REGULATION OF PULSATILE LH SECRETION BY OVARIAN STEROIDS IN THE HEIFER


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ABSTRACT

Two experiments were conducted to evaluate relationships among luteinizing hormone (LH), estradiol-17β (E₂) and progesterone secretion during the preovulatory period in the heifer after prostaglandin F₂α (PGF₂α)-induced regression of the corpus luteum. A second objective was to elucidate the effects of E₂ in regulating LH secretion. In Exp. 1, LH, E₂ and progesterone concentrations were determined in serial samples collected during the preovulatory period after PGF₂α-induced luteal regression in five Red Angus × Hereford heifers. Progesterone declined to 1 ng/ml by 12 h after the second injection of PGF₂α. Frequency of LH pulses increased linearly (P<.01), whereas no change in amplitude of LH pulses was detected before the preovulatory LH surge. This resulted in a linear increase (P<.01) in mean LH concentrations. Estradiol also increased in a linear manner (P<.01), and the rise in E₂ was parallel to the increase in mean LH concentrations. In Exp. 2, 12 Angus × Hereford heifers were ovariectomized and administered either 13.5- or 27-cm silastic implants containing E₂ at ovariectomy. Four heifers served as nonimplanted controls. Thirty-one days after ovariectomy all heifers were bled at 12-min intervals for 6 h. Frequency of LH pulses declined linearly (P<.03) while mean LH (P<.09) and pulse amplitude (P<.01) increased linearly as E₂ dose increased. These results indicate that a reduction in progesterone increases the frequency of LH pulses during the follicular phase of the estrous cycle in cattle. Estrogen seems to enhance total LH output from the pituitary during the preovulatory period by maintaining the amplitude of LH pulses during the period when frequency of LH pulses is increasing as a result of decreased progesterone brought on by the regression of the corpus luteum.

(Key Words: LH, Estradiol, Progesterone, Heifers, Estrous Cycle.)

Introduction

The concentration of plasma LH in cows exhibiting estrous cycles undergoes dynamic changes and the pattern of fluctuation varies, depending upon the phase of the estrous cycle (Rahe et al., 1980). Exogenous progesterone may reduce the frequency of luteinizing hormone-releasing hormone (LHRH) secretions in the ewe (Goodman and Karsch, 1980). It has been proposed that progesterone acts as a "brake" in preventing events necessary for ovulation in the cow (Walters et al., 1982). Removal of the "brake" (decrease in circulating progesterone) at luteolysis results in an increase in frequency of pulsatile LH discharge, eventually reaching a frequency of one pulse each hour (Baird, 1978; Rahe et al., 1980; Ireland and Roche, 1982; Walters and Schallengerger, 1984). There is limited evidence concerning the relationships between ovarian steroids and pituitary gonadotrophic hormones regulating the follicular phase of the estrous cycle of the cow (Chenuault et al., 1975; Walters and Schallengerger, 1984). Therefore, the objectives of the present studies were to evaluate the relationships of luteinizing hormone (LH), estradiol-17β (E₂) and progesterone secretions during the preovulatory period after prostaglandin F₂α (PGF₂α)-induced luteal regression, and to deter-
mine the effects of \( E_2 \) in regulation of LH secretion in the sexually mature heifer.

**Materials and Methods**

*Exp. 1.* Five sexually mature beef heifers (Red Angus × Hereford, 22 mo of age, 355 ± 7 kg body weight) were maintained in natural environmental conditions, and fed corn, oats and bromegrass hay. The \( \text{PGF}_2\alpha \) was used to synchronize stage of the estrous cycle.

Injections of 25 mg \( \text{PGF}_2\alpha \) were administered on d -10 and 0 (d 0 = day of initiation of blood collection regimen). All heifers were exhibiting estrous cycles at regular intervals at the time \( \text{PGF}_2\alpha \) was administered.

Secretion of LH and \( E_2 \) during the preovulatory period of the estrous cycle was monitored in serial blood samples collected at 12-min intervals for 4 h starting 0, 12, 24, 36, 48 and 60 h following the second \( \text{PGF}_2\alpha \) injection. Changes in progesterone concentrations after \( \text{PGF}_2\alpha \) administration were characterized by analyzing the first and last samples of each 4-h bleeding period. In addition, a single blood sample was collected every other day for the subsequent 21-d period and assayed for progesterone to monitor the estrous cycle length that occurred following the \( \text{PGF}_2\alpha \) injection. Blood samples (10 ml) were obtained by jugular venipuncture.

Observations for behavioral estrus were made during the 8-h periods between serial blood collections (0 to 60 h after the \( \text{PGF}_2\alpha \) injection) and at 12-h intervals up to 96 h.

*Exp. 2.* Estrous cycles were synchronized utilizing two injections of \( \text{PGF}_2\alpha \) at a 10-d interval in 12 Angus × Hereford and straightbred Hereford heifers (36 mo of age). This procedure was performed to synchronize the stage of the estrous cycle in all 12 heifers. All of the heifers were ovarioctomized (day of ovarioectomy = d 0 of experimental period) on d 12 of the synchronized estrous cycle. Eight of 12 heifers were implanted with a 13.5- or 27-cm long silastic tube \(^5\) (id = 3.35 mm, od = 4.65 mm) containing \( E_2 \) (n = 4/E2 dose group) at the time of ovarioectomy. The remaining four heifers were ovarioctomized but did not receive \( E_2 \). These heifers were maintained outdoors, except on the day of blood collection, and

pastured on high quality bromegrass. In both experiments, trace mineralized salt was provided ad libitum and heifers had free access to water. Thirty days after ovarioectomy, an indwelling jugular catheter was inserted. On d 31, heifers were restrained in a chute and blood was collected (6 ml) at 12-min intervals for 6 h. Serum was harvested from all samples, and was subsequently stored at -20°C until hormone concentrations were quantified by radioimmunoassay. Concentrations of \( E_2 \) were quantified in two samples collected at the second and fourth hours of blood collection, whereas LH was determined in all samples.

**Hormone Analyses.** Serum LH concentrations were determined by the double antibody radioimmunoassay as described by Golter et al. (1973), using rabbit antiserum against bovine LH (JFR-RABLH #5), highly purified iodinated ovine LH (LER-1056-C2) as labeled hormone and NIH-LH-B7 as standard. The sensitivity of the LH assay was .32 ng/ml. Intra- and inter-assay coefficients of variation were 2.8 and 9.7%, respectively. Serum progesterone concentrations were quantified by the method validated by Anthony et al. (1981). The assay sensitivity for progesterone was .15 ng/ml. Intra- and inter-assay coefficients of variation were 3.4 and 11.5%, respectively. The \( E_2 \) assay used was described by D'Occhio et al. (1982). The sensitivity for the assay was determined to be 1.9 pg/ml. Intra- and inter-assay coefficients of variation were 3.7 and 13.6%, respectively.

**Statistical Analyses.** Pulses of LH secretion were identified by the method described by Goodman and Karsch (1980). The concentrations of LH that were considered to be a part of the preovulatory LH surge (20 to 80 ng/ml) were not included in the analyses. Mean concentrations of LH for each bleeding period were calculated by averaging the 20 samples in each 4-h blood collection period (Exp. 1) and by averaging the 30 samples in the 6-h blood collection period (Exp. 2). Frequency of pulses of LH was calculated as the number of pulses detected in each blood collection period. Amplitude of LH pulses was the average height (preceding nadir to peak) of all pulses detected in each period.

An analysis of variance for a split-plot design was performed on the data for hormone concentration obtained from Exp. 1 (Steel and Torrie, 1980). Pairs of hormones (LH, \( E_2 \), progesterone) were considered to be effects in the

\(^5\) Dow-Corning, Midland, MI.
whole plot of the design. The analysis model contained the sources; heifer, hormone, heifer x hormone interaction (error term for hormone), blood collection period, collection period x heifer interaction and collection period x hormone interaction. The collection period x hormone interaction was used to test the hypothesis that two hormones followed parallel concentration patterns across collection periods; the test term was the split-plot error, which was the collection period x heifer x hormone interaction. Characterization of patterns of hormone secretions following the second PGF$_{2\alpha}$ injection was made using regression analysis (Draper and Smith, 1981). In addition, Pearson's correlations were used to characterize further relationships between hormone secretions (Draper and Smith, 1981). In Exp. 2, an analysis of variance for a completely random design was performed on endocrine features (Steel and Torrie, 1980). Regression analysis was used to characterize endocrine features in response to increased E$_2$ doses (Draper and Smith, 1981).

Results

Exp. 1. Behavioral estrus was observed in four of five heifers 53 ± 1.5 h (mean ± SE) after the second administration of PGF$_{2\alpha}$. Preovulatory LH surges were observed in four of five heifers 55 ± 2.9 h after the PGF$_{2\alpha}$ injection. Neither behavioral estrus nor a LH surge was detected in the remaining heifer during the 96-h period. However, all heifers had a functional corpus luteum, as assessed by serum progesterone concentrations during the subsequent luteal phase of the estrous cycle. The average length of the estrous cycle for all heifers following the PGF$_{2\alpha}$ injection was 19 ± 5 d.

Representative patterns of hormone secretion following the second PGF$_{2\alpha}$ administration in Exp. 1 are shown in figure 1. Serum progesterone concentrations decreased (P<.01) until 12 h after the PGF$_{2\alpha}$ injection (table 1) and remained low throughout the serial blood collection period. Concentrations of LH and E$_2$ increased linearly (P<.01). The increases in LH and E$_2$ were parallel (probability associated with hormone x period interaction, P>.15) until 52 h following the administration of PGF$_{2\alpha}$. The correlation coefficient for LH and E$_2$ was .35 (P<.10). The hormone x period interaction was significant for LH and progesterone, indicating that changes in the secretion of these hormones were not parallel. The correlation coefficient for LH and progesterone was -.48 (P<.02). Frequency of LH pulses increased linearly (P<.001) and reached a plateau (P<.001), whereas amplitude of LH pulses did not change (P>.20) during the 52-h period after the PGF$_{2\alpha}$ injection. Correlation coefficients for frequency of LH pulses with changes in E$_2$, and progesterone concentrations were .69 (P<.0003) and -.65 (P<.0009), respectively. However, amplitude of LH pulses was not correlated with changes in E$_2$ or progesterone concentrations (P>.20).

Exp. 2. Serum E$_2$ concentrations in ovariectomized heifers increased linearly (P<.05) as E$_2$ dose increased (table 2). Frequency of LH pulses decreased linearly (P<.03) as the dose of the E$_2$ administered increased, whereas amplitude of LH pulses increased linearly (P<.01) as the dose of E$_2$ increased. Mean concentrations of LH tended to increase in a linear fashion (P<.09) as dose of E$_2$ increased.

Discussion

The linear increases in mean LH concentrations during the preovulatory period in the present experiment agree with the results reported by Chenault et al. (1975). Concomitant increases in LH and E$_2$ after the decline in serum progesterone found in the present study were similar to those observed by Schallenberger et al. (1984). Walters and Schallenberger (1984) reported that the increase in E$_2$ concentrations in the jugular vein that occurred before and after the preovulatory LH surge in cows was a result of the frequent, high-amplitude pulses of E$_2$ observed in the vena cava. A possible association between LH and E$_2$ pulses was also suggested (Walters and Schallenberger, 1984). The E$_2$ pulses may be a result of stimulation of the preovulatory follicle by pulses of LH. Luteinizing hormone has been demonstrated to stimulate E$_2$ pulse secretion in the ewe (McNeilly et al., 1982). However, a direct relationship between LH and E$_2$ pulses has not been elucidated in cattle.

It has been suggested that the feedback effect of E$_2$ and progesterone in the cow may change the secretory pattern of plasma LH rather than to affect mean LH concentrations (Rahe et al., 1980). When high serum progesterone and small amounts of E$_2$ were present during the preovulatory period after the PGF$_{2\alpha}$-induced luteal regression in the present study,
Figure 1. Secretory patterns of LH, estradiol and progesterone (PROG) in two representative heifers following the second PGF$_{2\alpha}$ administration.
frequency of LH pulses was low. In the presence of decreased progesterone and increased E2 concentrations, the mean concentration of LH and frequency of LH pulses increased. Increases in mean concentrations of LH were observed without an altered amplitude of LH pulses in the present study. This pattern is different from that detected following the LH surge when concentrations of ovarian steroids (progesterone and E2) were low (Rahe et al., 1980). Frequency of LH pulses was high but a decreased amplitude of LH pulses resulted following the preovulatory surge of LH (Rahe et al., 1980).

Exogenous progesterone affects the pattern of LH secretion in ovariectomized ewes by decreasing the frequency and increasing the amplitude of LH pulses, whereas exogenous E2 decreases pulse amplitude without influencing the frequency of LH pulses (Goodman and Karsch, 1980). Progesterone is thought to act at the level of the hypothalamus to decrease the frequency of discharge of LHRH, whereas E2 apparently acts at the level of the pituitary to decrease the amount of LH released in response to LHRH in ewes (Goodman and Karsch, 1980). The question that arises from the present study is why the amplitude of LH pulses during the preovulatory period did not change significantly when progesterone concentration declined to less than 1 ng/ml in the serum. The increased frequency of pulses of LH (1/h)

### TABLE 1. MEAN CONCENTRATIONS OF HORMONES, FREQUENCY AND AMPLITUDE OF LUTEINIZING HORMONE (LH) PULSES IN SERIAL BLOOD SAMPLES COLLECTED DURING THE PGF$_{2\alpha}$-INDUCED PREOVULATORY PERIOD (EXP. 1) $^a$

<table>
<thead>
<tr>
<th>Hours after PGF$_{2\alpha}$</th>
<th>Mean LH, ng/ml</th>
<th>Amplitude, ng/ml</th>
<th>Frequency, number/4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>2.4 ± .4</td>
<td>8.3 ± 3.3</td>
<td>1.6 ± .4</td>
</tr>
<tr>
<td>12 to 16</td>
<td>3.5 ± .4</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± .4</td>
</tr>
<tr>
<td>24 to 28</td>
<td>3.3 ± .5</td>
<td>6.0 ± 1.9</td>
<td>4.0 ± .3</td>
</tr>
<tr>
<td>36 to 40</td>
<td>3.2 ± .4</td>
<td>5.4 ± .9</td>
<td>3.6 ± .4</td>
</tr>
<tr>
<td>48 to 52</td>
<td>4.0 ± .5</td>
<td>6.7 ± .1</td>
<td>4.0 ± .6</td>
</tr>
</tbody>
</table>

$^a$Mean ± SE.

$^b$Refers to the average height of all pulses detected in the 4-h blood collection period.

$^c$Refers to the average number of pulses detected in the 4-h period.

### TABLE 2. ENDOCRINE FEATURES IN OVARIECTOMIZED HEIFERS IMPLANTED WITH ESTRADIOL-17β (EXP. 2) $^a$

<table>
<thead>
<tr>
<th>Implant size, cm</th>
<th>Estradiol $^b$, pg/ml</th>
<th>LH $^c$, ng/ml</th>
<th>Frequency of LH pulses $^d$, pulses/6 h</th>
<th>Amplitude of LH pulses $^e$, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.1</td>
<td>2.4 ± .3</td>
<td>7.5 ± .5</td>
<td>1.0 ± .2</td>
</tr>
<tr>
<td>13.5</td>
<td>3.4</td>
<td>3.4 ± .2</td>
<td>6.0 ± .4</td>
<td>2.5 ± .4</td>
</tr>
<tr>
<td>27.0</td>
<td>4.7</td>
<td>3.6 ± .4</td>
<td>5.5 ± .7</td>
<td>3.2 ± .4</td>
</tr>
</tbody>
</table>

$^a$Mean ± SE.

$^b$Regression equation: $Y = 1.945 + .104X; P<.052$.

$^c$Regression equation: $Y = 2.735 + .035X; P<.09$.

$^d$Regression equation: $Y = 7.495 - .082X; P<.033$.

$^e$Regression equation: $Y = 1.349 + .074X; P<.011$. 
during the period subsequent to the decline in progesterone, and thus an increased number of observations, might have allowed for a more accurate estimate of amplitude of LH pulses during this period as compared with the period when progesterone concentrations were higher (frequency = .4/h). The relatively large standard error (3.3) associated with the mean for amplitude of LH pulses during the period when progesterone was increased as compared with the smaller standard errors (.1 to 1.9) during the period when progesterone was low support this point. However, the increased E2 during the preovulatory period might be acting to maintain the amplitude of LH pulses because in Exp. 2, E2 decreased frequency of LH pulses and increased amplitude of LH pulses in ovariectomized heifers implanted with E2 as compared with ovariectomized heifers without E2. After ovariectomy, mean concentrations of LH in heifers treated with E2 were higher than those in animals with no E2. Therefore, the mechanisms through which ovarian steroids regulate LH secretion in the heifer may not be the same as those reported in the ewe (Goodman and Karsch, 1980).

It has been suggested that the follicular phase rise in E2 in the ewe is driven by a sustained increase in tonic LH secretion (Goodman et al., 1981; McNeilly et al., 1982), and is terminated by the preovulatory surge of LH (Goodman et al., 1981). However, the initial increase in E2 after a decline of progesterone in estrous ewes and cows is independent of increases in LH (Fogwell et al., 1978; Gust et al., 1984). The data indicate that the initial increase in E2 during the decline in progesterone may be due to an increased responsiveness of follicles to circulating LH. Therefore, in the heifer progesterone decreases the frequency of pulses of LH and may decrease follicular response to LH. Thus, a decline in progesterone with the demise of the corpus luteum results in an increase in frequency of pulses of LH and an increase in E2 during the preovulatory period. Data from the present studies indicate that E2 contributes to maintenance of the amplitude of LH pulses during the time of the estrous cycle.

![Figure 2. Working model for an increase in mean LH concentrations during the preovulatory period in the estrual heifer.](image-url)
when progesterone concentrations are declining in the cow. Estradiol reduced the frequency of LH pulses in Exp. 2, which indicates that E$_2$ might be acting at the hypothalamic level to decrease frequency of discharge of LHRH in the cow. This is in contrast to data from the ewe, which indicate that E$_2$ does not influence frequency of secretion of LH (Goodman and Karsch, 1980).

Our working hypothesis concerning an increase in mean LH concentrations during the preovulatory period in the heifer is as follows (figure 2): Withdrawal of progesterone initiates the preovulatory events in the heifer by permitting an increase in the follicular response to circulating LH, enabling an initial increase in E$_2$. The decline of progesterone also increases frequency of LH pulses, which stimulates follicles to increase E$_2$ production. The increasing E$_2$ may, in turn, maintain amplitude of LH pulses. Therefore, the gradual increase in LH that occurs during the preovulatory period may not only be due to the increase in frequency of LH pulses, which results from the decline of progesterone, but also to the maintenance of amplitude of LH pulses by E$_2$.

Most research concerning the regulation of LH by ovarian steroids has been performed using silastic implants that give rise to a constant release of steroids. Schallenberger et al. (1984) and Walters and Schallenberger (1984) demonstrated that ovarian steroids were released in a pulsatile manner in the cow. This could suggest that fluctuant secretion of ovarian steroids is important in the regulation of pulsatile LH secretion. Using criteria similar to those used to identify LH pulses, E$_2$ pulses in jugular venous blood could not be determined in the present study; however, fluctuant secretions of E$_2$ seemed to be present. Thus, the data obtained using implants containing steroid hormones may oversimplify the physiological roles of steroids in vivo.

**Literature Cited**


