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Administration of GnRH at insemination in repeat breeding dairy cows: improved pregnancy rates, hormone secretion, and luteal function (1991)

Jeffrey S. Stevenson

M. O. Mee

Edward P. Call

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.)

Introduction

Dairy cattle that fail to conceive after several inseminations are a source of frustration and economic loss to the dairy enterprise. These so-called "repeat-breeders" are cows that fail to become pregnant after two or more services but continue to show estrus every 18 to 24 days. There are several potential causes of repeat breeding, including fertilization failure (29 to 41%), embryonic mortality (21 to 35%), defective luteal secretion of progesterone and other hormonal imbalances, errors in heat detection, various defects in sperm or egg function, and nutritional imbalances.

In three experiments conducted since 1981 involving over 2,200 repeat-breeding dairy cows located in Kansas, Oklahoma, and California, we have documented a 25% improvement in pregnancy rates when GnRH (100 µg Cystorelin®) was injected at the time of the third insemination (1988 Dairy Day, KAES Rep. Prog. 554, pp 16-18 and 1990 KAES Dairy Day Rep. Prog. 608, pp 34-37). A series of studies over the last 3 yr have been conducted to determine why GnRH is effective in improving pregnancy rates in these low fertility cows. Our purpose was to determine how GnRH affects hormone secretions and luteal function of cows treated at the time of insemination (12 hr after detected estrus), in order to better understand those components that influence and control fertility in cattle.

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 39^0C 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 -20^0C 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 48%	84/144 58%[a]
B	108/344 31%	74/169 44%[a]
C	113/353 32%	169/406 42%[a]
Total	296/854 35%	327/715 46%

[a]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

insemination were pregnant compared to only 14% of the control cows. This suggested that five (71%) pregnancies in the control cows and three (19%) pregnancies in the GnRH-treated groups were lost sometime between about day 25 and the time of pregnancy diagnosis.

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 50%	14 47%
No. with high P_4[2]	7 50%	16 53%
No. pregnant[3]	2 14%	13 43%[a]
No. embryos lost[4]	5 71%	3 19%[a]

[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 2, 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).

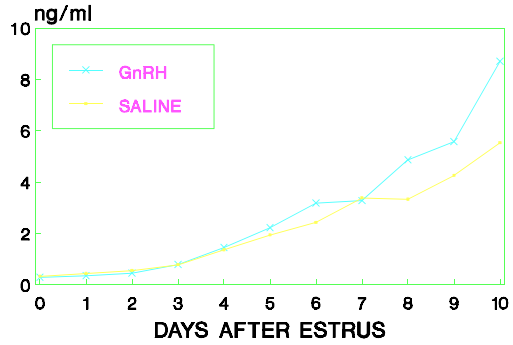

Figure 1. Concentrations of Progesterone in Serum on Days 0 to 10 after Estrus (Day 0) in Eight Repeat-breeding Cows Given Either GnRH or Saline 12 hr after Detected Estrus.

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	0.6[a]
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	0.5[a]
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	0.5[a]
No. LH pulses	1.3	1.5
Cows w/ pulses	4/6	5/6
Avg FSH, ng/ml	0.6	0.8[a]
No. FSH pulses	1	2.3[a]
Cows w/ pulses	2/6	6/6

[a]Different (P<.05) from control.

[1]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.

saline treatment. *In vitro* production of progesterone during 2 hr by luteal slices from the same cows described above was similar between cows previously treated with saline or GnRH, but after *in vitro* exposure to LH, production of progesterone was greater (P<.05) from day-10 corpora lutea of cows previously treated with saline. These differences in *in vitro* and *in vivo* progesterone production were anticipated, because GnRH-treated cows had higher peripheral concentrations of progesterone as a result of having more large luteal cells, which account for 85% of basal progesterone secretion (produced without the need for LH). In contrast, with fewer large luteal cells and proportionately more small luteal cells, which contain LH receptors, we expected to see more *in vitro* progesterone produced by the luteal slices from control cows.

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.