indicates that caution must be exercised in the ultrasonographic examination of the testes. During clinical breeding soundness evaluations, body temperature probably increases due to stress (e.g., novel environment, restraint, electroejaculation); therefore, if an ultrasonographic examination is planned, it should be conducted early in the course of the breeding soundness evaluation. Similarly, if infrared thermography is planned for the evaluation of SST, the images should be collected as soon as possible.

In both experiments, the magnitude of temperature changes was consistently greatest for bottom SST, corroborating a previous report that bottom SST was more labile than both top and average SST (6). In that regard, the top SST is the warmest area of the scrotum (due to the underlying testicular vascular cones) and hence has the least opportunity to change. High ambient temperatures increase 'basal' body temperatures (particularly bottom SST), making it difficult or impossible to detect increases in body temperature mediated by other mechanisms.

There were several differences between the 2 experiments that could have affected the outcome. Although GnRH from 2 different sources were used, these preparations had an identical amino acid sequence and, therefore, were expected to have similar effects. The bulls used in Experiment 1 were 4 to 6 mo older than the bulls used in Experiment 2. However, in a previous study (14), there was no difference in SST among bulls that were 0.5, 1, 2, or 3 y of age. Furthermore, based on thermographic examinations of scrotums of bulls from many breeds, there are no indications of differences in SST attributable to breed (unpublished observations).

In conclusion, it was apparent that stress, high ambient temperatures and GnRH treatment can all increase SST in bulls. Therefore, these factors should be taken into account when SST is being evaluated in bulls for clinical or research purposes.

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