Luteinizing Hormone Has a Role in Development of Fully Functional Corpora Lutea (CL) But Is Not Required to Maintain CL Function in Heifers

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INTRODUCTION

CL secrete progesterone and are a required ovarian structure for maintenance of pregnancy in all mammals [1]. The preovulatory surge of LH causes formation of CL by inducing functional and morphological changes in theca and granulosal cells of the ovulating follicle [1]. LH is characteristically released in a pulsatile fashion from the anterior pituitary during the estrous cycle of bovine females [2]. During the luteal phase, LH pulses are released relatively infrequently (1 pulse/6 h) compared to those during the follicular phase (1 pulse/1 h) of the estrous cycle. Differences in frequency of LH pulses are due to the effect of progesterone on the putative pulse generator in the hypothalamus. Little is known about the physiological importance of pulses of LH, particularly those that occur during the luteal phase of the estrous cycle. It is not clear how these pulses of LH affect development and subsequent maintenance of CL function. The notion that LH is the primary luteotropic agent in cattle was first introduced by Simmons and Hansel [3]. Several researchers subsequently reported that LH is important for normal luteal function in ewes [4-6]. Early research in ewes that were hypophysectomized at various stages of the estrous cycle demonstrated that the CL required LH support for secretion of progesterone [7]. CL were more independent of LH support during the early luteal phase, whereas on Day 10 (Day 0 = estrus), CL were more severely affected by withdrawal of luteotropic support. Further research in ewes indicated that in the absence of LH support, luteal weight was reduced [8], and concentration of progesterone in circulation declined [5, 8, 9].

Baird [1] provided evidence that CL were less dependent on LH support during the earlier portions of the estrous cycle than on Day 13, when ewes were treated with an antagonist of LH. Treatment with antagonist of LH on Day 13 of the estrous cycle caused immediate luteal regression and a subsequent decline in secretion of progesterone. Secrecion of progesterone declined minimally, however, in ewes that were administered an antagonist of LH on Day 6 of their estrous cycle. However, further research with ewes indicated that treatment of ewes with a GnRH agonist for 29 days beginning on Day 1 of the estrous cycle subsequently abolished pulsatile release of LH but did not influence secretion of progesterone on Day 10 and Day 14 of the luteal phase [10]. Research with gilts has indicated that during the estrous cycle CL are autonomous, with LH producing negligible effects on circulating concentration of progesterone [11, 12]. Conversely, pulses of LH during the luteal phase of the estrous cycle in ewes, women, and mon-
keys induced a concomitant increase in secretion of progesterone by CL [1, 13, 14].

Information available on the role of pulsatile secretion of LH in luteal function is scarce. Therefore, the specific aim of the present experiment was to assess the effects of suppression of endogenous LH during the luteal phase of the estrous cycle on development and maintenance of CL in heifers.

MATERIALS AND METHODS

All procedures and protocols in this experiment were approved by the Institution Animal Care and Use Committee at the University of Nebraska-Lincoln. Twenty postpubertal beef heifers of composite breeding (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, 1/4 Red Poll; 15 mo of age; 281 ± 66 kg of body weight) were used in this study. Heifers were synchronized to a common day of estrus (Day 0 = day of behavioral estrus) with two i.m. injections of prostaglandin F₂₀ (25 mg Lutalyse®; The Upjohn Co., Kalamazoo, MI) administered 11 days apart. Heifers were then randomly assigned to one of four treatments (n = 5/treatment). An antagonist to LHRH (LHRH-Ant SB-75: N-Ac-D-Nal[2],4Cl-D-Phe⁶,D-Pal[3],D-Cit³,D-Ala⁶⁰-LHRH) or vehicle (5% mannitol) was administered s.c. every 24 h: 1) LHRH-Ant Days 2–7, 2) LHRH-Ant Days 7–12, 3) LHRH-Ant Days 12–17, 4) no LHRH-Ant (control). This antagonist was synthesized at the Peptide Synthesis Facility at the University of Nebraska. Heifers received the LHRH-Ant SB-75 at 10 μg/kg of body weight. In a preliminary trial, this dose suppressed pulses of LH within 4 h and up to 24 h after treatment in an ovariectomized cow. All heifers were treated with the 5% mannitol vehicle on the days they were not treated with LHRH-Ant. Catheters were inserted into the jugular vein on Day 2 of the experiment. Blood samples were collected at 15-min intervals for 24 h on Days 2, 7, 12, and 17 of the estrous cycle to characterize patterns of pulsatile LH secretion. Blood was also collected twice daily from Day 0 to Day 24 of the experiment via jugular venipuncture or catheter depending on the day of collection to determine concentrations of progesterone in circulation throughout the estrous cycle.

Blood samples collected at 15-min intervals were allowed to clot at room temperature and then stored at 4°C until centrifugation at 1520 × g for 15 min. Serum was then decanted and stored at −20°C until assayed for concentrations of LH. Samples collected twice daily were placed in tubes treated with 30% EDTA (50 μl for 10 ml blood sample; Fisher Scientific Co., Fair Lawn, NJ). To minimize degradation of progesterone, these samples were immediately stored at 4°C and centrifuged within 4 h of collection at 1520 × g for 10–15 min. Plasma was then collected, and stored at −20°C until assayed for concentrations of progesterone.

Concentrations of LH in all samples collected serially were analyzed by RIA [15] using rabbit antiserum against ovine LH (TEA-RαoLH #35), highly purified ovine LH (LER-1374A) as radiolabeled tracer, and NIH-LH-B7 as standard [16]. Intra- and interassay coefficients of variation were 3.2% and...
11.4%, respectively. Concentrations of progesterone in plasma were analyzed by RIA [17]. This procedure used a monoclonal antibody (02-9B4-94) to P₄-11-BSA (Bios-Pacific, Emeryville, CA), progesterone-11α-hemisuccinate trimethyl ester (TME) as radiolabeled tracer, and progesterone (Sigma Chemical Co., St. Louis, MO) as standard. Intra- and interassay coefficients of variation for progesterone assays were 1.9% and 18.6%, respectively.

Function of CL was analyzed by measuring the area under the profile for concentrations of progesterone in circulation during the luteal phase of the estrous cycle, with use of the planimeter function of Bioquant beginning on the first day of LHRH-Ant treatment for each treated heifer and ending on either Day 24 or the first day of luteolysis, whichever occurred first. Luteolysis was considered to have occurred when three consecutive samples contained less than 1 ng progesterone/ml of plasma. These areas were then compared to areas from control heifers measured for the same time period.

Measurements of area under the profile for concentrations of progesterone in circulation during the luteal phase of the estrous cycle for heifers of each group treated with LHRH-Ant were compared statistically to those of controls by ANOVA using the general linear model procedure of SAS [18]. Mean and basal concentrations of LH (ng/ml) in serum, frequency of LH pulses (pulses/24 h), and amplitude of LH pulses were determined by using algorithms with G values of 4.8, 3.8, 2.6, 1.9, and 1.5 for G(1) through G(5), respectively (Pulsar software modified for the IBM-PC by J.F. Gitzen and V.D. Ramirez, Urbana, IL).

Data regarding secretion of LH on Days 2, 7, 12, and 17 of the estrous cycle were fitted to a mixed linear model containing the fixed effects of treatment, day, treatment × day, and the random effect of heifer. Variance components were estimated by means of the REML algorithms of PROC MIXED in SAS [18]. Repeated measures were accounted for by assuming an autocorrelation error structure for residual error that estimates the correlation among repeated measurements on an individual heifer. Each group treated with LHRH-Ant was compared only to the control group on Days 2, 7, 12, and 17. Differences are indicated as being significant at $p < 0.10$, $p < 0.05$, or $p < 0.01$.

**RESULTS**

Frequency of LH pulses was reduced in all heifers treated with LHRH-Ant beginning on the day of treatment (Days 2,
ROLE OF LH IN CORPUS LUTEUM FUNCTION

FIG. 5. Secretory profiles of LH (ng/ml) on Days 2, 7, 12, and 17 from individual representative animal (heifer 11) from heifers treated with LHRH-Ant on Days 12–17 of estrous cycle.

TABLE 1. Mean and basal concentrations of LH, LH pulse frequency, and pulse amplitude of heifers treated with a LHRH antagonist compared to control heifers.

<table>
<thead>
<tr>
<th>Day 2</th>
<th>LH pulse frequency (pulses/24 h)</th>
<th>LH pulse amplitude (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRH-Antb treatment</td>
<td>Mean LHc (ng/ml)</td>
<td>Basal LHc (ng/ml)</td>
</tr>
<tr>
<td>Day 2–7 (n = 5)</td>
<td>0.98***</td>
<td>0.96***</td>
</tr>
<tr>
<td>Day 7–12 (n = 5)</td>
<td>1.33</td>
<td>1.17</td>
</tr>
<tr>
<td>Day 12–17 (n = 5)</td>
<td>1.51</td>
<td>1.42</td>
</tr>
<tr>
<td>CONT (n = 5)</td>
<td>1.54</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Pooled SEMd 0.18 0.13 2.47 0.38

*aDay of the estrous cycle (Day 0 = behavioral estrus).
*bTreated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
*cDetermined with Pulsar software.
*dPooled SEM.

**Numbers with asterisks within column differ from CONT by p < 0.05.
***Numbers with asterisks within column differ from CONT by p < 0.01.

7, or 12) for each group, and suppression extended through Day 17 of the estrous cycle compared to controls (p < 0.05, Figs. 2–5 and Tables 1–4). Basal concentrations of LH were reduced on Day 2 in heifers treated with LHRH-Ant from Days 2–7 as compared to controls (p < 0.01, Table 1). Basal concentrations of LH were also lower on Day 17 in heifers treated with LHRH-Ant on Days 7–12 and Days 12–17 compared to that of controls (p < 0.05, Table 4). Amplitude of LH pulses was not different among treatment groups on Day 2 (p > 0.10, Table 1). Heifers given LHRH-Ant on Days 2–7 had reduced amplitudes of LH pulses on Day 7 as compared to controls (p < 0.01, Table 2). On Days 12 and 17, heifers in all groups treated with LHRH-Ant had lower am-

TABLE 2. Mean and basal concentrations of LH, LH pulse frequency and pulse amplitude of heifers treated with a LHRH antagonist compared to control heifers.

<table>
<thead>
<tr>
<th>Day 7</th>
<th>LH pulse frequency (pulses/24 h)</th>
<th>LH pulse amplitude (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRH-Antb treatment</td>
<td>Mean LHc (ng/ml)</td>
<td>Basal LHc (ng/ml)</td>
</tr>
<tr>
<td>Day 2–7 (n = 5)</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td>Day 7–12 (n = 5)</td>
<td>0.76*</td>
<td>0.74</td>
</tr>
<tr>
<td>Day 12–17 (n = 5)</td>
<td>1.06</td>
<td>0.80</td>
</tr>
<tr>
<td>CONT (n = 5)</td>
<td>1.08</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Pooled SEMd 0.18 0.13 2.47 0.38

*aDay of the estrous cycle (Day 0 = behavioral estrus).
*bTreated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
*cDetermined with Pulsar software.
*dPooled SEM.

**Numbers with asterisks within column differ from CONT by p < 0.05.
***Numbers with asterisks within column differ from CONT by p < 0.01.

TABLE 3. Mean and basal concentrations of LH, LH pulse frequency and pulse amplitude of heifers treated with a LHRH antagonist compared to control heifers.

<table>
<thead>
<tr>
<th>Day 12</th>
<th>LH pulse frequency (pulses/24 h)</th>
<th>LH pulse amplitude (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRH-Antb treatment</td>
<td>Mean LHc (ng/ml)</td>
<td>Basal LHc (ng/ml)</td>
</tr>
<tr>
<td>Day 2–7 (n = 5)</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Day 7–12 (n = 5)</td>
<td>0.77***</td>
<td>0.74</td>
</tr>
<tr>
<td>Day 12–17 (n = 5)</td>
<td>0.68***</td>
<td>0.67</td>
</tr>
<tr>
<td>CONT (n = 5)</td>
<td>1.26</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Pooled SEMd 0.18 0.13 2.47 0.38

*aDay of the estrous cycle (Day 0 = behavioral estrus).
*bTreated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
*cDetermined with Pulsar software.
*dPooled SEM.

**Numbers with asterisks within column differ from CONT by p < 0.05.
***Numbers with asterisks within column differ from CONT by p < 0.01.
TABLE 4. Mean and basal concentrations of LH, LH pulse frequency and pulse amplitude of heifers treated with a LHRH antagonist compared to control heifers.

<table>
<thead>
<tr>
<th>Day 17*</th>
<th>LHRH-Antb treatment</th>
<th>Mean LHc (ng/ml)</th>
<th>Basal LHc (ng/ml)</th>
<th>LH pulse frequency (pulses/24 h)</th>
<th>LH pulse amplitude (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2–7 (n = 5)</td>
<td>0.89**</td>
<td>0.82</td>
<td>4.6***</td>
<td>0.51***</td>
<td></td>
</tr>
<tr>
<td>Day 7–12 (n = 6)</td>
<td>0.64***</td>
<td>0.63**</td>
<td>1.8***</td>
<td>0.40***</td>
<td></td>
</tr>
<tr>
<td>Day 12–17 (n = 5)</td>
<td>0.61***</td>
<td>0.61**</td>
<td>0.2***</td>
<td>0.10***</td>
<td></td>
</tr>
<tr>
<td>CONT (n = 5)</td>
<td>1.34</td>
<td>0.90</td>
<td>11.2</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>Pooled SEMd</td>
<td>0.18</td>
<td>0.13</td>
<td>2.47</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

*Day of the estrous cycle (Day 0 = behavioral estrus).
**Treated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
***Numbers with asterisks within column differ from CONT by p < 0.05.
****Numbers with asterisks within column differ from CONT by p < 0.01.

The mean area under the profile for mean concentrations of progesterone during the luteal phase of the estrous cycle of heifers treated with LHRH-Ant on Days 2–7 was less (p < 0.05; 3.72 ± 0.93 and 7.56 ± 1.02 units, respectively) compared to that of controls (Fig. 1). Heifers treated with LHRH-Ant on Days 7–12 also had a reduced area under the profile for mean concentration of progesterone during the luteal phase of the estrous cycle (p < 0.01; 3.02 ± 0.33 and 6.75 ± 0.99 units, respectively) compared to controls (Fig. 1). However, mean areas under the profile of progesterone in circulation of heifers treated with LHRH-Ant from Days 12–17 did not differ from those of controls (p > 0.10; 3.97 ± 1.02 and 4.27 ± 0.80 units, respectively; Fig. 1).

**DISCUSSION**

Our objective of blocking pulsatile release of LH beginning on Days 2, 7, and 12 of the estrous cycle was accomplished by administration of a potent antagonist to LHRH. Our studies are the first to demonstrate the ability of LHRH-Ant that may have developed from repetitive injections, thus leading to a slow and prolonged release of the antagonist.

More importantly, data from the present experiment provide evidence that pulsatile secretion of LH is involved in normal development and function of bovine CL. This conclusion is supported by early work of Simmons and Hansel [3], who reported that LH serves as a luteotrophic agent in the bovine. In the present experiment, pulsatile LH support was needed by developing CL (Day 2 to Day 12) for normal progesterone production. However, pulsatile secretion of LH after Day 12 was not needed to maintain function of CL as indicated by concentrations of progesterone in circulation. In contrast, Denamur et al. [7] and Baird [1] reported that early developing CL in ewes are more independent of LH support than mid-luteal phase CL (Day 10 to Day 13), and McNeilly and Fraser [10] found that absence of pulsatile secretion of LH did not influence secretion of progesterone on Day 10 and Day 14 of the estrous cycle. However, Baird [1] also noted that pulses of LH are followed by pulses of progesterone during the mid- and late luteal phases of the ovine estrous cycle, suggesting a distinct role of pulses of LH in luteal function.

Since pulses of LH remained suppressed beyond the treatment periods in the heifers given LHRH-Ant on Days 2–7 and Days 7–12, the timing of pulsatile LH suppression as well as the duration of LH suppression differed among treatment groups. Duration of LH deprivation rather than timing could possibly explain the resultant depressed luteal function. However, the mean progesterone profile of heifers treated with LHRH-Ant on Days 7–12 (Fig. 1) indicates that progesterone secretion was suppressed within three days after treatment compared to that in controls. This indicates that suppression of pulses of LH alters luteal function in a relatively short time span and that six days of treatment with antagonist was not necessary to suppress luteal function if administration occurred during this part of the estrous cycle. Among those heifers treated with LHRH-Ant on Days 12–17, there appeared to be an enhanced secretion of progesterone on Days 21–24 of the estrous cycle compared to that of controls. This increased concentration of progesterone can be attributed to a single heifer given LHRH-Ant on Days 12–17 that exhibited an extended life span of the CL.

Interestingly, although endogenous-secretion of LH pulses was blocked from the beginning of the three time periods (Days 2, 7, and 12), basal levels of LH remained in circulation. Basal concentrations of LH were also detected in a biological assay of LH using mouse Leydig cells [22]. This basal secretion of LH probably contributed to development and maintenance of CL, although at a reduced level, in heifers treated with LHRH-Ant on Days 2–7 and Days 7–12. These basal concentrations of LH, however, were not sufficient luteotropically to develop fully functional CL.
crete LH in pulses and not in a tonic pattern, and on the basis of data from the present study, pulses of LH are important if fully functional CL are to develop in bovine females. Perhaps chronic supplementation of LH would be sufficient to promote normal function of CL, but chronic administration of LH does not mimic the physiological pattern of LH secretion in cows.

Numerous theories can be developed as to how function of CL is altered in the absence of pulsatile LH support. In addition to suppression of LH, the LHRH-Ant may have suppressed secretion of FSH, which in turn may have had an impact on luteal function. However, at present no research in cattle has demonstrated a role for FSH in luteal development or function. Similarly, unknown factors within the bovine endocrine system may have been altered by treatment with LHRH-Ant and subsequently may have affected luteal function.

A more logical explanation of how LH suppression affects luteal function requires focusing on the cellular and morphological components of bovine CL. In cattle and some other species, two distinct steroidogenic cell types, large and small luteal cells, are known to be the main steroidogenic components of CL [23–25]. In cattle and sheep, large luteal cells arise primarily from the granulosal cells of the follicle, whereas small luteal cells develop from theca interna cells and in turn can differentiate into large luteal cells as CL develop [23–28]. Small luteal cells in ovine species contain greater numbers of LH receptors than large luteal cells and respond by secreting more progesterone when stimulated with LH [24]. We speculate that in the present experiment, with the absence of LH support from Days 2 through 12 of the bovine estrous cycle, small luteal cells were not provided the stimulation to secrete progesterone and without LH were incapable of developing into large luteal cells. Because of reduced numbers of large steroidogenic luteal cells, the capacity of CL to secrete progesterone was hindered throughout the remainder of the luteal phase.

In the absence of pulsatile LH support from Days 12 through 17 in the present experiment, CL continued to function normally, secreting progesterone in concentrations similar to those of controls. This result is consistent with the expectation that an abundant population of large cells is derived from small luteal cells and that these large cells do not require LH for progesterone secretion [24]. Additionally, Milvae et al. [29] removed granulosal cells from preovulatory follicles and found a reduction in secretion of progesterone similar to that detected in heifers treated with LHRH-Ant on Days 2–7 and Days 7–12. Our research would then suggest that when pulsatile LH support is removed from the developing and early-formed CL (Days 2–12), insufficient numbers of large luteal cells are formed; therefore, CL function as indicated by progesterone secretion is reduced. We believe, then, that LH support is needed by CL from Days 2–12 of the estrous cycle so that CL can be formed that are capable of secreting progesterone at levels typical for a bovine female. The next step in elucidating the role of LH pulses in CL function is to determine what morphological changes of CL occur, such as shifts in populations of small and large luteal cells, in the absence of LH pulses at various stages of the bovine estrous cycle.

ACKNOWLEDGMENTS

We appreciate the assistance of Deb Clopton in performing LH and progesterone RIA and also of Robin Hopper in manuscript preparation. We thank Dr. Gautam Sarath and the Peptide Synthesis Facility of the Center for Biotechnology-University of Nebraska for synthesis of antagonist and to Dr. A.V. Schally for helpful advice regarding antagonist synthesis and use. We also thank Dr. Jerry Reeves for providing LH antiserum. Leo Reiehter, Jr., for providing purified LH, and Dr. Alain Belanger for the progesterone preparation for labeling. We greatly appreciate the countless hours of work by undergraduates in performing this experiment.

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