LHRH antagonist decreases LH and progesterone secretion but does not alter length of estrous cycle in heifers[1]

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ABSTRACT: The objective of this study was to evaluate the suppressive effect of an LHRH antagonist, Cetrorelix SB-75 (SB-75), on secretion of LH, FSH and ovarian function in beef heifers. In Exp. 1, heifers were treated with a single dose of 10 μg/kg body weight intramuscularly on d 3 of the estrous cycle. In Exp. 2, heifers received either a single injection (100 μg/kg) of SB-75 on d 3 of the estrous cycle or multiple injections of 20 μg/kg on d 3, 4, 5, 6, and 7. Serum LH, but not FSH, was suppressed from one to several days. However, neither FSH nor progesterone was significantly altered. In Exp. 3, heifers received an injection vehicle (5% mannitol) or 100 μg/kg BW of SB-75 on d 1 of the estrous cycle (30 h after first observed standing estrus). Injection of SB-75 suppressed LH pulse frequency on d 3, 5, and 7 ($P < 0.001$). The mean LH concentrations in the SB-75 treatment groups were lower on d 3 ($P < 0.01$) and 5 ($P < 0.05$). There were no differences ($P > 0.1$) between the two groups in the mean concentrations of LH on d 1, 7, or 14. Treatment did not affect the secretion pattern or concentration of FSH. Injection of SB-75 did not alter estradiol-17β concentrations ($P > 0.1$). Treatment reduced corpus luteum (CL) function as indicated by lower progesterone production. However, the length of the estrous cycle was not shortened. These data show that the CL can form and survive in the face of depressed LH concentrations during the early stages of the estrous cycle.

Key Words: Antagonist, Corpus Luteum, FSH, Heifers, LH, LHRH

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Introduction

Luteinizing hormone (**LH**) is considered to be the major hormone responsible for formation and maintenance of the corpus luteum (**CL**) in the cow (Milvae et al., 1996). The CL is an important temporary endocrine gland in supporting pregnancy and regulation of the estrous cycle. Both progesterone ($\mathbf{P_4}$) and estradiol-17β ($\mathbf{E_2}$) regulate pulsatile release of LHRH and subsequent pulses of LH. However, the LH requirement of the CL related to time of the cycle is a controversial issue. McNeilly and Fraser (1987), Baird (1992), and McNeilly et al. (1992) observed that suppression of LH during the early luteal phase in the ewe did not alter CL function during the subsequent period. Peters et al. (1994) and Kojima et al. (1996), using the LHRH antagonist SB-75 (10 μg/kg BW), demonstrated that lack of LH support during early days of the luteal phase decreased P_4 production but did not abolish its effects.

In some estrus synchronization programs, it is necessary to have a CL at least 5 d old to respond to a prostaglandin $F_{2\alpha}$ ($\mathbf{PGF_{2\alpha}}$) injection for luteolysis. We hypothesized that if LHRH antagonist is given early in the cycle, it could possibly prevent the CL from developing. If the LHRH antagonist prevented early CL from developing, then a combination of $PGF_{2\alpha}$ and LHRH antagonist may be an effective single injection synchronization program. Therefore, the objective of this study was to evaluate the effective dose of the LHRH antagonist Cetrorelix SB-75 on gonadoptropin secretion and ovarian function in beef heifers.

Materials and Methods

Animals

Experiment 1. In Exp. 1, eight beef heifers were used. Heifers were synchronized for estrus (d 0 = day of behavioral estrus) with two injections of $PGF_{2\alpha}$ 11 d apart. Heifers were randomly assigned to either control or treatment (n = 4) groups. Control heifers received the

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vehicle (5% mannitol) and treatment animals received an intramuscular injection of 10 μg/kg of BW of Cetrorelix SB-75 (provided by ASTA Medica, Frankfurt, Germany) on d 3 of the estrous cycle. On this same day, immediately following SB-75 injection, blood samples were collected every 15 min for 24 h from all heifers. Blood samples were also collected at 15-min intervals for 6 h on d 5, 7, 10, 20, and 25 following estrus. All heifers were bled once every other day via the tail vein for progesterone analyses until animals exhibited signs of estrus.

Experiment 2. Twelve $PGF_{2\alpha}$-synchronized heifers were assigned to three groups (n = 4): control, single LHRH antagonist injection, or multiple LHRH antagonist injection. Heifers in the single-injection group received 100 μg/kg of BW of SB-75 on d 3 of the estrous cycle. The multiple-injection heifers received 20 μg/kg of BW of SB-75 (total 100 μg/kg) on d 3, 4, 5, 6, and 7 of the estrous cycle. Control heifers received the vehicle (5% mannitol) injection on the same days as the multiple-injection heifers. Heifers in all groups were serially bled: 25 samples were taken at 15-min intervals for 6 h on d 3, 5, 7, 9, 14, and 20 to determine pulsatile secretory pattern of LH and follicle-stimulating hormone (FSH). All heifers were bled every day via the tail vein for progesterone analyses until d 29 and then every other day until animals exhibited signs of the second estrus.

Experiment 3. Ten Synchromate B-synchronized heifers were assigned to two groups (n = 5). Heifers in the treatment group received 100 μg/kg of BW of SB-75 on d 1 (30 h after first observed standing estrus). Control group heifers received the carrier vehicle (5% mannitol) injection on the same day. Heifers in all groups were serially bled: 25 blood samples were taken at 15-min intervals for 6 h on d 1, 3, 5, 7, 14, and 20. In order to determine daily FSH, E_2, and P_4 concentrations, all heifers were bled every other day via the tail vein for two consecutive estrous cycles.

Assays

The pattern of LH secretion in circulation was characterized by determining mean concentration of LH and frequency of LH pulses. Serum samples were assayed for LH by a double-antibody RIA (Golter et al., 1973) using antiserum (JJR#35) USDA-b-LH-B6 as a standard. The FSH was determined by RIA described by Acosta et al. (1983). The E_2 serum concentrations were assayed using a radioimmunoassay kit (Coat-A-Count Estradiol, Diagnostic Products, Los Angeles, CA). Intra- and interassay CV were both 20%. Assay sensitivity was 0.5 pg/mL. Concentrations of P_4 were assayed using a radioimmunoassay kit (Coat-A-Count Progesterone). The function of CL was analyzed by measuring the area under the curve for concentrations of P_4. The area under the P_4 curve for the entire estrous cycle was determined for each heifer using the trapezoidal summation method (Thomas, 1953). Intra- and interassay CV were 5 and 9%, respectively. Assay sensitivity was 0.1 ng/mL.

Table 1. Effects of Cetrorelix SB-75 (SB-75) treatment of heifers on d 3 of the estrous cycle on LH pulse frequency and concentration (Exp. 1)

	Control		Treatment (SB-75, 10 μg/kg)	
Day	LH, ng/mL (mean ± SE)	LH pulses (pulses/6 h, mean)	LH ng/mL (mean ± SE)	LH pulses (pulses/6 h, mean)
3	0.4 ± 0.05	4.7	0.06 ± 0.03*	1.4**
5	0.2 ± 0.04	3.0	0.1 ± 0.05	2.7
7	0.2 ± 0.09	2.5	0.1 ± 0.05	1.7
10	0.1 ± 0.05	2.5	0.1 ± 0.07	2.8
20	0.6 ± 1.9	—	0.3 ± 0.17	—
25	0.2 ± 0.08	—	0.3 ± 0.16	—

*Different from control ($P < 0.05$).
**Different from control ($P < 0.01$).

Statistical Analyses

The LH pulse was defined by the following criteria: 1) an increase of hormone greater than 50% of the previous nadir (Acosta et al., 1983); 2) the hormone level must have remained elevated for two sampling periods (Goodman and Karsh, 1980); and 3) the peak must be followed by two declining values or one declining value of at least 30% of pulse amplitude (Acosta et al., 1983). Mean ± SE of LH and FSH pulse frequencies and amplitude and LH, FSH, E_2 concentrations and area under the P_4 curve were analyzed by analysis of variance (ANOVA) using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC). Least significant difference was used to evaluate differences between means (Steel et al., 1997).

Results

Experiment 1

The 10-μg/kg dose of the LHRH antagonist SB-75 on d 3 of the estrous cycle significantly suppressed LH pulses from 4.7/6 h to 1.4/6 h (Table 1) and mean concentrations of LH from 0.4 ± 0.06 ng/mL to 0.06 ± 0.03 ng/mL. The single injection of the 10-μg/kg dose of SB-75 did not significantly alter the CL function during d 3 to 25 after treatment (data not shown).

Experiment 2

The 100-μg/kg dose of LHRH antagonist altered the LH secretory pattern and reduced the LH pulse frequency in both treatment groups compared to the control group (Figure 1 and Table 2). The mean serum LH concentrations on d 3 in both treatment groups were significantly suppressed (0.23 ± 0.1 ng/mL for single-injection heifers and 0.22 ± 0.1 ng/mL for multiple-

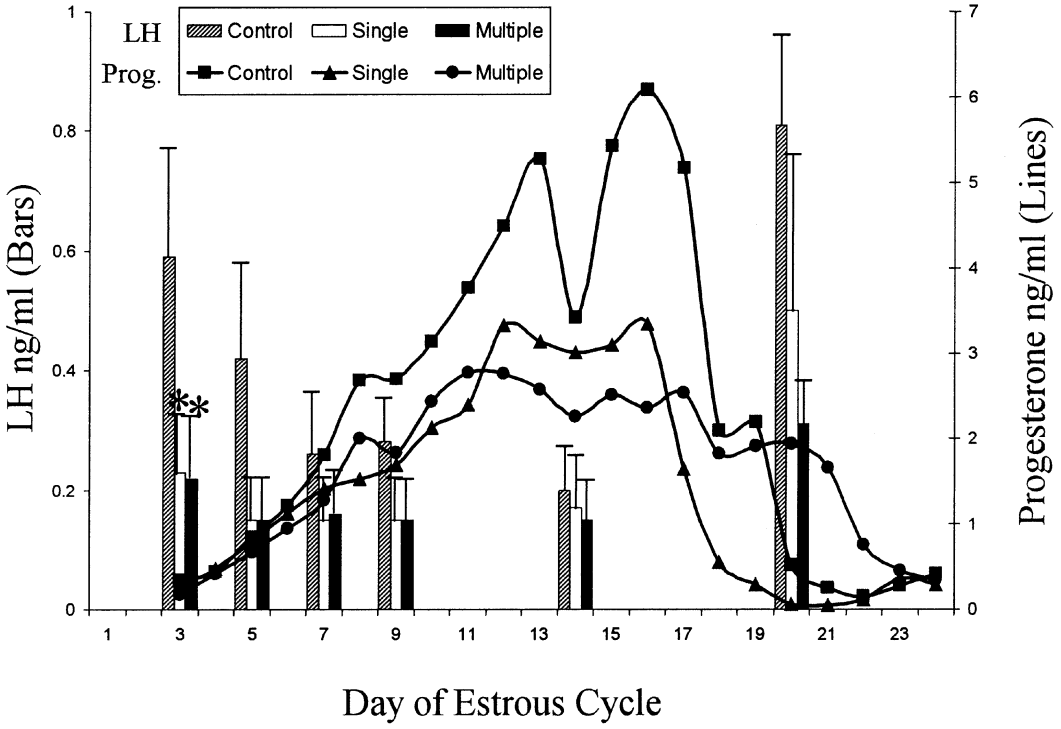

Figure 1. Serum LH and progesterone concentrations in heifers administered Cetrorelix SB-75 (SB-75) as a single injection of 100 μg/kg BW on d 3 of the estrous cycle or multiple injections of 20 μg/kg BW on d 3, 4, 5, 6, and 7 of the estrous cycle. *Different from control mean on that day ($P < 0.05$). Administration of SB-75 tended ($P < 0.10$) to decrease progesterone concentrations.

injection heifers) compared to control values of 0.6 ± 0.2 ng/mL (Figure 1). There were no differences ($P > 0.1$) between any groups for mean concentrations of LH on d 5, 7, 9, and 14. The LHRH antagonist treatment did not alter ($P > 0.05$) the secretory pattern or mean serum concentrations of FSH (data not shown). The mean areas under the P_4 curve in treatment groups (3.0 ± 0.5 arbitrary units for single injection; 3.6 ± 1.4 units for multiple injection) tended to be smaller but were not different ($P > 0.5$) from that of the control group (5.2 ± 1.4 units; Figure 1). The progesterone profiles among the groups during the second cycle were similar to each other compared to the first cycle (data not shown). Estrous cycle lengths of 20 ± 1 d for control and 21 ± 1 and 23 ± 1 d for single- and multiple-injection groups, respectively, were not significantly different due to treatment. The limited animal numbers associated with the amount of variance noted in P_4 profiles is probably the reason for nonsignificance of P_4 after SB-75 treatment.

Table 2. Effect of Cetrorelix SB-75 treatment as a single or multiple injection on LH pulse frequency in heifers (Exp. 2)

	Control	Single injection of SB-75 (100 μg/kg) on d 3	Multiple injections of SB-75 (20 μg/kg) on d 3, 4, 5, 6, and 7
Day	LH pulse (pulses/6 h, mean)	LH pulse (pulses/6 h, mean)	LH pulse (mean)
3	6.3	1.3***	1.0***
5	5.0	0.0***	0.0***
7	2.0	0.0*	0.0*
9	2.5	0.0*	0.0*
14	0.0	0.0	0.0

*Different from control ($P < 0.05$).
***Different from control ($P < 0.001$).

Experiment 3

Serum LH pulse frequency was depressed ($P < 0.001$) on d 3, 5, and 7 after a single injection of Cetrorelix at 100 μg/kg BW, 30 h after estrus, compared to LH pulse frequency in control animals (data not shown). Mean LH concentrations in the treatment group were lower only on d 3 ($P < 0.01$) and 5 ($P < 0.05$; Figure 2). There were no differences ($P > 0.1$) in the mean concentrations of LH between the two groups on d 1, 7, 14, or 20. Cetrorelix SB-75 treatment did not significantly affect the secretory pattern of FSH or mean serum concentrations of FSH during the estrous cycle (data not shown). The FSH concentrations during the second estrous cycle were similar to the values observed in the first cycle in both groups. Serum estradiol-17β concentrations fluctuated in both groups throughout the cycle. We could not see a treatment effect ($P > 0.1$) either when data were pooled or when evaluated on an individual animal

basis. Treatment reduced CL function, as indicated by lower P_4 concentrations (Figure 2). The mean area under the P_4 curve in the treatment group (1.0 ± 0.3, arbitrary units ± SEM) tended to be lower ($P < 0.1$) than that of the control group (2.5 ± 0.5, arbitrary units ± SEM). Progesterone profiles of groups during the second cycle were not different ($P > 0.1$) between groups. The LHRH antagonist treatment did not shorten ($P > 0.1$) the length of the estrous cycle. Mean length of the estrous cycle was 21 ± 1 d in the control group and 19 ± 1 d in the treatment group.

Discussion

A single injection of 10 μg/kg BW of LHRH antagonist (Cetrorelix SB-75) was effective in suppressing LH pulses and mean LH concentrations only during the injection day. Therefore, we decided to use a higher dose administered as a single injection or a multiple injection over 5 d. Treatment with a 100-μg/kg BW dose abolished LH pulse frequency for at least 7 d and reduced mean serum LH concentration. Suppression of serum LH pulse numbers and mean LH concentrations by the LHRH antagonist has been documented in cows by Rieger et al. (1989), Peters et al. (1994), and Madill et al. (1994). We could not detect any LH pulses on d 1 of the cycle. It seems that pulsatile release of LH secretion in cattle is abolished after ovulation because the releasable pool of pituitary LH is depleted during the postovulatory period (Convey et al., 1977). Pulsatile LH release was observed on d 3 in the control group; however, we are not certain whether LH pulsatility resumed on d 2. Peters et al. (1994) reported the presence of LH pulses on d 2 in heifers. In all cases, while LH pulses were suppressed by treatment, basal LH concentrations remained in the circulation at the same concentration as in control animals, indicating that the LHRH antagonist affected pulsatile LH release more severely than basal LH release.

In Exp. 2 and 3, LH pulses were suppressed for at least 7 d. Furthermore, a single injection of 100 μg of antagonist/kg BW had the same suppressive effect on LH secretion as did multiple injections of 20 μg/kg BW antagonist (total dose = 100 μg/kg). A high dose of antagonist may have formed a depot store of the antagonist that was released gradually, causing the extended suppression of LH pulses, or this concentration of SB-75 may have caused severe down-regulation of LHRH receptors that extended for at least 7 d. Alternatively, the prolonged duration of inhibition may be aided by the antagonist's being bound to serum proteins (Danforth et al., 1990). Although antagonist suppressed serum LH concentrations, there was always a detectable amount of LH in the serum.

In contrast to LH, the pulsatile release pattern and mean serum concentrations of FSH were unaffected

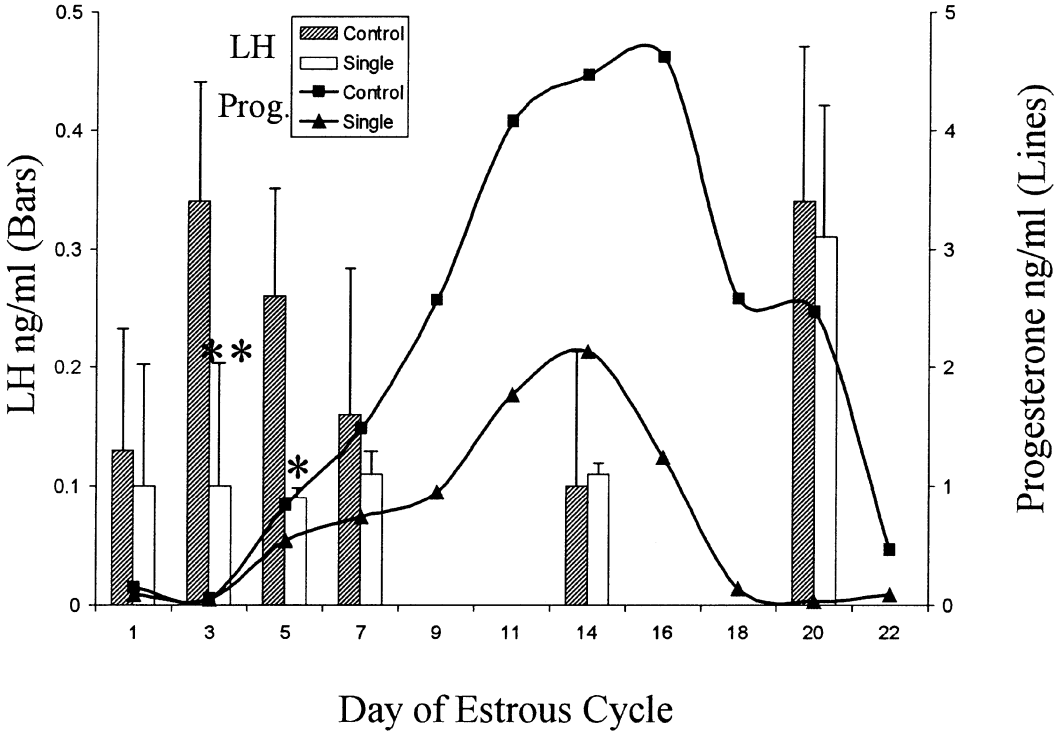

Figure 2. Serum LH and progesterone concentrations in heifers treated with either Cetrorelix SB-75 (SB-75) (100 μg/kg BW) or carrier medium 30 h after first detected estrus. Histogram shows mean serum LH concentrations. The lines show serum progesterone concentrations. *Different from control mean on that day ($P < 0.05$). **Different from control mean on that day ($P < 0.01$). Administration of SB-75 significantly decreased progesterone concentrations.

by treatment with SB-75 in all experiments. A similar observation has been reported by Manikam et al. (1995) in heifers. These observations seem inconsistent with the well-known phenomenon that LHRH regulates the synthesis and release of gonadotropins, LH, and FSH (Schally et al., 1971). The present data would suggest the regulation of the secretion of LH and FSH by LHRH is divergent when challenged with an LHRH antagonist. The mechanism of this divergent regulation of secretion is unknown. This may be associated with the lack of change in serum estrogen during low LH concentrations. There was an increase in estrogen during luteolysis in both treatment and control animals, showing that the assay was sensitive enough to evaluate bovine serum estrogen samples.

The SB-75 treatment in this study did not alter E_2 production when administered during the early luteal phase of the estrous cycle. In the present study, E_2 concentrations of heifers in both treated and the control groups followed a pattern indicative of 2 to 3 follicular waves during the estrous cycle. It is not clear why follicular development was not affected in the presence of low concentrations of LH. In most studies, the effect of LHRH antagonist on E_2 concentrations was studied during the follicular phase. Suppression of E_2 production during the follicular (Campbell et al., 1990) or mid-luteal phases (Brooks and McNeilly, 1994) was demonstrated in sheep. Rieger et al. (1989) also delayed the preovulatory E_2 peak when an LHRH antagonist was administered during the late follicular phase. Similarly, E_2 concentrations were suppressed in a dose-dependent manner in superstimulated heifers (Madill et al., 1994).

Injecting SB-75 at a dose of 100 μg/kg tended ($P < 0.10$) to decrease P_4 output from the CL when administered on d 3 of the estrous cycle (Figure 1) and significantly decreased circulating concentrations of P_4 when administered on d 1 of the estrous cycle (Figure 2). In all cases, the life span of the CL lasted for a normal luteal phase. Using the same LHRH antagonist, progesterone production decreased during the late luteal phase in sheep (McNeilly et al., 1992) and mid-luteal phase in cattle (Kojima et al., 1996) due to a lack of LH support during the early days (d 2 to 7) of the luteal phase (Peters et al., 1994). However, SB-75 injections on d 12 to 17 of the estrous cycle (Peters et al., 1994) in cattle failed to decrease progesterone secretion.

Pulsatile LH secretion seems to be more important in CL development and function than is basal LH secretion. Even though CL function was lowered, complete regression of the CL did not occur. Peters et al. (1994) proposed that the absence of LH support due to an LHRH antagonist at the start of the estrous cycle through d 12 may not have provided enough LH to develop the small luteal cells into large luteal cells. The reduced numbers of large luteal cells thus reduced the amount of progesterone produced during the later stages of the estrous cycle. Apparently, complete regression of the CL is difficult, and a CL that is producing subnormal concentrations of serum P_4 can survive for its normal duration in the estrous cycle. It seems that the secretory pattern of LH during the very early days of the estrous cycle is important for CL development, and once the CL is exposed to adequate levels of LH during this period it can maintain itself for the rest of the cycle.

In conclusion, LH support during d 1 to 2 of the estrous cycle is important for the development of a fully mature CL; however, it is almost impossible to obtain a complete regression of CL by suppression of LH support during this period.

Implications

Luteinizing hormone is important in development of a fully mature corpus luteum in the cow. However, it is almost impossible to prevent the corpus luteum from developing or stop functioning "prematurely" by using the LHRH antagonist Cetrorelix SB-75. Cetrorelix SB-75 will not be useful in a $PGF_{2\alpha}$ synchronization scheme in causing repression of a d 0 to 5 corpus hemorrhagicum.

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