Serum Gonadotropins After Gonadotropin-Releasing Hormone Injection in Bulls Subjected to Spacial Restriction

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ABSTRACT

Response patterns of luteinizing hormone, follicle-stimulating hormone, and testosterone after injection of gonadotropin-releasing hormone were investigated in bulls grouped by weight (250 to 459 kg body weight) and confined five per pen in 9.2 or 6.4 m² space per bull in two replicates. Blood samples were collected for 1 h prior to injection of 100 µg gonadotropin releasing hormone and 5 h after injection at 15-min intervals. Overall mean luteinizing hormone concentrations were not affected by spacial restriction or replicate. Interaction of treatment by time revealed that luteinizing hormone response curves were not similar. Restricted bulls had a higher response of luteinizing hormone to gonadotropin-releasing hormone. Follicle-stimulating hormone increased in all groups within 15 min and peaked at 219.4 ng/ml at 45 min. Both gonadotropin responses returned to preinjection concentrations by 4 h. Testosterone was affected by treatment, replicate, and time of sampling. Testosterone was higher in restricted bulls and higher in replicate 2. Mean testosterone peak following gonadotropin-releasing hormone was 3.86 ng/ml and occurred between 105 and 120 min which was approximately 90 min after the gonadotropin peaks. It appears that hormone responses to gonadotropin-releasing hormone were not depressed by spacial restriction, and additional spacial restriction of young bulls could be used commercially.

INTRODUCTION

Effects of social dominance, feed bunk stocking density, and availability of housing in confinement conditions as they affect production characteristics in dairy cattle have been documented (7, 9, 16). Severe restriction of personal space usually leads to increases of social conflict and reduction of productive responses, such as weight gain and milk yield. The stress response due to high density housing, social disruption, and free stall competition has been evaluated (6, 8). Adrenocorticotropin (ACTH) injections in cows 3x daily for 3 days resulted in maximal adrenal glucocorticoid output on the 2nd day and reduction of corticoid output on the 3rd day (12). Thus, the adrenal gland will release greater amounts of glucocorticoids following ACTH challenge under conditions of increasing stress.

Behavioral and productive capabilities of the dairy bull under conditions of group confinement are less known. According to Viljoen (17), bulls housed in all-male groups tend to develop "monosexual" tendencies. Submissive bulls become timid, lose masculinity, and become undesirable for breeding purposes. Larson (18) reported that bulls less than 14-mo old were allowed between 9.3 to 12.4 m² space during progeny testing and anticipated more than 20% losses as a result of group housing. Agonistic behaviors were greatest during the first 48 h of grouping. The hormonal changes associated with confinement stress in dairy bulls have not been documented. In the bull (14) and ram (10), ACTH injections suppressed episodic secretion of lutenizing hormone (LH); however, Barnes et al. (3) did not observe altered pituitary response to gonadotropin-releasing hormone (GnRH) following GnRH injection. There is no information available on changes of gonadotropin following GnRH in young dairy bulls under spacial restriction.
The objective of this research was to evaluate the response pattern of the gonadotropins, LH, and FSH (follicle-stimulating hormone), following injection of GnRH in bulls under two acute confinement regimens.

MATERIALS AND METHODS

Ten Holstein bulls (250 to 459 kg body weight and 7.5 to 15.1 mo of age), grouped five per pen, were allotted either 46.2 or 32.0 m$^2$ space in two replicates. Bulls were housed in pens with concrete floors and approximately one-half the area under a roof. The feed bunk also was enclosed. A corn silage-based complete ration was provided to bulls for ad libitum intake. Control bulls had 9.2 m$^2$ space per bull compared to 6.4 m$^2$ space for restricted bulls. Initially, bulls were grouped by weight for 3 wk and assigned randomly to spacial allotments. After the initial period, bulls were regrouped by weight three additional times for three spacial allotments for a nutrition study. The first and last groupings of the most and least restricted pens were used for this study. Six bulls appeared in both replicates. Control bulls in the first replicate had an average of 2.94 m$^2$/kg body weight compared with 2.56 m$^2$/kg for restricted bulls. In the second replicate, control bulls had 2.14 m$^2$/kg compared with 1.86 m$^2$/kg body weight for restricted bulls.

Because adrenal response to ACTH is maximal on the 2nd day (12), this day was chosen to evaluate acute spacial restriction. Thus, on the 2nd day of spacial restriction in each replicate and grouping, all bulls except one were catheterized for collection of jugular blood samples. Blood samples (10 ml) were collected at 15-min intervals for 1 h prior to i.v. injection of 100 $\mu$g GnRH until 5 h postinjection. Serum samples were analyzed by radioimmunoassay for LH (5), FSH (19), and testosterone (15). Within assay variations for LH, FSH, and testosterone were 6.8, 9.4, and 7.4%. Variation between assays for testosterone was 18.7%. Because of low sample volume, testosterone was quantified in pooled samples at -60 and -45, -15 and 0 min pre-GnRH and at 15 and 30, 60, and 75, 105 and 120, 150 and 165, 195 and 210, 240 and 255, and 285 and 300 min following GnRH. Statistical analysis was by least squares techniques, and nonorthogonal Bonferroni contrasts (11) were used to detect differences in means. Area under response curves was calculated for each bull by:

$$\Sigma [(H_s + H_{s+1})/2(I)]$$

where $H$ is hormone concentration of serum in ng/ml for sample $s$ and the next sample $s+1$ with an interval of 1 h between them.

RESULTS AND DISCUSSION

Overall statistical analyses revealed no significant effects of space restriction on LH or FSH concentrations. Additional analyses of variance on the post-GnRH response data revealed no significant effects of treatment or replicate on LH or FSH (Table 1). Significant bull variability was evident for LH and FSH concentrations. There was an elevation in LH by 15 min following GnRH injection (Figure 1), and an interaction of treatment by time ($P<.01$) revealed that the curves were not parallel. Peak responses occurred at 30 min post-GnRH. Restricted bulls had an LH peak of 32.9 ng/ml compared with 26.9 ng/ml for control bulls. The higher LH response to GnRH in restricted than control bulls may be related to body weight and age as the restricted bulls were 66 kg lighter (373 vs. 307 kg body weight) and 2.6 mo younger (12.9 vs. 10.3 mo old). A linear increase of basal LH between 7 and 13 mo was reported (20); however, episodic peaks of LH were generally lower in 12-mo-old bulls than in 10-mo-old bulls (13), whereas testosterone surges were greater in older bulls, suggesting that alterations of gonadotropic control of testicular function occur with age. When area under the response curve was tested, there were no significant effects from treatment, bull within treatment, replicate, or interaction of replicate by treatment. Nonorthogonal contrasts were not different for interactions. Concentrations of FSH were not influenced by treatment, replicate, or interactions but were different ($P<.01$) over time (Table 1). Bulls responded to GnRH with increased concentrations at 15 min after treatment (Figure 2). Peaks were attained by 45 min (219.4 ng FSH/ml serum) after GnRH administration. The lack of interaction of treatment by time ($P>.05$) suggested that responses of control and restricted bulls were similar. Mean concentrations of both gonadotropins did
TECHNICAL NOTE

TABLE 1. Analysis of variance for serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone in bulls following injection with gonadotropin-releasing hormone.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>LH</th>
<th>FSH</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (T)¹</td>
<td>1</td>
<td>391</td>
<td>2,204</td>
<td>1</td>
<td>50.4*</td>
</tr>
<tr>
<td>Bull T</td>
<td>13</td>
<td>249**</td>
<td>14,297**</td>
<td>13</td>
<td>5.8</td>
</tr>
<tr>
<td>Replicate (R)²</td>
<td>1</td>
<td>255</td>
<td>64</td>
<td>1</td>
<td>113.4*</td>
</tr>
<tr>
<td>T X R</td>
<td>1</td>
<td>161</td>
<td>674</td>
<td>1</td>
<td>19.3</td>
</tr>
<tr>
<td>Bull T X R</td>
<td>2</td>
<td>165**</td>
<td>1,061</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>Time (Tt)</td>
<td>19</td>
<td>1,698**</td>
<td>73,929**</td>
<td>6</td>
<td>9.4*</td>
</tr>
<tr>
<td>T X Tt</td>
<td>19</td>
<td>49**</td>
<td>914</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>Error</td>
<td>317</td>
<td>14</td>
<td>1,069</td>
<td>98</td>
<td>3.6</td>
</tr>
</tbody>
</table>

¹Treatment tested by Bull T.

²Replicate and treatment by replicate tested by Bull T X R; R² = .89 for LH, .83 for FSH, and .56 for testosterone.

*P<.05.

**P<.01.

not return to preinjection baseline until 4 to 5 h post-GnRH. Gonadotropin peaks attained following GnRH agree with Barnes et al. (2, 3), but were earlier than those reported by Schanbacher and Echternkamp (23), who evaluated gonadotropin response to GnRH in 2-yr-old Hereford bulls. Response areas were not different.

Testosterone concentrations were influenced by treatment (P<.05), replicate (P<.05), and time of sampling (P<.05, Table 1). Post-GnRH testosterone concentrations in control bulls average 2.65 ± .24 (n = 68) ng/ml, whereas restricted bulls had a mean testosterone concentration of 3.93 ± .27 (n = 61) ng/ml serum. In addition, response area after GnRH was

![Graph](https://via.placeholder.com/150)

Figure 1. Mean and SE luteinizing hormone concentrations in bulls before and after injection of gonadotropin-releasing hormone.

Journal of Dairy Science Vol. 67, No. 12, 1984
different for control (12.12 ± 1.38 ng h/ml) and restricted (16.99 ± 1.47 ng h/ml) bulls ($P<.05$). These data suggest that the brief stress of regrouping and spacial restriction had no overall effect to inhibit testosterone secretion following GnRH and also suggest a stimulatory action to improve testicular responsiveness to GnRH. Post-GnRH testosterone means for replicates 1 and 2 were 1.86 ± .23 (n = 68) and 4.72 ± .24 (n = 61) ng/ml. Area of response curves following GnRH also was affected by replicate ($P<.01$). Replicate 1 response area was 8.71 ± 1.38 ng h/ml compared with 20.41 ± 1.47 ng h/ml in replicate 2. The higher testosterone concentrations of replicate 2 would support greater testicular steroidogenesis and higher androgen production with advancing weight (21) and age (2, 4, 20). Even though interaction of treatment by replicate for testosterone response area was not significant,

**TABLE 2. Mean testosterone area under response curves (ng/ml per h) following gonadotropin-releasing hormone in control and restricted bulls.**

| Replicate | Control | Restricted
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>9.2 m² per bull</td>
<td>Mean body weight</td>
</tr>
<tr>
<td>1</td>
<td>7.11 ± 1.96₁</td>
<td>314</td>
</tr>
<tr>
<td>2</td>
<td>10.30 ± 1.96</td>
<td>432</td>
</tr>
</tbody>
</table>

₁X ± SE.

²Contrasts of replicate 1 restricted vs. replicate 2 restricted and replicate 2 control vs. restricted groups were significantly different at $P<.05$.

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the nonorthogonal comparisons showed that testosterone area was greater for restricted bulls in replicate 2 compared with replicate 1, and this testosterone response area was greater than control bulls of replicate 2 (Table 2). Although data are confounded, the large response area for restricted bulls of replicate 2 would suggest that brief stress did not inhibit testicular secretion of testosterone in response to GnRH. The absence of interaction of treatment by time (P>.05) suggested that responses to GnRH in all bulls were not different. Mean testosterone responses (Figure 3) peaked at 105 to 120 min following GnRH and had not reached pre-injection concentrations by 5 h after injection. Testosterone peaked at 3.86 ng/ml serum approximately 90 min after LH and FSH peaks. These data are consistent with those reported by others (2, 3, 13, 23) who showed that, in the absence of spacial restriction, an increase in gonadotropin is followed by an increase of peripheral testosterone.

Decreased LH response to GnRH was reported in starved male rats (22). Welsh and Johnson (24) found that elevated corticosteroids associated with the stress of electroejaculation temporarily altered secretion of LH in the bull did not prevent increases of LH, FSH, or testosterone (3). This suggests that brief spacial restriction, which may be associated with the elevated corticosteroids temporally (8, 12), reduces gonadotropin and testicular responses to GnRH. Although glucocorticoids altered LH testicular receptor content and steroidogenesis in the rat (1), injections of ACTH followed by GnRH in the bull did not prevent increases of LH, FSH, or testosterone (3). This suggests that group housing of young bulls could be more restricted spacially than has been commercially (18).

This study did show changes of responses of testosterone that probably were associated with body weight and age and, thus, would reflect changes of pituitary and testicular function. It is possible that the spacial restriction did not provide stress sufficient to elevate glucocorticoids to maximize adrenal output and have adverse effects on the hypothalamic-hypophyseal-testicular axis. Thus, further study on bull housing and spacial requirements is needed to delineate hormonal aberrations that may be

Figure 3. Mean and SE testosterone concentrations in bulls before and after injection of gonadotropin-releasing hormone.
associated with transient increase of abnormal spermatozoa production when young bulls enter a collection program.

REFERENCES


