University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Faculty Papers and Publications in Animal Science

Animal Science Department

4-1-1994

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

K. E. Peters
University of Nebraska-Lincoln

E. G.M. Bergfeld
University of Nebraska-Lincoln, ebergfeld@sciencesocieties.org

Andrea S. Cupp
University of Nebraska-Lincoln, acupp2@unl.edu

F. N. Kojima
University of Nebraska-Lincoln

V. Mariscal
University of Nebraska-Lincoln

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/animalscifacpub

 Part of the Animal Sciences Commons

Peters, K. E.; Bergfeld, E. G.M.; Cupp, Andrea S.; Kojima, F. N.; Mariscal, V.; Sanchez, T.; Wehrman, M. E.; Grotjan, H. E.; Hamernik, D. L.; Kittok, Roger J.; and Kinder, J. E., "Luteinizing Hormone Has a Role in Development of Fully Functional Corpora Lutea (CL) But Is Not Required to Maintain CL Function in Heifers" (1994). *Faculty Papers and Publications in Animal Science*. 182.
https://digitalcommons.unl.edu/animalscifacpub/182

This Article is brought to you for free and open access by the Animal Science Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Papers and Publications in Animal Science by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

K. E. Peters, E. G.M. Bergfeld, Andrea S. Cupp, F. N. Kojima, V. Mariscal, T. Sanchez, M. E. Wehrman, H. E. Grotjan, D. L. Hamernik, Roger J. Kittok, and J. E. Kinder

This article is available at DigitalCommons@University of Nebraska - Lincoln: https://digitalcommons.unl.edu/animalscifacpub/182

BIOLOGY OF REPRODUCTION 51, 1248–1254 (1994)

Luteinizing Hormone Has a Role in Development of Fully Functional Corpora Lutea (CL) But Is Not Required to Maintain CL Function in Heifers[1]

K.E. PETERS, E.G. BERGFELD, A.S. CUPP, F.N. KOJIMA, V. MARISCAL, T. SANCHEZ[3]
M.E. WEHRMAN, H.E. GROTJAN, D.L. HAMERNIK,[4] R.J. KITTOK, and J.E. KINDER[2]

Department of Animal Science, University of Nebraska-Lincoln, Lincoln, Nebraska 68583–0908

ABSTRACT

We tested the hypothesis that endogenous pulses of LH have a role in development and maintenance of CL during the estrous cycle of the bovine female. Twenty heifers were synchronized to estrus by treating two times with prostaglandin $F_{2\alpha}$ 11 days apart (Day 0 = behavioral estrus). Heifers were then randomly assigned to one of four treatments (n = 5/group). Heifers were treated with an antagonist to LHRH (LHRH-Ant; *N*-Ac-D-Nal[2]^1,4Cl-D-Phe^2,D-Pal[3]^3,D-Cit^6,D-Ala^{10}-LHRH; 10 μg/kg body weight) or vehicle (5% mannitol) once 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). Blood samples were collected from the jugular vein twice daily on Days 0–24, and area under the profile of progesterone in circulation during the luteal phase of the estrous cycle was characterized from the start of each treatment period until the demise of CL or Day 24, whichever came first. Luteolysis was considered to have occurred when three consecutive samples contained less than 1 ng progesterone/ml plasma. Areas under the profile of progesterone in circulation during the luteal phase of the estrous cycle were compared to those of heifers from the control group for the same period. LHRH-Ant treatment diminished LH pulses in all treatment groups compared to control ($p < 0.05$). Treatment with LHRH-Ant on Days 2–7 diminished function of CL (3.72 ± 0.93 vs. 7.36 ± 1.02 units, respectively; $p < 0.05$). Heifers treated with LHRH-Ant on Days 7–12 also had reduced function of CL (3.02 ± 0.33 vs. 6.75 ± 0.99 units, respectively; $p < 0.01$). However, treatment with LHRH-Ant on Days 12–17 did not influence function of CL (3.97 ± 1.02 vs. 4.27 ± 0.80 units, respectively; $p > 0.10$). The data support our hypothesis that endogenous pulses of LH have a role in development and maintenance of CL during the estrous cycle of bovine females.

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 thecal 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 LHRH. Treatment with antagonist of LHRH on Day 13 of the estrous cycle caused immediate luteal regression and a subsequent decline in secretion of progesterone. Secretion of progesterone declined minimally, however, in ewes that were administered an antagonist of LHRH 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-

Accepted August 5, 1994.
Received April 4, 1994.
[1]Published as paper no. 10765, Journal Series, Nebraska Agr. Res. Div. This research was supported by USDA Animal Health Formula Funding, USDA CRGO 90-37240-5724 and appropriated funding of the State of Nebraska.
[2]Correspondence: James E. Kinder, A224j Animal Sciences, University of Nebraska, Lincoln, NE 68583–0908. FAX: (402) 472-6362.
[3]Current address: Colegio de Postgraduados, Texococco, Mexico.
[4]Current address: Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE 68583-0905.

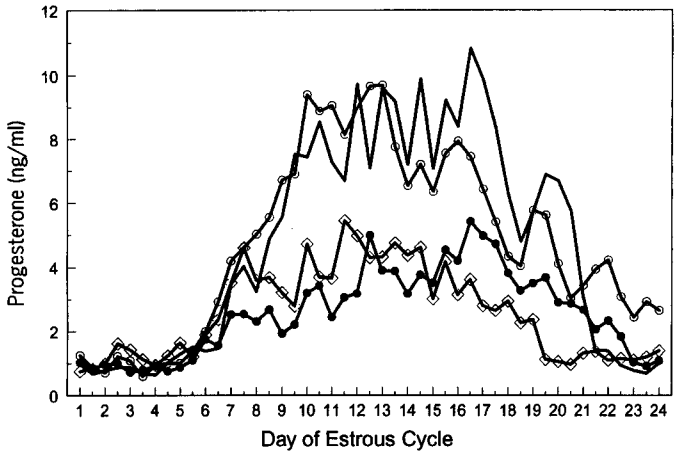

FIG. 1. Mean concentrations of progesterone (ng/ml) in plasma from heifers treated with an antagonist of LHRH and untreated control heifers. Solid line, control; solid line with solid circles, LHRH-Ant Days 2–7; solid line with open diamonds, LHRH-Ant Days 7–12; solid line with open circles, LHRH-Ant Days 12–17).

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.

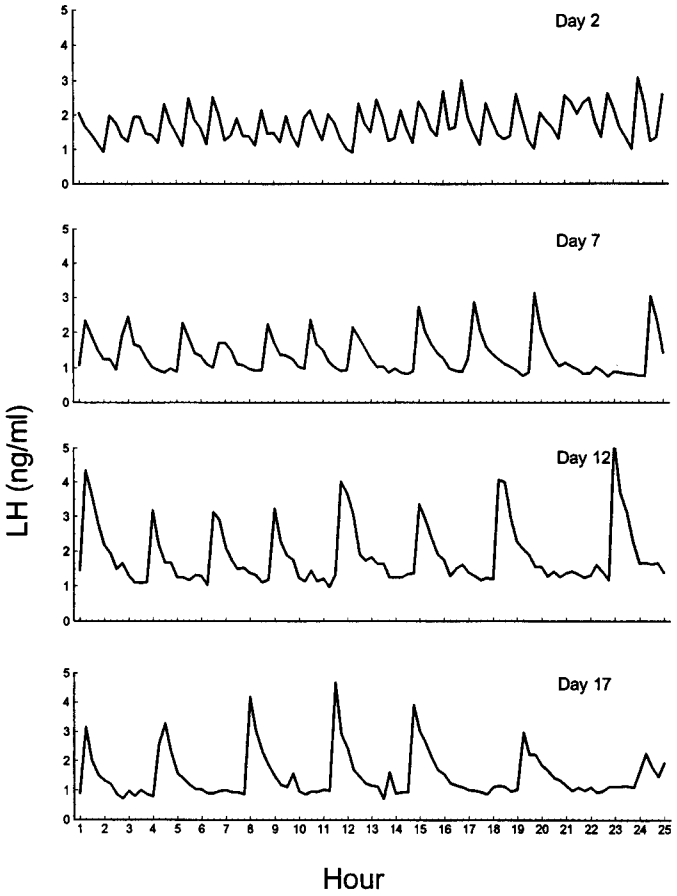

FIG. 2. Secretory profiles of LH (ng/ml) on Days 2, 7, 12, and 17 from individual representative animal (heifer 16) from untreated control group.

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 $F_{2\alpha}$ (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]^1,4Cl-D-Phe^2,D-Pal[3]^3,D-Cit^6,D-Ala^{10}-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-RAoLH #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

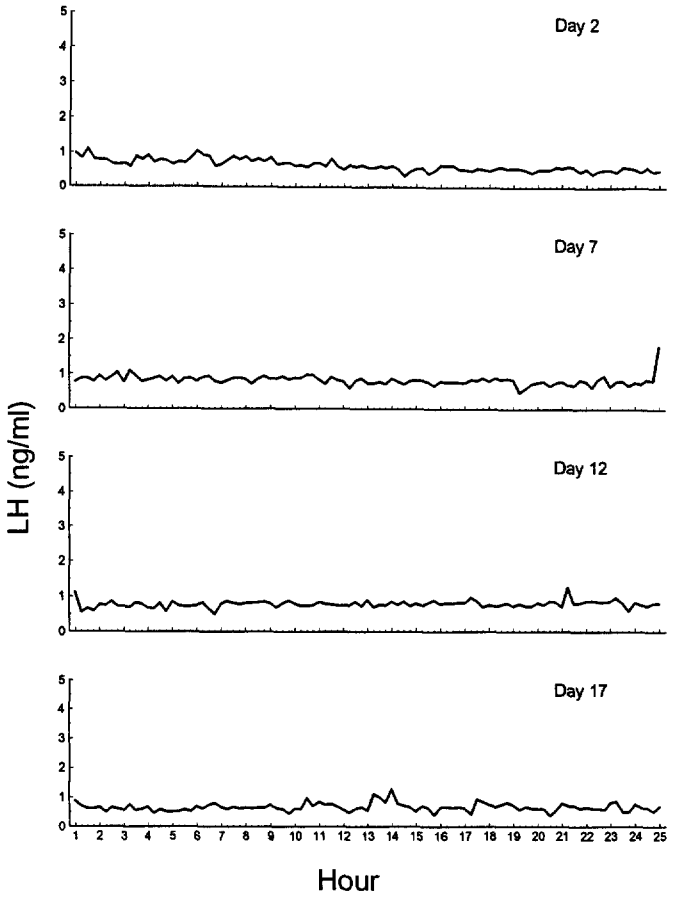

FIG. 3. Secretory profiles of LH (ng/ml) on Days 2, 7, 12, and 17 from individual representative animal (heifer 2) from heifers treated with LHRH-Ant on Days 2–7 of the estrous cycle.

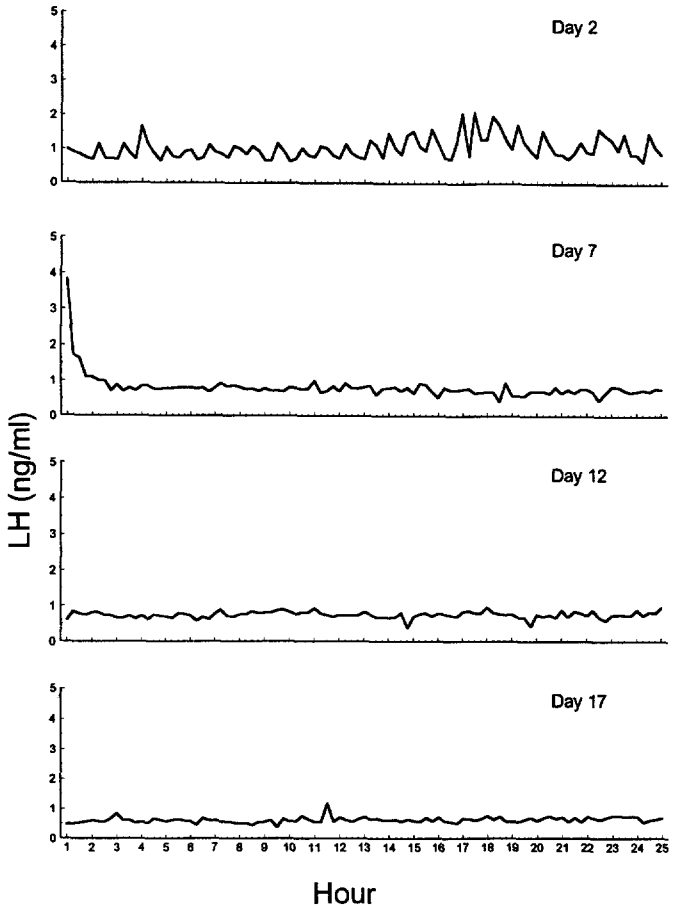

FIG. 4. Secretory profiles of LH (ng/ml) on Days 2, 7, 12, and 17 from individual representative animal (heifer 9) from heifers treated with LHRH-Ant on Days 7–12 of estrous cycle.

11.4%, respectively. Concentrations of progesterone in plasma were analyzed by RIA [17]. This procedure used a monoclonal antibody (02–9B4–94) to P_4-11-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,

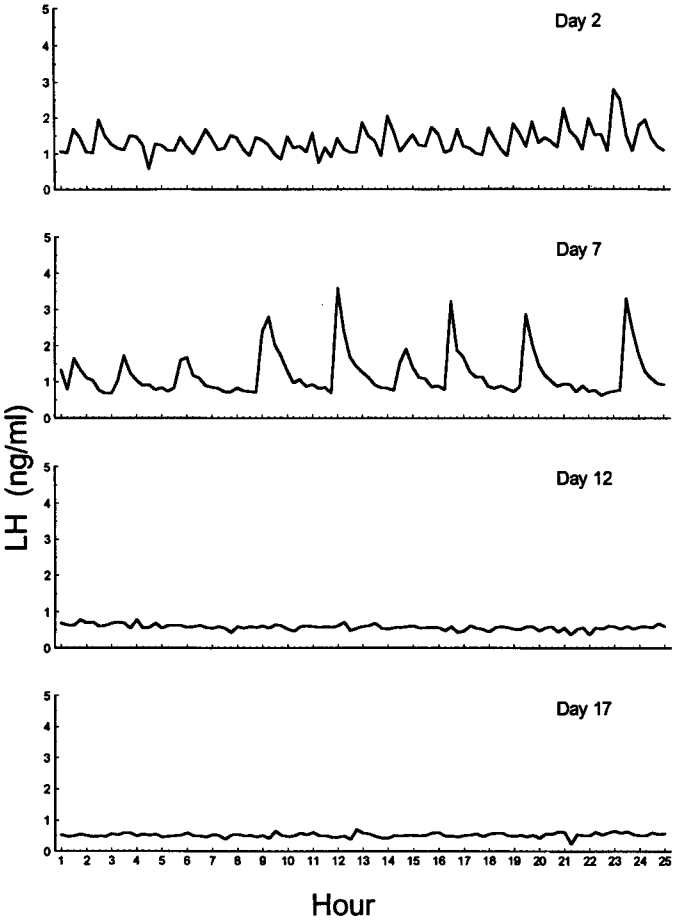

FIG. 5. Secretory profiles of LH (ng/ml) on Days 2, 7, 12, and 17 from individual representative animal (heifer 11) from heifers treated with LHRH-Ant on Days 12–17 of estrous cycle.

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

	Day 7[a]			
LHRH-Ant[b] treatment	Mean LH[c] (ng/ml)	Basal LH[c] (ng/ml)	LH pulse[c] frequency (pulses/24 h)	LH pulse[c] amplitude (ng/ml)
Day 2–7 (n = 5)	0.91	0.90	0.20***	0.21***
Day 7–12 (n = 5)	0.76*	0.74	0.80***	0.96
Day 12–17 (n = 5)	1.06	0.80	7.80	1.47
CONT (n = 5)	1.08	0.79	7.60	1.51
Pooled SEM[d]	0.18	0.13	2.47	0.38

[a]Day of the estrous cycle (Day 0 = behavioral estrus).
[b]Treated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
[c]Determined with Pulsar software.
[d]Pooled SEM.
*Numbers with asterisks within column differ from CONT by $p < 0.10$.
***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 1. Mean and basal concentrations of LH, LH pulse frequency, and pulse amplitude of heifers treated with a LHRH antagonist compared to control heifers.

	Day 2[a]			
LHRH-Ant[b] treatment	Mean LH[c] (ng/ml)	Basal LH[c] (ng/ml)	LH pulse[c] frequency (pulses/24 h)	LH pulse[c] amplitude (ng/ml)
Day 2–7 (n = 5)	0.98***	0.96***	1.0***	0.52
Day 7–12 (n = 5)	1.33	1.17	12.6	0.85
Day 12–17 (n = 5)	1.51	1.42	7.0**	0.91
CONT (n = 5)	1.54	1.32	13.4	0.94
Pooled SEM[d]	0.18	0.13	2.47	0.38

[a]Day of the estrous cycle (Day 0 = behavioral estrus).
[b]Treated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
[c]Determined with Pulsar software.
[d]Pooled 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.

	Day 12[a]			
LHRH-Ant[b] treatment	Mean LH[c] (ng/ml)	Basal LH[c] (ng/ml)	LH pulse[c] frequency (pulses/24 h)	LH pulse[c] amplitude (ng/ml)
Day 2–7 (n = 5)	0.99	0.98	0.60**	0.28***
Day 7–12 (n = 5)	0.77***	0.74	1.00**	0.29***
Day 12–17 (n = 5)	0.68***	0.67	0.80**	0.22***
CONT (n = 5)	1.26	0.88	6.2	2.18
Pooled SEM[d]	0.18	0.13	2.47	0.38

[a]Day of the estrous cycle (Day 0 = behavioral estrus).
[b]Treated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
[c]Determined with Pulsar software.
[d]Pooled 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.

	Day 17[a]			
LHRH-Ant[b] treatment	Mean LH[c] (ng/ml)	Basal LH[c] (ng/ml)	LH pulse[c] frequency (pulses/24 h)	LH pulse[d] amplitude (ng/ml)
Day 2–7 (n = 5)	0.88**	0.82	4.6***	0.51***
Day 7–12 (n = 5)	0.64***	0.63**	1.8***	0.40***
Day 12–17 (n = 5)	0.61***	0.61**	0.2***	0.10***
CONT (n = 5)	1.34	0.90	11.2	1.89
Pooled SEM[d]	0.18	0.13	2.47	0.38

[a]Day of the estrous cycle (Day 0 = behavioral estrus).
[b]Treated with an antagonist to LHRH every 24 h from Days 2–7, Days 7–12, Days 12–17 and control (vehicle injections).
[c]Determined with Pulsar software.
[d]Pooled SEM.
**Numbers with asterisks within column differ from CONT by $p < 0.05$.
***Numbers with asterisks within column differ from CONT by $p < 0.01$.

plitudes of LH pulses than controls ($p < 0.01$, Tables 3 and 4).

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 7.36 ± 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 SB-75 to suppress pulsatile release of LH in heifers. This result is in agreement with Szende et al. [19] and Korkut et al. [20], who detected a reduction in serum LH in athymic male nude mice and male rats treated with LHRH-Ant SB-75. Pulses of LH were not expected to be blocked beyond 24 h after the last treatment with LHRH-Ant. Pulses of LH remained suppressed, however, in all heifers treated with LHRH-Ant through the time of the last serial blood collection on Day 17 compared to LH pulses of controls. Repeated treatment with LHRH-Ant SB-75, may have resulted in a severe down-regulation of LHRH receptors at the anterior pituitary that extended beyond the treatment period [21]. Alternatively, suppression of pulses of LH beyond the treatment period may have resulted from depot stores 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 luteotropic 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 luteotropic support to develop fully functional CL. Cows se-

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 thecal 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 RIAs 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 antisera, Dr. Leo Reichert, 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.

REFERENCES

1. Baird DT. Luteotrophic control of the corpus luteum. Anim Reprod Sci 1992; 28:95–102.
2. Rahe CH, Owens RE, Fleeger JL, Newton HL, Harms PG. Patterns of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endocrinology 1980; 107:498–503.
3. Simmons KR, Hansel W. Nature of the luteotropic hormone in the bovine. J Anim Sci 1964; 23:136–141.
4. Domanski E, Skrzeczkowski L, Stupnicka E, Fitko R, Dobrowolski W. Effect of gonadotrophins on the secretion of progesterone and oestrogens by the sheep ovary perfused *in situ*. J Reprod Fertil 1967; 14:365–372.
5. Kaltenbach CC, Graber JW, Niswender GD, Nalbandov AV. Luteotrophic properties of some pituitary hormones in non-pregnant or pregnant hypophysectomized ewes. Endocrinology 1968; 82:818–824.
6. Hixon JE, Clegg MT. Influence of the pituitary on ovarian progesterone output in the ewe: effects of hypophysectomy and gonadotrophic hormones. Endocrinology 1969; 84:828–834.
7. Denamur R, Martinet J, Short RV. Secretion of progesterone by corpus luteum of the ewe after hypophysectomy, pituitary stalk section and hysterectomy. Acta Endocrinol (Copenh) 1966; 52:72–90.
8. Fuller GB, Hansel W. Regression of sheep corpora lutea after treatment with antibovine luteinizing hormone. J Anim Sci 1970; 31:99–103.
9. McCracken JA, Baird DT, Goding JR. Factors affecting the secretion of steroids from the transplanted ovary in the ewe. Recent Prog Horm Res 1971; 27:557–647.
10. McNeilly AS, Fraser HM. Effect of gonadotrophin-releasing hormone agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe. J Endocrinol 1987; 115:273–282.
11. Hunter MG. Responsiveness *in vitro* of porcine luteal tissue recovered at two stages of the luteal phase. J Reprod Fertil 1981; 63:471–476.
12. Buhr MM. Effect of lipoproteins and luteinizing hormone on progesterone production by large and small luteal cells throughout the porcine estrous cycle. J Anim Sci 1987; 65:1027–1033.
13. Backstrom CJ, McNeilly AS, Leask RM, Baird DT. Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the menstrual cycle. Clin Endocrinol 1982; 17:29–42.
14. Filicori M, Butler JP, Crowley WF. Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. J Clin Invest 1984; 73:1638–1647.
15. Adams TE, Kinder JE, Chakraborty PK, Estergreen VL, Reeves JJ. Ewe luteal function influenced by pulsatile administration of synthetic LHRH/FSHRH. Endocrinology 1975; 97:1460–1467.
16. Wolfe MW, Stumpf TT, Roberson MS, Wolfe PL, Kittok RJ, Kinder JE. Estradiol influences on pattern of gonadotropin secretion in bovine males during the period of changed responses to estradiol feedback in age matched females. Biol Reprod 1989; 41:626–634.
17. Roberson MS, Wolfe MW, Stumpf TT, Kittok RJ, Kinder JE. Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. Biol Reprod 1989; 41:997–1003.
18. SAS. SAS User's Guide, Statistics. Cary, NC: SAS Inst Inc.;1985.

19. Szende B, Redding TW, Schally AV. Suppression of meiosis of male germ cells by an antagonist of luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 1990; 87:901–903.
20. Korkut E, Bokser L, Comaru-Schally AM, Groot K, Schally AV. Inhibition of growth of experimental prostate cancer with sustained delivery systems (microcapsules and microgranules) of the luteinizing hormone-releasing hormone antagonist SB-75. Proc Natl Acad Sci USA 1991; 88:844–848.
21. Srkalovic G, Bokser L, Radulovic S, Korkut E, Schally AV. Receptors for luteinizing hormone-releasing hormone (LHRH) in Dunning R3327 prostate cancers and rat anterior pituitaries after treatment with a sustained delivery system of LHRH antagonist SB- 75. Endocrinology 1990; 127:3052–3060.
22. Hejl KM, Wolfe MW, Kinder JE, Grotjan HE. Bioactive and immunoreactive concentrations of circulating luteinizing hormone during sexual maturation in the bovine. Biol Reprod 1992; 46:1205- 1210.
23. Donaldson L, Hansel W. Histological study of bovine corpora lutea. J Dairy Sci 1965; 48:905–909.
24. Fitz TA, Mayan MH, Sawyer HR, Niswender GD. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol Reprod 1982; 27:703–711.
25. Alila HW, Hansel W. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 1984; 31:1015–1025.
26. McClellan MC, Diekman MA, Abel JH, Niswender GD. LH, progesterone and the morphological development of normal and superovulated corpora lutea in sheep. Cell Tissue Res 1975; 164:291–307.
27. O'Shea JD, Cran DG, Hay MF. Fate of the theca interna following ovulation in the ewe. Cell Tissue Res 1980; 210:305–319.
28. Farin CE, Moeller CL, Mayan H, Gamboni F, Sawyer HR, Niswender GD. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. Biol Reprod 1988; 38:413–421.
29. Milvae RA, Alila HW, Bushmich SL, Hansel W. Bovine corpus luteum function after removal of granulosa cells from the preovulatory follicle. Domest Anim Endocrinol 1991; 8:439–443.