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Abstract
Administering saline to 14 repeat breeders or 100 μg GnRH to 38 repeat breeders resulted in a greater (P=.07) proportion of pregnancies at 42 to 56 days after third service and fewer (P<.05) lost pregnancies during the 25- to 38-day period of placentation. Concentrations of LH in serum of six repeat breeders previously given 100 μg GnRH 12 hr after detected estrus (day 0) were reduced (P<.05) on days 1, 3, and 8 after estrus compared to those in six cows previously given saline at estrus. Concentrations of FSH were similar among treatment groups on days 1 and 3, but were elevated (P<.05) on day 8 in GnRH-treated cows. Furthermore, all six GnRH-treated cows had detectable FSH pulses on day 8 compared to two of six control cows. Concentrations of progesterone in serum were elevated (P<.05) on days 8 to 10 after estrus in GnRH-treated cows, and their corpora lutea obtained on day 10 after estrus and treatment contained a greater (P<.05) proportion (31 vs. 14%) of large-diameter (21 to 37 μm) luteal cells and a lesser (P<.05) proportion (69 vs. 86%) of small-diameter (10 to 17 μm) luteal cells than corpora lutea from control cows. In vitro production of progesterone in response to LH was reduced (P<.05) in luteal tissue obtained on day 10 after estrus from cows previously treated with GnRH at estrus compared to cows given only saline. It appears that pregnancy rates are improved in repeat breeders given GnRH at the time of insemination as a result of increased secretion of progesterone related to alterations in the morphology and function of the corpus luteum, as well as possible influences of FSH secretion from the pituitary gland.; Dairy Day, 1991, Kansas State University, Manhattan, KS, 1991;

Keywords
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ADMINISTRATION OF GnRH AT INSEMINATION IN REPEAT BREEDING DAIRY COWS: IMPROVED PREGNANCY RATES, HORMONE SECRETION, AND LUTEAL FUNCTION

J.S. Stevenson, M.O. Mee, and E.P. Call

Summary

Administering saline to 14 repeat breeders or 100 µg GnRH to 38 repeat breeders resulted in a greater (P=.07) proportion of pregnancies at 42 to 56 days after third service and fewer (P<.05) lost pregnancies during the 25- to 38-day period of placentation. Concentrations of LH in serum of six repeat breeders previously given 100 µg GnRH 12 hr after detected estrus (day 0) were reduced (P<.05) on days 1, 3, and 8 after estrus compared to those in six cows previously given saline at estrus. Concentrations of FSH were similar among treatment groups on days 1 and 3, but were elevated (P<.05) on day 8 in GnRH-treated cows. Furthermore, all six GnRH-treated cows had detectable FSH pulses on day 8 compared to two of six control cows. Concentrations of progesterone in serum were elevated (P<.05) on days 8 to 10 after estrus in GnRH-treated cows, and their corpora lutea obtained on day 10 after estrus and treatment contained a greater (P<.05) proportion (31 vs. 14%) of large-diameter (21 to 37 µm) luteal cells and a lesser (P<.05) proportion (69 vs. 86%) of small-diameter (10 to 17 µm) luteal cells than corpora lutea from control cows. In vitro production of progesterone in response to LH was reduced (P<.05) in luteal tissue obtained on day 10 after estrus from cows previously treated with GnRH at estrus compared to cows given only saline. It appears that pregnancy rates are improved in repeat breeders given GnRH at the time of insemination as a result of increased secretion of progesterone related to alterations in the morphology and function of the corpus luteum, as well as possible influences of FSH secretion from the pituitary gland.

(Key Words: Pregnancy Rates, Progesterone, LH, FSH, GnRH, Luteal Function.)
Procedures

In Experiment 1, 32 cows eligible for third service were assigned randomly as they were detected in estrus to receive (i.m.) either saline or 50, 100, or 250 µg GnRH (eight cows per group; Cystorelin, SANOFI Animal Health, Inc., Overland Park, KS) immediately following insemination (12 hr after detected estrus). Blood was collected on alternate days for 30 days beginning 4 days after estrus (day 0) to measure serum concentrations of progesterone.

In Experiment 2, an additional 12 cows were assigned to receive either saline or 100 µg GnRH immediately following insemination (12 hr after detected estrus). Blood was collected daily from estrus (day 0) until 40 days after estrus to measure concentrations of progesterone in serum. In addition, blood was collected for 8 hr at 15-min intervals on days 1, 3, and 8 after estrus to characterize average concentrations and pulse frequency of LH and FSH.

In Experiment 3, eight cows were treated with either 100 µg GnRH or saline 12 hr after detected estrus. Blood was collected daily from estrus until day 10 of the estrous cycle to measure serum concentrations of progesterone. On day 10, the ovary containing the corpus luteum was removed and both ovary and corpus luteum were weighed. A slice of the corpus luteum was fixed for histological analysis of large and small luteal cells and the remaining luteal tissue was sliced into 0.3 mm slices to determine in vitro production of progesterone. Duplicate flasks containing 200 to 300-mg slices of luteal tissue were incubated in a Dubnoff metabolic incubator at 39°C for 2 hr without LH or 2 hr with LH (10 ng/ml) in Dulbecco’s Modified Eagles Deficient Medium supplemented with glucose and antibiotics. Following the incubation period, 6 ml cold ethanol were added to each flask to terminate synthesis of progesterone. Tissue samples, including the unincubated control, were transferred to culture tubes and stored at -20°C until homogenized, extracted, and assayed for progesterone.

Results and Discussion

Results of our previous work demonstrating improved pregnancy rates at repeat services in three different studies are illustrated in Table 1. These studies were conducted in one 5,000-cow dairy herd in Oklahoma, five 500-cow dairy herds in California, and in our KSU Dairy Teaching and Research herd in Manhattan. In each study, one injection (i.m.) of 100 µg or 2 ml of GnRH improved (P<.05) pregnancy rates by approximately 25% or 11 percentage points.

Table 1. Pregnancy Rates in Dairy Cows When Given GnRH at the Time of Repeat Inseminations.

| Study | Control | GnRH |
|-------|---------|------|
| A     | 75/157  | 84/144 |
|       | 48%     | 58% a |
| B     | 108/344 | 74/169 |
|       | 31%     | 44% a |
| C     | 113/353 | 169/406 |
|       | 32%     | 42% a |
| Total | 296/854 | 327/715 |
|       | 35%     | 46%   |

*Different (P<.05) from control.

In Experiment 1, we examined concentrations of progesterone and pregnancy rates in order to determine how pregnancy rates might be improved in cows given GnRH. Three doses of GnRH (50, 100, and 250 µg) were tested and the results were similar, so data from the three doses are combined in Table 2. The proportion of cows that recycled or returned to estrus 18 to 24 days after insemination and treatment with GnRH or saline (control) was similar, as was the proportion of cows with high concentrations of progesterone from 30 to 40 days after insemination and treatment. However, when cows were palpated to determine pregnancy status at 42 to 56 days after that service, 43% of the cows receiving GnRH at
Table 2. Reproductive Characteristics in Repeat Breeders after GnRH Given at the Time of Insemination

| Item                              | Control | GnRH |
|-----------------------------------|---------|------|
| No. cows                          | 14      | 30   |
| No. returned to heat\(^1\)        | 7       | 14   |
|                                  | 50%     | 47%  |
| No. with high P\(_4\)\(^2\)       | 7       | 16   |
|                                  | 50%     | 53%  |
| No. pregnant\(^3\)               | 2       | 13   |
|                                  | 14%     | 43%  |
| No. embryos lost\(^4\)           | 5       | 3    |
|                                  | 71%     | 19%  |

\(^{a}\)Different (P<.05) from control.

\(^{1}\)Number of cows returning to estrus 18 to 24 days after insemination and treatment.

\(^{2}\)Number of cows with high progesterone until 30 to 40 days after insemination (assumed to be pregnant).

\(^{3}\)Number of cows pregnant by palpation at 42 to 56 days after insemination.

\(^{4}\)Number of potential embryos lost sometime from 25 days after insemination until palpation.

In Experiment 1, we examined the concentrations of the pituitary gonadotropins, LH and FSH, which are increased by the injection of GnRH. We examined these hormonal changes on days 1, 3, and 8 after estrus (day 0), when either GnRH or saline was given 12 hr after detected estrus. GnRH reduced (P<.05) average concentrations of LH on days 1, 3, and 8 compared to controls (Table 3), although the number of LH pulses was unaffected by treatment. There were no treatment effects on concentrations of FSH or number of FSH pulses on days 1 and 3, but GnRH treatment at estrus increased (P<.05) concentrations of FSH and the number of FSH pulses on day 8 compared to controls. All six treated cows had detectable pulses of FSH, whereas only two of six control cows had FSH pulses.

In Experiment 3, concentrations of progesterone were determined in eight cows treated with GnRH or saline 12 hr after estrus. Concentrations of progesterone were increased (P<.05) on days 8 to 10 after treatment with GnRH compared to saline (Figure 1).

In each of those same eight cows, the ovary bearing the corpus luteum was removed on day 10 after estrus (GnRH was administered at 12 hr after detected estrus). Then the procedure was repeated 33 days later when the same eight cows were treated at estrus with saline, and the remaining ovary and the corpus luteum were removed on day 10 of a subsequent estrous cycle. Weights of the ovaries and corpora lutea were unaffected by treatments, but the day-10 corpora lutea of cows previously treated with GnRH at estrus contained a greater (P<.05) proportion (31 vs. 14%) of large-diameter (21 to 37 µm) luteal cells and a lesser (P<.05) proportion (69 vs. 86%) of small-diameter (10 to 17 µm) luteal cells than corpora lutea of control cows obtained on day 10 after
Table 3. Characteristics of LH and FSH in Serum on Days 1, 3, and 8 after Treatment with GnRH or Saline at Estrus

| Item                | Control | GnRH |
|---------------------|---------|------|
| **Day 1**           |         |      |
| Avg LH, ng/ml       | 1.2     | 0.6a |
| No. LH pulses       | 0       | 0.5  |
| Cows w/ pulses      | 0/6     | 1/6  |
| Avg FSH, ng/ml      | 0.4     | 0.4  |
| No. FSH pulses      | 0       | 0    |
| Cows w/ pulses      | 0/6     | 0/6  |
| **Day 3**           |         |      |
| Avg LH, ng/ml       | 0.8     | 0.5a |
| No. LH pulses       | 1.8     | 1    |
| Cows w/ pulses      | 4/6     | 2/6  |
| Avg FSH, ng/ml      | 0.5     | 0.5  |
| No. FSH pulses      | 0.1     | 0.5  |
| Cows w/ pulses      | 1/6     | 1/6  |
| **Day 8**           |         |      |
| Avg LH, ng/ml       | 0.8     | 0.5a |
| No. LH pulses       | 1.3     | 1.5  |
| Cows w/ pulses      | 4/6     | 5/6  |
| Avg FSH, ng/ml      | 0.6     | 0.8a |
| No. FSH pulses      | 1       | 2.3a |
| Cows w/ pulses      | 2/6     | 6/6  |

aDifferent (P<.05) from control.

Blood was collected for 8 h at 15-min intervals on days 1, 3, and 8 after estrus (day 0). Treatment with saline (control) or 100 µg GnRH was given 12 h after detected estrus.

In summary, GnRH treatment at the time of insemination resulted in improved pregnancy rates because of higher embryonic survival in the 25 to 38-day period. This is the period when the placenta attaches to the uterine wall of the cow and generally corresponds to what is called the "late embryonic period." These improvements appear to result from increased secretion of progesterone and perhaps altered secretion of both LH and FSH. Further research is needed to determine what role FSH is playing in this process.