SUMMARY

Forty-eight Holstein and Guernsey cows were assigned according to age and breed to one of seven groups based on days following parturition. Cows in Groups II to VII received an intramuscular injection of 100 μg gonadotropin releasing hormone (GnRH) on either 1 or 2, 3 or 4, 5 or 6, 7 or 8, 12 or 13, or 18 or 19 days postpartum, respectively (six cows per group). An injection of the carrier vehicle for GnRH was given to cows in Group I (controls), which included two cows per each postpartum treatment period (12 cows).

Prior to treatment, plasma LH increased (P<.05) from a mean concentration of 1.1 ± .1 ng/ml at 1 or 2 days postpartum to 3.5 ± .6 ng/ml at 18 or 19 days postpartum. Preinjection estradiol-17β concentrations tended to increase with days postpartum (r = .31; P<.07), however, plasma progesterone did not change during the postpartum interval studied.

Following treatment, plasma LH did not increase (P>.05) for cows in Groups I, II, III and IV but was significantly increased in cows of Groups V, VI and VII (P<.05). Peak LH concentrations following GnRH were higher (P<.05) for cows in Groups V to VII (14.1 ± 2.7, 11.2 ± 2.1 and 13.6 ± 2.3 ng/ml, respectively) than those in Groups II to IV (3.1 ± 1.4, 2.8 ± .8 and 4.2 ± .6 ng/ml, respectively).

Combining all treatment groups, peak LH concentrations increased with days postpartum (r = .61; P<.01), increasing preinjection estradiol-17β concentrations (r = .64; P<.01) and increasing preinjection LH concentrations (r = .50; P<.01). Preinjection plasma progesterone concentrations were not related to peak LH levels (r = .09; P>.10). The inclusion of both days postpartum and preinjection estradiol-17β in step-up multiple regression significantly improved the coefficient of determination (R² = .59) in comparison to either variable alone. Preinjection LH and progesterone, however, did not significantly improve the model.

(Key Words: GnRH, LH, Progesterone, Estradiol-17β, Postpartum Dairy Cows.)

INTRODUCTION

Pituitary and systemic plasma luteinizing hormone (LH) concentrations are low at calving and gradually increase for 8 to 30 days postpartum (Erb et al., 1971; Garverick et al., 1973; Labhsetwar et al., 1964; Saiduddin et al., 1968). Furthermore, plasma LH fluctuated within individuals during the early postpartum period (Garverick et al., 1973; Echternkamp and Hansel, 1973). Goodale et al. (1975) showed more fluctuation in LH of plasma in dairy cows 7 or 8 and 12 or 13 days postpartum as compared to 2 or 3 days when samples were collected at hourly intervals for 10 hours.

Early studies on the effects of synthetic gonadotropin releasing hormone (GnRH) in domestic animals have been reviewed (Convey, 1973; Schally et al., 1972). More recently, GnRH has been shown to stimulate an LH release in: 1) dairy cows with ovarian cysts (Cantley et al., 1975; Kittock et al., 1973); 2) cows following prostaglandin F₂α treatment or progesterone withdrawal for estrus synchronization (Convey et al., 1976; Kaltenbach et al., 1974); and, 3) dairy cows on day 14 post-
partum (Britt et al., 1974). In another study with postpartum dairy cows, GnRH stimulated a greater release of LH on days 7 or 8 and 12 or 13 postpartum as compared to 2 or 3 days (Goodale et al., 1975). In addition, exogenous estrogens have been shown to increase the responsiveness of the pituitary to GnRH stimulation (Reeves et al., 1971b; Beck and Convey, 1974).

Following parturition, the regressing corpus luteum of pregnancy, systemic plasma and plasma from the ovarian vein contain very low concentrations of progesterone (Labhsetwar et al., 1964; Hunter et al., 1970; Erb et al., 1968b). Systemic estradiol-17\(^\beta\) concentrations in plasma decline rapidly within 24 hr following parturition (Robertson, 1974). However, ovarian follicles are detectable 5 to 7 days following calving (Callahan et al., 1971; Marion and Gier, 1968; Morrow et al., 1966) and could be a significant source of estradiol-17\(^\beta\) or progesterone during the early postpartum period (Marion et al., 1968).

The time when postpartum dairy cows regain the ability to release LH in response to GnRH has not been determined. Therefore, the objective of this study was to determine when the pituitary of postpartum lactating dairy cows can release LH in response to GnRH stimulation. An additional objective was to determine the relationships between pre-injection endogenous plasma hormone concentrations and GnRH-induced LH release in postpartum dairy cows.

### MATERIALS AND METHODS

Forty-eight Holstein and Guernsey cows from the University of Missouri dairy herds were assigned according to age and breed to one of seven groups. The distribution of breeds and age among groups was proportional. Cows in Groups II, III, IV, V, VI and VII received an intramuscular injection of 100 µg GnRH\(^2\) at 1 or 2, 3 or 4, 5 or 6, 7 or 8, 12 or 13 and 18 or 19 days postpartum, respectively. There were six cows per treatment group. Controls (Group I) received an intramuscular injection of carrier vehicle for GnRH (sterile water with a .9% benzyl alcohol preservative). Group I consisted of two cows per each postpartum treatment period (Groups II to VII) for a total of 12 cows. Blood for hormone analysis was collected via jugular venipuncture with heparinized vacu-tainers prior to treatment (time 0) and .5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 6.0 hr following treatment. Following collection, blood was chilled in ice water until centrifugation at 10,000 \(\times\) g for 10 min at 4 C. Plasma was stored at −20 C until assayed.

**Assay of Hormones.** Plasma LH\(^3\) was quantified by double antibody radioimmunoassay as reported by Niswender et al. (1969) and Cantley et al. (1975) except \(^{125}\)I was used instead of \(^{131}\)I as the radioactive label. Samples were assayed in duplicate in two assays. Each assay included all samples (0 to 6 hr) for one half the cows in each group.

Plasma progesterone\(^4\) was quantified by radioimmunoassay using procedures reported by Cantley et al. (1975) except that progesterone was extracted from duplicate plasma aliquots of 50 and 200 microliters. Standard curves were established for each assay in triplicate at concentrations of 0, 20, 50, 100, 200, 300, 500, 700 and 1,000 pg of crystalline progesterone.

Plasma estrogens were extracted from duplicate aliquots of 1 ml of plasma. Following addition of 5 ml of diethyl ether, samples were vigorously shaken on a mechanical shaker for 7 minutes. The extracted mixture was stored overnight at −20 C and further cooled over a mixture of solid CO\(_2\) and ethanol before decanting the organic phase into disposable culture tubes. Extracts and standards were dried under a stream of N\(_2\) at 40 C prior to radioimmunoassay for estradiol-17\(^\beta\) using procedures similar to those described by Erb et al. (1976). However, a different antisera\(^5\) which has a high specificity to estradiol-17\(^\beta\) (Mason and March, 1975) was used at a dilution of 1:70,000. Standard curves were established for each assay in triplicate at concentrations of 0, 5, 10, 20, 30, 50, 100, 150 and 200 pg of crystalline estradiol-17\(^\beta\).

Cross reaction of estradiol-17\(^\beta\) antisera was

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\(^{3}\)GnRH was generously supplied by Dr. M. D. Brown, Abbott Laboratories.

\(^{4}\)Antisera for radioimmunoassay of luteinizing hormone (LH; B-225) was generously supplied by Dr. G. D. Niswender. Purified bovine LH (LER-1072-2) was generously supplied by Dr. L. E. Reichert.

\(^{5}\)Antisera for radioimmunoassay of progesterone (RDR-9-P) was generously supplied by Drs. R. D. Randel and R. E. Short.

\(^{6}\)Antisera for radioimmunoassay of estradiol-17\(^\beta\) was generously supplied by Dr. Norman Mason, Eli Lilly Co.
evaluated using estrone, estradiol-17\alpha, estradiol benzoate, progesterone, testosterone, corticosterone and cortisol (.01, .05, .1, .2, .5, 1.0 and 10.0 ng per reaction tube in triplicate). Only estrogens were found to cross react. Cross reactivity of estrone, estradiol-17\alpha and estradiol benzoate as compared to estradiol-17\beta was 9\%, 2.2\% and 70.8\%, respectively. When 5, 10 and 20 pg of exogenous estradiol-17\beta were added to 1 ml of plasma from an ovariectomized cow, 6.9 \pm .24, 10.4 \pm .20 and 19.3 \pm .60 pg/ml, respectively, were recovered (\bar{X} = 112\%; n = 10). The sensitivity, i.e., the least amount of estradiol-17\beta which resulted in less (P<.05) binding of \textsuperscript{3}H-estradiol-17\beta to antibody, was 1 pg (n = 6).

Samples were assayed for progesterone and estradiol-17\beta in duplicate in six assays. Each assay included plasma samples from two control cows and one cow per treatment group (Groups II to VII). Intraassay coefficients of variation were 2.5\% and 10.2\%, respectively, for progesterone and estradiol-17\beta. Interassay coefficients of variation were 15.0\% and 14.1\%, respectively, for progesterone and estradiol-17\beta. Each day unknowns were determined, three recovery aliquots were included by adding the appropriate \textsuperscript{3}H-steroid to plasma from an ovariectomized cow. Subsequently, all samples were adjusted for recovery. Recoveries of \textsuperscript{3}H-progesterone and \textsuperscript{3}H-estradiol-17\beta averaged 85.1 \pm .9\% and 80.8 \pm 1.8\%, respectively. The coefficients of variability for recovery were 2.8\% and 5.4\% for progesterone and estradiol-17\beta, respectively.

Duplicate estimates of hormone concentrations were averaged prior to analysis. Following treatment (0 to 6 hr), data from all groups were analyzed by split plot analysis of variance as described by Gill and Hafs (1971) and a multiple range test described by Kramer (1956) for identification of differences between means. Controls (Group I) were excluded when comparisons among peak LH and preinjection hormone concentrations were made since control cows were 1 to 19 days postpartum and did not receive GnRH. Correlation (Snedecor and Cochran, 1967) was used to analyze interrelationships among days postpartum and hormone concentrations with all treatment groups combined (Groups II to VII). Step-up multiple regression (Draper and Smith, 1966) was used to evaluate effects of days postpartum and preinjection LH, progesterone and estradiol-17\beta on peak LH in response to GnRH treatment. In addition, a chi square test described by Cochran and Cox (1957) was used to test differences in enumerative data.

**RESULTS AND DISCUSSION**

Prior to treatment, plasma LH increased from 1.1 \pm .1 ng/ml at 1 or 2 days postpartum to 3.5 \pm .6 ng/ml at 18 or 19 days postpartum (table 1). Mean preinjection plasma LH concentration was higher (P<.05) for cows in Group VII than those in Groups II to V but did not differ from cows in Group VI. With all treatment groups combined, preinjection plasma LH was significantly correlated with days postpartum (r = .73; P<.01; table 2). These results are consistent with previously reported studies (Erb et al., 1971; Garverick et al., 1973; Goodale et al., 1975; Labhsetwar et al., 1964).

Preinjection estradiol-17\beta concentrations tended to increase with days postpartum (r = .31; P<.07). During late pregnancy, fetal cotyledons are a rich source of estradiol-17\beta (Gorski and Erb, 1959; Veenhuizen et al., 1960); however, within 24 hr following parturition, systemic estradiol-17\beta concentrations decrease rapidly (Robertson, 1974). Follicular growth is detectable by 5 to 7 days postpartum (Callahan et al., 1971; Morrow et al., 1966) which may explain the increasing estradiol-17\beta concentrations with days postpartum observed in this study. As estradiol-17\beta concentrations increased, preinjection LH concentrations also tended to increase (r = .33; P<.06).

Plasma progesterone, however, did not change as the postpartum interval increased. The corpus luteum of pregnancy appears to be the major source of progesterone during pregnancy (Gorski et al., 1958; Erb et al., 1968a). However, following parturition, progesterone is very low in ovarian venous plasma (Erb et al., 1968a), in the regressing corpus luteum (Labhsetwar et al., 1964; Erb et al., 1968b) and in systemic plasma (Hunter et al., 1970). Since first ovulation occurs at about 20 days postpartum in dairy cows (Callahan et al., 1971; Morrow et al., 1966), little difference between groups would be expected.

Following treatment, plasma LH concentrations did not increase significantly for cows in Groups I, II, III and IV (figure 1). In contrast, plasma LH for cows in Groups V to VII increased following treatment (P<.05) and were higher at .5 hr post-treatment than preinjection concentrations (P<.05). Plasma LH in cows of
Group VII gradually declined from .5 to 6.0 hr following treatment. In Groups V and VI, however, the elevation in plasma LH at .5 hr was followed by a decline and a further elevation (P<.05) 2 to 3 hr following treatment. Arimura et al. (1972) reported a biphasic LH response in ewes following a large dose of GnRH (250 μg). Other reports with cows having ovarian cysts or heifers indicate that plasma LH gradually increases to peak concentrations at 1.5 to 2.5 hr following GnRH treatment (Cantley et al., 1975; Zolman et al., 1973). At 4 and 6 hr following treatment, plasma LH levels were not different from preinjection levels for cows in Groups V to VII.

Peak LH concentrations were higher (P<.05) for cows in Groups V to VII than Groups II to IV (table 1). The mean time to the LH peak was not different between groups. One of six, two of six and five of six cows for Groups II to IV, respectively; and six of six cows for Groups V to VII showed a peak in LH of over 3.0 ng/ml following GnRH. One of six cows for Groups II to IV in contrast to six of six, five of six and six of six cows in Groups V to VII, respectively, showed a peak in LH following GnRH over 5.0 ng/ml (P<.05). An increase in plasma LH fluctuations was reported to occur on 7 or 8 and 12 or 13 days postpartum compared to 2 or 3 days when samples were collected at hourly intervals for 10 hr (Goodale et al., 1975).

Combining all treatment groups, peak plasma LH concentrations increased with days postpartum (r = .61; P<.01), increasing preinjection estradiol-17β concentrations (r = .64; P<.01) and increasing preinjection LH concentrations (r = .50; P<.01; table 2). In addition, preinjection estradiol-17β concentrations in the control cows (Group I) were correlated (r = .72; P<.01) with increasing fluctuation in plasma LH (0 to 6.0 hr). Preinjection plasma progesterone concentrations were not correlated to peak LH levels (r = .09; P<.10). This would be expected since early postpartum cows were used in this study and all cows had relatively low progesterone concentrations prior to GnRH treatment. In multiple regression with peak LH as the dependent variable, days postpartum and preinjection estradiol-17β significantly improved (P<.01) the coefficient of determination in comparison to either variable alone (table 2). Inclusion of preinjection LH and progesterone, however, did not significantly improve the model.
## General Discussion

Progesterone and estrogens in plasma are elevated during late stages of pregnancy, decrease rapidly at parturition and are low during the early postpartum period. Oxender et al. (1972) have shown that concentrations of LH are low during gestation in the dairy cow. In ovariectomized cows, Beck et al. (1976) have shown that exogenous progesterone and estrogen given together suppressed LH in plasma. Each hormone given singly, however, was less effective. With progressing gestation, pituitary responsiveness to GnRH stimulation is reduced in ewes (Chamley et al., 1974). Our results indicate that systemic LH increases during the early postpartum period and that pituitary responsiveness to GnRH as evidenced by plasma LH concentrations, appears to be regained by 7 or 8 days postpartum. Thus, it appears that high concentrations of progesterone and estrogen in combination are involved in reducing pituitary responsiveness to GnRH stimulation during gestation. Following parturition, systemic concentrations of LH increase and pituitary responsiveness to GnRH is rapidly regained. In addition, the magnitude of the LH response is associated with systemic concentrations of estradiol-17β as well as days postpartum.

Estrogens have been shown to modify responsiveness of the pituitary to exogenous GnRH by other investigators. Reeves et al. (1971a) demonstrated that LH release in ewes in response to GnRH stimulation was greatest during an 8 hr period at estrus. In another study, ewes pretreated with estradiol benzoate prior to GnRH administration responded with a greater LH release than control ewes (Reeves et al., 1971b). Beck and Convey (1974) demonstrated in ovariectomized heifers that GnRH induced a LH release of longer duration when exogenous estradiol was administered. Zolman et al. (1974) reported that endogenous serum estradiol and estrone concentrations in heifers were related to the magnitude of the LH release on day 15 and day 20 of the estrous cycle.

The influence of progesterone on pituitary responsiveness to GnRH stimulation is conflicting. Zolman et al. (1974) found no relationship between preinjection progesterone levels and peak LH levels when GnRH was administered on day 15 and 20 of the estrous cycle.

![Figure 1. Mean concentration of plasma LH in dairy cows following 100 µg GnRH as the time from parturition increases.](image-url)
However, Kittok et al. (1973) observed a two-fold greater LH increase following GnRH in cows with ovarian cysts compared to cows treated during the luteal phase of the estrous cycle. Additionally, Debeljuk et al. (1972) reported that progesterone injection inhibited LH release after GnRH treatment in rats and ewes and completely masked the increased responsiveness of the pituitary to GnRH pretreated with estradiol.

The results of this experiment indicate that pituitary responsiveness, as evidenced by systemic LH concentrations, is regained by 7 or 8 days postpartum. The ability to release LH in lactating dairy cows does not appear to be the limiting factor in reestablishment of cyclic ovarian activity. In addition to days postpartum, estradiol-17β is associated with the magnitude of LH response.

**LITERATURE CITED**


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