LUTEINIZING HORMONE CONCENTRATIONS IN SERUM OF POSTPARTUM BEEF COWS INJECTED WITH MICROENCAPSULATED LUTEINIZING HORMONE-RELEASING HORMONE ANALOG

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ABSTRACT

Twenty-five cows were divided equally into five groups to determine whether [D-Trp⁶]-luteinizing hormone releasing hormone (LHRH-A) microencapsulated in poly (DL-lactide co-glycolide) would increase basal serum concentrations of LH during the postpartum period. On d 5 postpartum, cows were injected i.m. with 2 ml of vehicle alone (Group 1) or vehicle containing microcapsules calculated to release .4, 1.6, 6.4 or 25.6 μg LHRH-A per day for approximately 30 d (Groups 2, 3, 4 and 5, respectively). Cows were bled every 15 min for 4 h immediately before and after injection and every 15 min for 4 h at weekly intervals for the next 4 wk to evaluate serum profiles of LH. Estrus was determined by twice daily observations and confirmed by serum progesterone. More cows in Groups 2, 3, 4 and 5 exhibited pulsatile patterns of LH after LHRH-A injection than in Group 1 (P < .06). More pulses of LH were observed after LHRH-A injection in Groups 4 and 5 than in Group 1 (P < .01). Mean concentrations of LH within treatment groups did not change during the initial injection, except in Group 5. All cows in Group 5 had a surge of LH immediately after injection. The induced surge of LH in two cows in Group 5 resulted in progesterone profiles similar to those during a normal luteal phase. Days to first postpartum estrus were not different among the five treatment groups. Microencapsulated LHRH-A given at a dose estimated to release 25 μg LHRH-A/d was effective in elevating LH concentrations following injection. However, effectiveness of this hormonal treatment in shortening postpartum anestrus was not substantiated.

(Key Words: LHRH, Postpartum Anestrous, LH, Cows.)


Introduction

Many factors influence the duration of postpartum intervals in beef cows. Low concentrations of circulating LH appear to be an important factor influencing the return of ovarian cyclicity following parturition. Concentrations of LH are depressed from immediately prepartum until about 5 to 10 d after parturition, at which time a gradual rise in circulating LH occurs until d 20 to 30 after parturition (Lamming et al., 1981; Riley et al., 1981). Distinct pulsatile secretion of LH is not detected in suckling beef cows until d 20 after parturition (Peters et al., 1981). Pulsatile
injections of LHRH have been used successfully to increase pulsatile secretion of LH and thereby to shorten the postpartum anestrous period in suckling cows (Riley et al., 1981; Walters et al., 1982). However, this type of treatment is impractical for treating postpartum anestrous. McLeod et al. (1984) reported that continuous infusion of LHRH in postpartum anestrous cows reduced the postpartum interval similarly to that brought about by pulsatile administration of LHRH. Therefore, an increase in mean concentration of LH by continuously infused LHRH may be an adequate stimulus to initiate ovarian cyclicity in postpartum anestrous cows. With this in mind, it was the objective of this study to determine whether an analog of LHRH would increase basal serum concentrations of LH and thereby shorten the postpartum anestrous period of suckling beef cows.

**Materials and Methods**

*Animals and Treatments.* Twenty-five crossbred beef cows maintained under the same nutritional and management conditions were assigned randomly to one of five treatment groups (five cows/group) with respect to calving date. On d 5 after parturition, cows were injected i.m. with vehicle alone (control) or vehicle containing .54, 2.16, 4.74, 34.56 mg (Groups 2, 3, 4 and 5, respectively) and microcapsules containing poly (DL-lactide co-glycolide) [D-Trp6]-luteinizing hormone-releasing hormone (LHRH-A). These quantities were calculated to deliver LHRH-A at the rate of .4, 1.6, 6.4 or 25.6 μg/d for approximately 30 d (Groups 2, 3, 4 and 5, respectively). Vehicle consisted of 2 ml water containing 2% carboxymethyl cellulose and 1% Tween 20. Microencapsulation of LHRH-A and evaluation of LHRH-A release from the microcapsules were described previously in rats (Redding et al., 1984; Asch et al., 1985; Mason-Garcia et al., 1985). All cows were kept with their calves throughout the study.

*Sampling and Hormone Assays.* Jugular venous blood samples were collected through indwelling cannulas every 15 min for 4 h before and after injection and every 15 min for 4 h at weekly intervals for 4 wk. Serum from each blood sample was subjected to RIA to determine circulating concentrations of LH (Adams et al., 1975). Pulses of LH were determined as described by Acosta et al. (1983). Briefly, a pulse was defined as an increase in LH that was 50% higher than its adjacent nadir with a minimum of two descending points.

All cows were observed twice daily for estrus from d 5 after parturition until each cow exhibited her first postpartum estrus. Progesterone concentrations from weekly blood samples were quantitated by a previously validated RIA (Chang et al., 1987) to confirm estrus and ensure that concentrations of progesterone remained elevated for the 2-wk period following estrus.

*Statistical Analysis.* The number of pulses of LH observed during all bleedings following injections were transformed (the square root of the number of pulses plus one) and subjected to analysis of variance for a completely random design. Least significant difference (LSD) was used to determine whether mean number of pulses of LH observed in each LHRH-A treated group differed from mean number of pulses observed in controls. Chi-square analysis was used to determine differences between the proportion of cows that exhibited pulsatile secretion of LH in LHRH-A treated groups and the control group. Mean concentrations of LH observed during each bleeding for each cow was used in a repeated measures design in order to analyze changes in LH secretion over the bleeding periods. The model for this analysis included treatment effect, cow within treatment, week, and treatment x week interaction. Pairwise comparisons (LSD) were made within treatments, using cow within treatment as the error term to determine whether mean concentrations of LH during the postinjection period increased above those in the preinjection periods. Analysis of variance for a completely random design was used to determine treatment differences in days to first postpartum estrus.

**Results**

Pulsatile patterns of LH were observed in only one of five control cows during the 4-wk period after injection, whereas four cows from each of the LHRH-A treated groups demonstrated pulsatile patterns of LH during this period (P < .06; Table 1). The number of pulses of LH observed in cows treated with microcapsules calculated to release .4 or 1.6 μg LHRH-A/d during the five bleedings following injection did not differ from the
TABLE 1. EFFECTS OF LHRH-A DOSES ON PULSES OF LH (FIVE COWS/DOSE)

<table>
<thead>
<tr>
<th>Dose of LHRH-A per day</th>
<th>No. of cows exhibiting pulses of LHa</th>
<th>Average pulses of LH in a 4-h periodb</th>
<th>Mean days to first estrusc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>.1</td>
<td>74</td>
</tr>
<tr>
<td>.4</td>
<td>4d</td>
<td>.4</td>
<td>37</td>
</tr>
<tr>
<td>1.6</td>
<td>4d</td>
<td>.2</td>
<td>60</td>
</tr>
<tr>
<td>6.4</td>
<td>4d</td>
<td>.6e</td>
<td>54</td>
</tr>
<tr>
<td>25.6</td>
<td>4d</td>
<td>.6e</td>
<td>54</td>
</tr>
</tbody>
</table>

aTotal number of cows that had pulses of LH at some time during the five bleeding periods after injection of LHRH-A.
bAverage number pulses/cow for each 4-h period for the five bleedings after injection. Transformed SE = .05.
cSE = 37.
dDifferent from control (P < .06).
eDifferent from control (P < .01).

number of pulses observed in control cows. Cows injected with microcapsules estimated to release 6.4 or 25.6 μg LHRH-A/d had more (P < .01) pulses of LH than control cows did during the sampling periods following injection (Table 1).

Mean concentrations of LH within treatment groups did not increase over time compared with preinjection concentrations in any of the groups except the group treated with the highest dose of microcapsules. All cows injected with microcapsules calculated to release 25 μg LHRH-A/d had preovulatory-like surges of LH immediately following injection with fewer (P < .01) surges preinjection than postinjection. Mean LH in this group returned to concentrations similar to those observed in the preinjection period by wk 1 after injection and then slowly increased during the following 3 wk (Table 2). No differences in the time to first postpartum estrus were observed between any of the treatment groups (Table 1).

Discussion

The range of calculated doses used in this study was chosen based on previous studies in postpartum anestrous cows that demonstrated that natural LHRH (which has a lower biopotency than the analog) must be given at a dose equivalent to at least 6 μg/d to increase basal concentrations of LH (Walters et al., 1982; Edwards et al., 1983) and of 60 μg/d resulted in surges of LH (McLeod et al., 1984) and pituitary desensitization (Riley et al., 1981). Collectively, these studies indicated that a dose range suitable for treating postpartum cows would be between 6 and 60 μg LHRH/d. The analog of LHRH used in this study is approximately 13 times more potent than the naturally occurring LHRH molecule in the rat (Coy et al., 1975). Therefore, logarithmic doses calculated to release .4, 1.6, 6.4 or 25.6 μg LHRH-A/d were chosen for use in this study. It was speculated that the low dose would have little or no effect on circulating concentrations of LH and that the high dose possibly could result in pituitary desensitization.

The number of pulses of LH observed in cows that received the high doses, 6.4 and 25.6 μg/d, of microencapsulated LHRH-A indicate that this type of delivery system is adequate to increase pulse frequency of LH in postpartum cows. However, this increase in pulse frequency is lower than the 2 to 5 pulses/4 h observed in postpartum cows that were close to resuming estrous activity (Peters et al., 1981; Walters et al., 1982).

Only the cows injected with a dose calculated to release 25 μg LHRH-A/d had postinjection concentrations of LH that were higher than preinjection concentrations. These cows all had preovulatory-like surges of LH immediately (beginning within 15 min) following injection of microcapsules. Two cows in this group had progesterone profiles indicative of normal luteal function following the induced surges of LH. Estrus was not detected at the time of the induced surges but did occur 23 and 26 d later in the two cows that had elevated progesterone after injections. Continuous infusion of natural LHRH in postpartum cows at a rate of 30 or 60 μg/d (Jagger et al., 1987) or 480 μg/d (Lamming and McLeod, 1988) also resulted in surges of LH within 6 h after onset of infusion that were followed either by transient rises of progesterone or by progesterone profiles indicative of normal luteal phases.

The fact that the surge of LH in the cows receiving the high dose of microcapsules occurred immediately after injection indicates that there was a burst of release from the microcapsules. This burst of LHRH-A release maintained elevated LH throughout the 4-h postinjection period. Concentrations of LH then declined to preinjection levels by 1 wk after injection and then gradually increased
TABLE 2. COMPARISON OF MEAN PRE- AND POST-INJECTION CONCENTRATION OF LH (NG/ML)

<table>
<thead>
<tr>
<th>Bleeding period</th>
<th>0</th>
<th>1.6</th>
<th>6.4</th>
<th>25.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min to 4 h postinjection</td>
<td>2.9</td>
<td>4.5</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td>1 wk postinjection</td>
<td>2.8</td>
<td>4.5</td>
<td>3.9</td>
<td>25.6*</td>
</tr>
<tr>
<td>2 wk postinjection</td>
<td>2.7</td>
<td>4.5</td>
<td>3.5</td>
<td>3.9</td>
</tr>
<tr>
<td>3 wk postinjection</td>
<td>2.7</td>
<td>4.3</td>
<td>3.6</td>
<td>4.5b</td>
</tr>
<tr>
<td>4 wk postinjection</td>
<td>3.4</td>
<td>4.7</td>
<td>3.3</td>
<td>4.5b</td>
</tr>
<tr>
<td>SE</td>
<td>1.02</td>
<td>1.08</td>
<td>1.02</td>
<td>1.02</td>
</tr>
</tbody>
</table>

\*Different from preinjection concentration (P < .001).
\d Different from preinjection concentration (P < .1).
\d Different from preinjection concentration (P < .05).

... during the next 3 wk (Table 2). This biphasic response of LH may be a direct response to LHRH-A release from the microcapsules. Release of LHRH-A from microcapsules is biphasic in rats (Mason-Garcia et al., 1985). The biphasic release of LHRH-A perhaps was due to diffusion of LHRH-A out of the microcapsules, resulting in a burst of LHRH-A release followed by enzymatic degradation of the microcapsules, which release LHRH-A at a much slower rate. Thus, biphasic release of LHRH-A may be responsible for the biphasic response of LH observed in cows treated with the high dose.

An alternative explanation for the biphasic LH response is an adjustment in pituitary sensitivity to LHRH-A. Lamming and McLeod (1988) reported that continuous infusion of LHRH increased concentrations of LH initially, but that by 2 to 3 d after initiation of infusion, concentrations of LH returned to values similar to those observed prior to infusion. These researchers observed that the pituitary gland still was capable of releasing LH in response to an injection of LHRH after 14 d of continuous infusion. However, the response was less than that observed when an injection was given prior to infusion. Therefore, perhaps the pituitary gland of cows injected with the high dose of LHRH-A may have been temporarily desensitized to the continuous exposure to LHRH-A but recovered as time progressed.

The information obtained in this study supports the theory that continuous infusion of low doses of LHRH can be used to increase concentrations of LH in the postpartum cow. Although the effectiveness of this treatment in reducing the postpartum anestrous period of beef cows was not substantiated in this study, further research is needed to evaluate this type of delivery system for LHRH and its analogs.

**Literature Cited**


