



## The effect of GnRH at time of insemination on initiation of LH pulses and subsequent progesterone<sup>1</sup>

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### Summary

Research has indicated that luteinizing hormone (LH) pulses play a vital role in corpus luteum (CL) formation and subsequent progesterone concentrations. Therefore, our objectives were to determine: 1) when LH pulses begin following onset of estrus, 2) the effect an injection of gonadotropin releasing hormone (GnRH) would have on initiation of LH pulses, and 3) the effect LH pulse initiation had on subsequent plasma progesterone concentrations. Cows were synchronized with the Select Synch + Controlled Internal Drug Releasing device (CIDR) protocol (d -7 100 µg GnRH and CIDR; d 0 25 mg prostaglandin (PG) and removal of CIDR; estrus detected with HeatWatch). Following detection in estrus, a jugular catheter was inserted in each cow (n = 10). Based on initiation of estrus, cows were allotted into two treatments: 1) GnRH given 12 h (12.5 ± 1.2 h) after the initiation of estrus (n = 5; 100 µg) and 2) Control (n = 5). Blood samples were collected at 15-min intervals for 6 h at 12 h (bleed 1), 26 h (bleed 2), 40 h (bleed 3), 54 h (bleed 4), and 68 h (bleed 5) after the onset of estrus. The interval from onset of estrus to bleed 1 and ovulation was similar between treatments. The GnRH cows tended to have a greater area under the LH curve for bleed 1 compared to control cows. No differences were detected in bleeds 2, 3, 4, or 5. Average concentration of LH for GnRH cows in bleed 1 tended to be greater than control. No differences were detected in bleeds 2, 3, 4, or 5. No differences were detected in pulse frequency between treatments in bleeds 1, 3, 4, or 5, but in bleed 2, control tended to have more pulses than GnRH (2.5 ± 0.5 vs 1.4 ± 0.4). The GnRH-treated cows tended to have greater subsequent progesterone concentrations; however, GnRH-treated cows that had no LH pulses during bleed 2 had lower progesterone concentrations than cows with pulses (control or GnRH). In summary, injecting cows with GnRH approximately 12 h after the onset of estrus tended to reduce LH pulses 26-32 h following initiation of estrus, and elimination of LH pulses between 26-32 h resulted in decreased concentrations of progesterone during the subsequent cycle.

### Introduction

A single injection of GnRH results in the release of LH. A surge of LH results in ovulation and the formation of a CL which produces progesterone. Progesterone is essential for embryo development and maintenance of pregnancy. It has been proposed that giving an injection of GnRH at the time of insemination could increase CL function; thereby, increasing subsequent concentrations of progesterone and embryo survival. Unfortunately, researchers have had conflicting results. In dairy cows, Mee et al. (1993) reported that an injection of GnRH given at the time of insemination caused an increase in subsequent concentrations of progesterone. However, Lucy et al. (1986) reported that an injection of GnRH at time of insemination decreased subsequent concentrations of progesterone. Furthermore, a study done in our laboratory with beef heifers also resulted in a decrease in subsequent concentrations of progesterone (Perry, 2006). Therefore, the question arises, why have different studies reported conflicting results?

Release of LH from the pituitary has been reported to be necessary for CL formation and function (Peters et al., 1994). Furthermore, pituitary LH content returned to normal within 1 d following an ovulatory surge of LH (Nett et al., 1986). Therefore, it can be expected that LH pulses return to normal within 1 d of an ovulatory surge of LH. However, Peters et al. (1994) found that utilizing a luteinizing hormone releasing hormone (LHRH) antagonist to inhibit LH pulses following an ovulatory surge of LH resulted in decreased

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CL function; thereby, resulting in decreased subsequent concentrations of progesterone. Based on these studies, we hypothesized that GnRH would delay the initiation of LH pulses, and by changing the timing of the initiation of LH pulses, would influence subsequent concentrations of progesterone. Our objectives were to determine: 1) when LH pulses begin following ovulation, 2) the effect an injection of GnRH would have on the initiation of LH pulses, and 3) the effect the timing of LH pulse initiation would have on subsequent concentrations of progesterone.

## Materials and Methods

Thirty-two non-lactating, open, mature cows were synchronized with the Select Synch + CIDR protocol. An injection of GnRH (100 µg as 2 mL of Ovasynch i.m.; IVX, St. Joseph, Missouri) was given at the time of the CIDR insertion. The CIDRs were left in for 7 days. All cows were given an injection of prostaglandin (PGF<sub>2α</sub>; 25 mg as 5 mL of Prostamate i.m., IVX, St. Joseph, Missouri) at the time of CIDR removal. The Heat Watch estrous detection system was used to determine when the cows initiated standing estrus. Onset of estrus was determined as the first of 3 mounts within a 4-h period of time lasting 2 s or longer in duration. After 10 cows were determined in standing estrus within a 6-h period of time, indwelling jugular catheters were inserted into each of the cows. At an average of 12 h after the onset of estrus, 5 of the cows were given an injection of GnRH (treatment group) and 5 did not receive treatment (control group).

Blood samples were collected via jugular catheters every 15 min for 6 h from 12-18 (bleed 1), 26-32 (bleed 2), 40-46 (bleed 3), 54-60 (bleed 4), and 68-74 (bleed 5) h after the onset of estrus. Cows then were bled daily for 15 d until the 18<sup>th</sup> d of their estrous cycle. Blood was allowed to coagulate at room temperature then stored at 4° C for 24 h. Samples were centrifuged at 1,200 x g for 30 min, and the serum was harvested and frozen at -20° C until analyzed. Serum samples from the intensive bleeds were analyzed for LH concentrations, and daily blood samples were analyzed for serum concentrations of progesterone using radio immunoassays (RIA).

Transrectal ultrasonography was used to determine ovulation using an Aloka 500V ultrasound with a 7.5 MHz linear probe (Aloka, Wallingford, CT). Ovulation was defined as the disappearance of a dominant follicle from an ovary.

Cluster was used to determine LH total content, average concentration of LH, and LH pulse frequency. Differences between the treatment groups in total content of LH, average concentration of LH, LH pulse frequency, and subsequent concentrations of progesterone were determined by analysis of repeated measures in SAS.

## Results

There were no differences between treatments for the interval from the onset of estrus to bleed 1 ( $P = 0.82$ ; Table 1). The GnRH treated group tended to have a greater total content of LH ( $P = 0.075$ ) and greater average concentration of LH ( $P = 0.068$ ) compared to the control group (Table 1). There was no difference ( $P = 0.65$ ) in LH pulse frequency. During bleed 2, there were no differences in LH total content ( $P = 0.48$ ) or average concentrations of LH ( $P = 0.53$ ). However, the control group tended ( $P = 0.095$ ) to have a greater LH pulse frequency compared to the GnRH-treated group (Table 2). All animals ovulated by 32 h after the onset of estrus. During bleeds 3, 4, and 5 there were no differences ( $P > 0.10$ ) in LH total content, average concentration of LH, or LH pulse frequency (Tables 3, 4, and 5).

Table 1. Influence of an injection of GnRH on LH concentration and pulse frequency 12 - 18 h after the onset of standing estrus.

	GnRH	Control	<i>P</i> -value
Interval from onset of estrus	12.5 ± 1.2	12.1 ± 1.2	0.82
Total LH content ng/6 h	2091.2 ± 158.4	1647.6 ± 182.9	0.075
Average concentration of LH; ng/mL	5.6 ± 0.4	4.4 ± 0.5	0.068
LH pulse frequency; pulses/6 h	2.0 ± 0.5	2.3 ± 0.6	0.65

Table 2. Influence of an injection of GnRH on LH concentration and pulse frequency 26 – 32 hours after the onset of standing estrus.

	GnRH	Control	<i>P</i> -value
Total LH content ng/6 h	1259.5 ± 141.6	1411.5 ± 158.4	0.48
Average concentration of LH; ng/mL	3.5 ± 0.4	3.8 ± 0.4	0.53
LH pulse frequency; pulses/6 h	1.4 ± 0.4	2.5 ± 0.5	0.095

Table 3. Influence of an injection of GnRH on LH concentration and pulse frequency 40 – 46 h after the onset of standing estrus.

	GnRH	Control	<i>P</i> -value
Total LH content ng/6 h	1316.3 ± 141.6	1296.5 ± 141.6	0.92
Average concentration of LH; ng/mL	3.5 ± 0.4	3.4 ± 0.4	0.87
LH pulse frequency; pulses/6 h	2.0 ± 0.4	2.2 ± 0.4	0.74

Table 4. Influence of an injection of GnRH on LH concentration and pulse frequency 54 – 60 h after the onset of standing estrus.

	GnRH	Control	<i>P</i> -value
Total LH content ng/6 h	1306.7 ± 141.6	1352.7 ± 158.4	0.83
Average concentration of LH; ng/mL	3.5 ± 0.4	3.6 ± 0.4	0.83
LH pulse frequency; pulses/6 h	1.8 ± 0.4	2.3 ± 0.5	0.49

Table 5. Influence of an injection of GnRH on LH concentration and pulse frequency 68 – 74 h after the onset of standing estrus.

	GnRH	Control	<i>P</i> -value
Total LH content ng/6 h	1595.3 ± 141.6	1697.5 ± 158.4	0.63
Average concentration of LH; ng/mL	4.3 ± 0.4	4.5 ± 0.4	0.67
LH pulse frequency; pulses/6 h	1.6 ± 0.4	2.3 ± 0.5	0.32

For subsequent concentrations of progesterone, the GnRH-treated group tended ( $P = 0.07$ ) to have greater concentrations than the control group, but there was not a treatment x time interaction (Figure 1). Two of the GnRH-treated animals did not have any LH pulses during bleed 2, while 3 animals did have LH pulses. All of the control animals had LH pulses. The GnRH-treated animals that did have LH pulses during bleed 2 had greater ( $P < .0001$ ) concentrations of progesterone compared to animals that did not have LH pulses. The control group falls intermediate between the two (Figure 2).

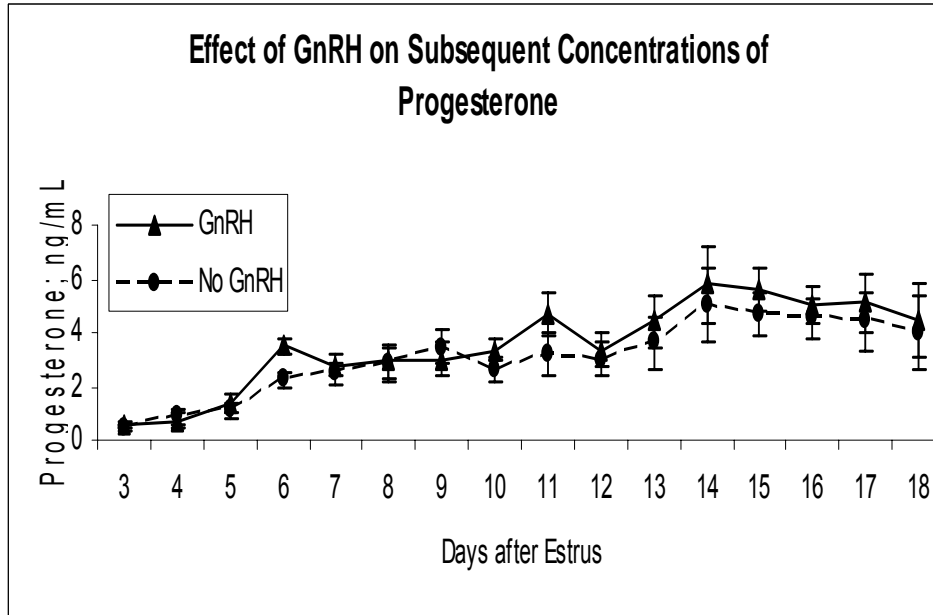


Figure 1. Influence on an injection of GnRH 12 hours after the onset of standing estrus on subsequent concentrations of progesterone. (Treatment  $P = 0.07$ ; Time  $P < 0.0001$ ; Treatment x time  $P = 0.72$ ).

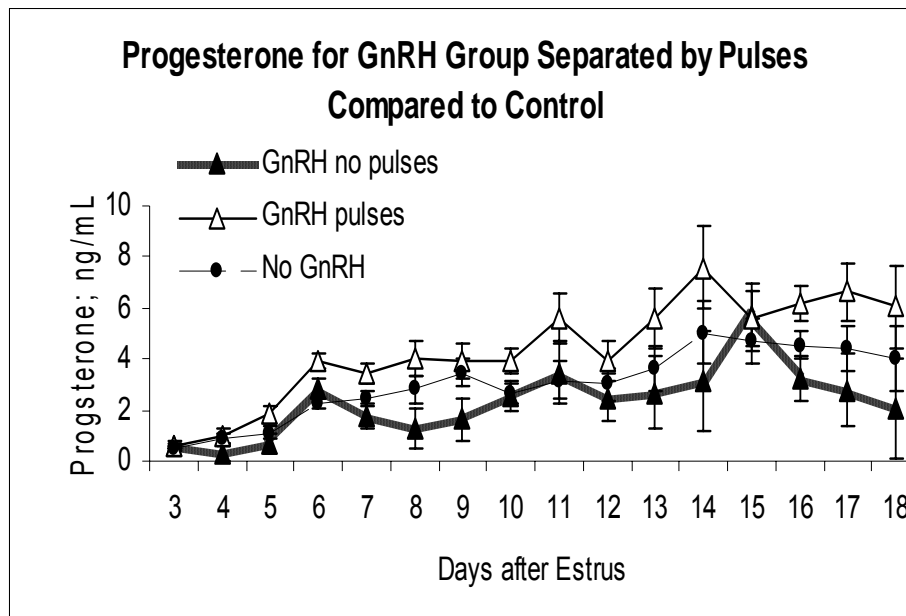


Figure 2. A comparison among the GnRH animals which had LH pulses during bleed 2, GnRH which did not have LH pulses during bleed 2, and control. (Treatment  $P < 0.0001$ ; Time  $P < 0.0001$ ; Treatment x time  $P = 0.014$ ).

## Discussion

Adequate production of progesterone by the CL must occur in order to establish and maintain pregnancy; therefore, it is important to have a well formed CL. In this study, GnRH was given at the time of insemination and tended to increase LH content and average concentrations from 12 to 18 h after the onset of estrus. However, it tended to reduce LH pulse frequency during bleed 2 (26 to 32 h after the onset of estrus). This may be due to the increased dumping of LH from the pituitary, down regulation of GnRH receptors in the pituitary, or another unknown mechanism. Bleed 2 may be an important time point for LH pulses because it is during the time that ovulation occurs. Ovulation occurs at about 30 h after the onset of estrus, and bleed 2 was 26-32 h after the onset of estrus. Animals that pulsed during that time period had greater subsequent concentrations of progesterone compared to animals that did not have LH pulses. Furthermore, the control animals had concentrations of progesterone intermediate between the other two groups. This may explain why sometimes research has indicated increases in subsequent concentrations of progesterone, whereas other research has reported decreases in subsequent concentrations of progesterone when given GnRH at the time of insemination.

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