Changes of Luteinizing Hormone and Progesterone for Dairy Cows After Gonadotropin-Releasing Hormone at First Postpartum Breeding

C. N. LEE, J. K. CRITSER, and R. L. AX

1675 Observatory Drive
University of Wisconsin
Madison 53706

ABSTRACT

Gonadotropin-releasing hormone administered at breeding enhances fertility of dairy cows, so a study was designed to evaluate the mechanism for enhanced fertility following administration of gonadotropin-releasing hormone at first postpartum breeding. Twenty-four cows were assigned randomly to one of two treatments, 100 μg of gonadotropin-releasing hormone intramuscular or saline vehicle intramuscular at insemination. Blood samples for luteinizing hormone assay were taken at 2-h intervals prior to breeding and .5-h intervals for 3 h after insemination. Composite morning milk samples for progesterone assay were collected for 30 days after insemination or until next estrus. Cows given gonadotropin-releasing hormone had higher luteinizing hormone concentrations in blood serum following treatment than cows given saline, 13.2 versus 3.0 ng/ml. There was no relationship between luteinizing hormone and subsequent conception. Progesterone for cows that became pregnant was higher throughout sampling days. Mean progesterone concentrations were 4.6 versus 2.2 ng/ml in pregnant and nonpregnant cows during the first 4 days after insemination. Cows treated with gonadotropin-releasing hormone that conceived had higher progesterone than other cows, and that was evident at the first 4 days postbreeding.

INTRODUCTION

The therapeutic application of gonadotropin-releasing hormone (GnRH) to cure follicular cystic conditions (3, 9, 13, 24, 35) has led to its use in reproductive management of dairy cows. Collectively, those studies showed that GnRH was about 80% effective in causing follicular cysts to luteinize. Animals returned to estrus approximately 18 to 23 days after GnRH treatments. Endogenous luteinizing hormone (LH) released from the adenohypophysis following GnRH reaches a peak in about 2 h (3, 24). Responsiveness of the pituitary for LH release after GnRH is restored by 8 to 10 days after calving (15).

Several studies have stressed the economic importance of reproductive efficiency of dairy cattle (2, 8, 36). Thatcher and Wilcox (40) reported that fertility for dairy cows was enhanced by increased number of estrous cycles before first service. The number of ovulations before 65 days postpartum was improved after GnRH (5). Administration of GnRH (100 μg) 2 wk postpartum contributed to a numerical advantage in conception rates at first service (27, 30).

Studies of GnRH in postpartum cows provided inconclusive results on fertility rates (20, 34). Recent reports showed that administration of GnRH at first postpartum breeding resulted in higher conception rates (27, 29). This phenomenon also was observed when GnRH was administered to repeat breeder cows (27, 37). Our study was designed to investigate changes of LH and progesterone (P) in cows given GnRH at first postpartum breeding to examine hormonal profiles that might account for higher fertility.

Received July 6, 1984.

1This research was supported in part by the University of Wisconsin College of Agricultural and Life Sciences Cooperative Research Grant 049, the United States Department of Agriculture, and CEVA Laboratories, Overland Park, KS.

2Department of Dairy Science.

3Department of Meat and Animal Science.

4To whom reprint requests should be addressed.
MATERIALS AND METHODS

From the University of Wisconsin-Madison dairy herd, 24 cows at least 60 days postpartum were assigned randomly to one of two treatments, either 100 µg of GnRH intramuscular or 2 ml of .15 M saline solution intramuscular at first postpartum breeding. All cows had at least one cycle where standing estrus was observed. Cows (14 Holstein, 4 Jersey, 4 Guernsey, and 2 Ayrshire) were housed in stanchion barns and were observed three times daily for estrus: early morning (0300 to 0630 h), after the morning milking (0800 to 1030 h), and during the afternoon (1400 to 1700 h). Cows were inseminated approximately 10 h after standing estrus. Pregnancy diagnoses were by palpation per rectum 45 to 60 days and 100 days after breeding.

Luteinizing Hormone Assay

Blood was collected daily from the jugular vein 1 wk before the expected day of estrus for each cow to monitor basal LH. On the day of standing estrus, blood samples were taken at 2-h intervals prior to insemination and at .5-h intervals for 3 h after breeding. Serum was stored at -20°C until assayed for LH by radioimmunoassay (RIA). Serum LH concentrations were quantitated by a modified double antibody RIA (31). Iodinated antigen was LER-1702-2 and NIAMDD-bLH-4 was the standard. Anti-ovine LH (GDN #15) was first antibody. Addition of 200 µl of a 1:30,000 working dilution gave 25.7 ± 3.6% binding (SE, n=6). A second antibody (antirabbit gamma globulin prepared in sheep) of 200 µl at a 1:60 dilution was used. All samples from each animal were assayed concurrently in duplicate. Serum pooled from ovariectomized heifers was an interassay standard. Pooled serum (range of 50 to 400 µl) yielded displacement of the tracer LH parallel to the standard curve. Sensitivity of the assay was 43 ± 9 pg/tube (SE, n = 6). The interassay and intraassay coefficients of variation were 4.9 and 2.2% (n = 5 assays).

Progesterone Assay

Composite milk samples for progesterone (P) assay by RIA were taken daily at a.m. milking for 30 consecutive days following breeding or until the cow returned to estrus. Milk was preserved with potassium dichromate and stored at 4°C until assayed by RIA (26). Antiserum for the single antibody P RIA was obtained from castrated adult male goats immunized with 4-pregnen-3, 20-dione 3-CMO:BSA as an emulsion in Freund’s complete adjuvant. The antiserum (L-422) was at 1:1000 dilution. Sensitivity of the assay was 19 ± 3.7 pg. The L-422 crossreacts with 5 dihydroprogesterone (3.1%), 17α, 20α, and 20 hydroxyprogesterone (10.2, 2.8, and 1.5%) and deoxycorticosterone (2.8%). Intraassay coefficients of variation were 6.7, 5.3, and 6.4% at doses of 125,500, and 2,000 pg P from the standard curve (n = 50).

Petroleum ether (5 vol) was used for extractions of P from milk samples. Radioactive recoveries yielded 85% of labeled P added to samples (n = 8). Cold recoveries showed 90% of the P could be recovered (n = 20). For routine assays 60,000 dpm of 1, 2, 6, 7-tritiated P (New England Nuclear) were added to each assay tube. Charcoal (1.25 mg) coated with dextran T-70 (.125 mg) was used to separate free steroid from bound steroid for each assay tube. The interassay and intraassay coefficients of variation for the P RIA were 8.9 and 4.7% for a sample of pooled milk as internal standard (n = 9 assays).

Statistical Analysis

Data were analyzed for treatment and pregnancy by analysis of variance for split-plot in time (18). The test model included tests for treatment and pregnancy and all possible interactions. All data were normalized by log transformation before analyses. Arithmetic means were plotted. Breeds of the animals were not included in the statistical model or experimental design because enhanced fertility by GnRH was not affected by breed (27).

RESULTS

All results are arithmetic means ± standard error of the mean. Figure 1 shows concentrations of serum LH on the day of estrus and following breeding. The split-plot analysis showed effects of treatment (P<.01) and treatment × time (P<.02). Averages of LH following treatments were 13.2 ± 2.5 ng/ml and 3.0 ± .6 ng/ml for GnRH and saline treatments. However, there were no interactions of preg-
Partitioning of LH data for treatment and pregnancy is in Figure 2. Variance was homogeneous. Cows treated with gonadotropin-releasing hormone that did not conceive had the highest overall mean LH in serum (16.7 ± 4.9 ng/ml). Means of LH for the GnRH-pregnant, saline pregnant, and nonpregnant groups were 9.8 ± 1.6, 2.3 ± .4, and 4.0 ± 1.4 ng/ml. Concentrations of LH 1 h after injection for both GnRH groups were different from saline groups (P<.03). Large variations of LH for GnRH-treated nonpregnant cows are in Figure 3. Three cows responded to GnRH with surges of LH, and three did not respond to treatment.

Figure 4 illustrates P concentrations in milk for cows that were diagnosed pregnant or nonpregnant. Progesterone for cows that became pregnant was higher throughout the sampling period than for cows that did not conceive (P<.01). The higher P was evident as early as the first 4 days post breeding, 4.6 ± .5 vs. 2.2 ± .3 ng/ml for pregnant and nonpregnant groups. Analyses for split-plot in time showed interaction of pregnancy × day (P<.01) but no interaction of treatment × day (P>.1).

Figure 5 contains P concentrations in milk partitioned for treatment and conception. Cows treated with GnRH that conceived had higher P in the 1st wk postbreeding than other groups (P<.01). The difference in P remained significant through the sampling period except days 13 to 20 for the saline pregnant group and days 9 to 16 for the saline nonpregnant group. Cows treated with GnRH that did not conceive had the lowest P throughout the sampling period. The split-plot analyses showed interaction of treatment × pregnancy (P<.01). Average P concentrations in milk (ng/ml) for the four
Figure 2. Profiles of luteinizing hormone (LH) for cows given 100 μg gonadotropin-releasing hormone (GnRH) or saline at first postpartum service with respect to subsequent pregnancy diagnoses (n = 6 per group). Time of breeding is indicated by the arrow.

DISCUSSION

The endogenous surge of LH at estrus is vital for ovulation and luteinization of granulosa and theca cells to luteal cells whose progesterone production is necessary for maintenance of pregnancy (22). The ability of GnRH to induce an endogenous surge of LH approximately 2 h following its administration is documented (3, 9, 24). Sensitivity of the pituitary response to GnRH seems to be related to concentrations of estrogen (32, 39) or previous exposure to endogenous P (15). Postpartum dairy cows regained the ability to respond to GnRH 8 to 10 days after calving (15).
PROGESTERONE AND LUTEINIZING HORMONE CHANGES

Onset of estrus preceded the surge of LH by approximately 3 h (10, 23, 39). Injection of GnRH at the time of artificial insemination, 10 h after the first sign of estrus, was capable of eliciting an additional surge of LH (Figure 1). This surge of LH was not the LH surge of estrus as the 2-h blood sampling indicated that the endogenous estrus surge of LH had occurred 10 h earlier. That observation confirmed findings that GnRH was capable of eliciting a surge of LH at onset of estrus or 10 h after the first sign of estrus (1). Injections of GnRH resulted in higher LH for treated animals than control (saline injection).

Three types of LH responses to GnRH were observed. Cows that conceived responded to GnRH with intermediate LH (10 to 20 ng/ml, Figure 2). Cows that did not conceive responded with higher mean LH (Figure 2). However, variation about the mean was also large. Nonpregnant cows treated with GnRH either did not respond to GnRH treatment or responded with an enormous increase of LH (>30 ng/ml) (Figure 3). For the two groups of GnRH-

nonpregnant cows (those with and without LH release following treatment), P production was lower. From the joint data we suggest pituitary and ovarian dysfunction in those cows. Edgerton and Hafs (12) reported that infertile cows had higher postbreeding LH than cows that conceived. The causal factor for this type of pituitary and ovarian malfunction remains to be determined. Extremely high LH could result in desensitization of granulosa cells and their subsequent P production (25, 38).

Cows that responded to GnRH with intermediate LH had higher production of P (Figure 5) than other groups. This was evident as early as the first 4 days postbreeding. Although the saline-pregnant animals also had higher mean P at the first 4 days postbreeding, this advantage was not statistically different from the nonpregnant groups. Hence, higher P produced by the GnRH-pregnant group was probably due to the surge of LH following administration of GnRH which recruited more granulosa cells to become luteal cells for production of P, or

Journal of Dairy Science Vol. 68, No. 6, 1985
enhancement of P production by existing cells. This might explain the higher fertility in cows receiving GnRH (27, 29, 37).

Studies involving the use of human chorionic gonadotropin (hCG) at insemination resulted in higher fertility rates (6, 41) or no advantage in conception rates (21). The administration of hCG at breeding increased serum P (28). Injections of hCG to embryo transfer recipients at transfer increased concentrations of P of heifers but not cows (11). In other studies, administration of hCG to improve conception rates of embryo transfer recipients provided inconclusive results (11, 19). Generally, hCG was given at embryo transfer (5 to 8 days after estrus) to provide a luteotrophic stimulus to the already developing corpus luteum (CL). The ability of hCG (which has LH-like activity) to prolong CL life is established (42). Hence, a possible reason why administration of hCG or GnRH at breeding increases conception rates could be additional LH for recruitment of more
granulosa and theca cells to be luteinized to form luteal cells for production of P. In addition, Seguin et al. (35) reported that both hCG and GnRH were capable of stimulating production of P following their administration, the latter being more effective.

Cows that were diagnosed pregnant following AI also had higher P, 4.6 vs. 2.2 ng/ml, and that observation agreed with (16, 17). Those higher P were contributed by the GnRH-pregnant cows. Repeat breeders had lower P concentrations in the luteal phase (7, 14). Remsen et al. (33) reported a significant relationship between progesterone concentrations and subsequent pregnancy in recipient heifers. The importance of P concentrations to support pregnancy has been discussed by Boice (4), who reported that 14% of cows in the early postpartum period did not produce sufficient P following ovulation to support pregnancy had it occurred. Progesterone concentrations during the estrous cycle prior to breeding also may be an important indicator of the cow's subsequent fertility (17).

In summary, GnRH administration at insemination, 10 h after the onset of estrus, was capable of eliciting an endogenous surge of LH in most animals. Cows that responded with an intermediate surge of LH and subsequently became pregnant produced higher P. Cows with extremely higher LH responses or no LH response to GnRH and that did not conceive had lower P production. Higher conception rates in cows injected with GnRH are probably the result of increased progesterone production to support pregnancy.

ACKNOWLEDGMENT

The authors acknowledge the gift of LH (NIAMDD-bLH-4) from the pituitary hormone distribution program, NIAMDD. Gratitude is expressed to L. E. Reichert, Jr., Albany Medical College, Union University, Albany, NY, for the purified LH (LER-1702-2) for iodination; G. D. Niswender, Colorado State University, Fort Collins, CO, for the LH antiserum (GDN, anti-ovine LH-15); S. Brantmeier for assisting in sample collection; and P. Crump for statistical analyses.

REFERENCES

16 Folman, Y., M. Rosenberg, Z. Herz, and M. David.


