ABSTRACT

Data from artificial insemination, rectal palpation, and hormone assays were used to characterize postpartum reproductive activity in 54 dairy cows. Progesterone and estradiol-17β were measured in milk samples collected for 120 d (Trial 1) or 65 d (Trial 2). Progesterone was higher and estradiol was lower in milk than in serum. Values for both hormones in milk were highly correlated with those in serum. Most cows (64%) had short first luteal phases (<12 d). First rise (28 d) in progesterone was later (33.4 vs. 24.9 d) for cows having short rather than normal (>12 d) luteal phases. Cows were classified as having a short luteal phase followed by a normal luteal phase or as having normal luteal phases for the first two estrous cycles. Estradiol for the 6 d prior to each luteal phase was higher preceding the second phase than the short phase or those preceding both phases of cows with normal phases. Follicular function prior to ovulation, as measured by estradiol, was not responsible for short-lived corpora lutea. Concentrations of progesterone in milk in the late luteal phase prior to insemination were related to fertility.

INTRODUCTION

Calving interval is indicative of overall reproductive performance of dairy cows (6), and financial loss due to prolonged calving intervals is substantial (8). Postpartum intervals to first ovulation, first estrus, completion of uterine involution, first breeding, or conception (3) are critical components of calving interval. Better understanding of postpartum ovarian endocrine events may lead to better control of postpartum intervals. Measurement of hormones in milk aids study of ovarian physiology due to relative ease of collection of samples. Close correlations between concentrations of estradiol-17β and progesterone in plasma and milk have been shown (1). Concentrations of estradiol-17β in milk have been used with ovarian palpation to demonstrate errors in timing of artificial insemination associated with poor conception (16).

Concentrations of progesterone and estradiol-17β (hereafter referred to as estradiol) in milk were used in the current study to monitor postpartum ovarian events in dairy cows. The relationship between progesterone in milk during the luteal phase prior to insemination and conception was determined. In addition, the relationship between concentrations of estradiol around estrus and the duration of the subsequent luteal phase was examined.

MATERIALS AND METHODS

Reproductive status was monitored in dairy cows in two trials. In trial 1, samples of milk (20 ml) were collected daily (p.m. milking) from weigh jars and frozen immediately for the first 120 d following calving in 22 Holstein and 4 Ayrshire pluriparous cows. Reproductive tracts were palpated weekly via the rectum. Uterine and ovarian characteristics, estrous behavior, and conception rate to AI were recorded for each cow. Herd personnel were responsible for detection of estrus and insemination of cows >40 d postpartum. Cows (18 Holstein, 10 Ayrshire) in trial 2 were monitored for the first 65 d postpartum and were not inseminated during the collection period.
Concentration of progesterone in milk was measured by radioimmunoassay (RIA). Profiles of progesterone were constructed for each cow and were combined with palpation and estrous cycle information to estimate: 1) interval to onset of ovarian cyclicity, defined as the first rise in progesterone (>4 ng/ml), 2) duration of luteal (milk progesterone >4 ng/ml for >3 d) phases, classified as short (<12 d), normal (13 to 20 d), or prolonged (>20 d), 3) association of progesterone and subsequent conception, and 4) incidences of apparent embryonic loss. Milk samples collected between 7 and 4 d prior to AI were used to determine the relationship between this 4-d average concentration of milk progesterone and subsequent conception rate. An average concentration of progesterone in serum of 5 ng/ml for this period was related to conception (5). Using a derived regression equation [progesterone in milk = 1.53 (progesterone in serum + 3.46)], 5 ng/ml in serum was equivalent to 11 ng/ml in milk. With this criterion, cows were classified as having a concentration of progesterone >11 ng/ml or <11 ng/ml. Conception rates were determined for each class based on a palpable embryo at 40 to 50 d after insemination. Absence of a palpable embryo was taken as evidence of embryonic mortality for cows deemed pregnant by having milk progesterone >8 ng/ml at 22 to 24 d after insemination.

Based on milk progesterone profiles of the first luteal phase for cows from both trials, two subgroups were formed based on the duration of the first two luteal phases. Cows in group 1 (SN, n=7) had short first luteal phases followed by a luteal phase of normal duration. Cows in group 2 (NN, n=7) had consecutive normal luteal phases. Daily concentrations of estradiol were measured by RIA in milk samples collected on the 6 d prior to each luteal phase to ascertain if follicular function affected subsequent luteal function.

Hormonal Assays

Duplicate milk samples were assayed for progesterone by a modification of the method of (13). Twenty-five microliters of milk were extracted with 2 ml of petroleum ether. Lipid and aqueous phases were separated by freezing in a dry ice-acetone bath and the aqueous phase was reextracted. Lipid phases were combined and evaporated to dryness. Two milliliters of 70% methanol were added to each dried extraction tube and placed at −20°C for 12 h. One milliliter of the methanol was removed, dried under air, and extracted twice with 2 ml of petroleum ether. The final lipid extract was evaporated and assayed for progesterone (14).

Extraction recoveries for increasing concentrations of unlabelled progesterone from 2.5 to 50 pg/tube were greater than 90%. Values were not corrected for extraction losses. Intraassay and interassay coefficients of variation were 17 and 18%, respectively, for 30 assays, and assay sensitivity was 10 pg/tube.

Milk samples were assayed in duplicate for estradiol. Four milliliters of milk and 8 ml of 100% methanol were placed in 16 x 125-mm extraction tubes. Contents were mixed for 30 s, placed at −20°C for 12 h, and then centrifuged at 1000 x g for 10 min. Six milliliters of supernatant were removed and air-dried to evaporate the methanol. The residue was extracted twice with 5 ml of diethyl ether. The final extract was chromatographed on a Sephadex LH-20 column and assayed for estradiol (14). Two standard curves were run in triplicate. In one curve 1 ml of milk was extracted and standards were added to the dried extraction tubes. In the second curve, standards were added to blank tubes. Resulting curves were essentially parallel with similar slopes (−2.26 and −2.22), Y intercepts (2.91 and 3.07), and picograms of estradiol displaced at 50% bound (19.5 and 22.6) for the unextracted and extracted curves, respectively. Assay sensitivity was 1.2 pg/ml. Intraassay and interassay CV were 14.8 and 12.4%, respectively, for 20 assays.

Statistical Analyses

Mean values for intervals to AI, rises in progesterone, numbers of estrous cycles prior to first service, and intervals to peak estradiol were compared using Student's t distribution (15). Contingency chi-square analysis was used to compare conception rates. Concentrations of estradiol in milk were examined for effects on length of subsequent luteal phases by analysis of variance for a split-plot design and Fisher's least significant difference test.
RESULTS

Concentrations of progesterone in milk and concurrent serum samples were correlated ($r=0.95$, $P<0.001$; Figure 1). Representative profiles of milk progesterone and related palpation and insemination data of cows in different postpartum states of trial 1 are in Figure 2. The top panel illustrates prolonged anestrus. Ovarian

![Graph]

Figure 1. Daily concentrations of progesterone in whole milk and serum in two cows sampled over two estrous cycles.

cyclicity was resumed within 120 d in trial 1 by 25 of the 26 cows and by 22 of the 28 cows in trial 2 by 65 d postpartum. In panel 2, embryonic death presumably occurred following second service based on high progesterone 22 d after AI and absence of a palpable embryo at

Figure 2. Representative milk progesterone profiles and associated information from palpations and inseminations.
40 to 50 d after AI. Incidence of apparent embryonic death based on those criteria was 17.4%. The third panel illustrates early onset of ovarian activity followed by conception to first service. Sixty-four percent (30/47) of the cows that began to cycle exhibited short (6.8 ± .5 d) first luteal phases. Interval from parturition to the first rise in milk progesterone was longer in cows that showed a short rather than a normal first luteal phase (33.4 ± 3.9 and 24.9 ± 2.2 d, respectively; P<.05). Panel 4 depicts failure to detect estrus for two consecutive, apparently normal estrous cycles. Based on milk progesterone profiles and estrous detection information, only 51% of the possible estruses were detected, and improper timing of insemination (as indicated by the second service in panel 2) occurred in 9.8% of the inseminations.

Concentrations of estradiol in milk and serum were correlated (r=.88, P<.01); however, the concentration in milk was lower (Figure 3). Concentrations of estradiol in milk was measured for 14 cows assigned to either SN or NN groups depending on duration of the first luteal phase. First phases averaged 6.2 ± .8 d (SN) and 16.3 ± 1.3 d (NN) and second phases averaged 15.0 ± .5 (SN) and 15.5 ± 1.0 (NN) d. Interval from parturition to the first peak of estradiol was less in the NN than the SN group (14.6 ± 1.2 vs. 30.6 ± 6.1 d, respectively; P<.05). Second peaks of estradiol occurred at comparable times (42.6 ± 6.0 d and 38.4 ± 1.2 d for SN and NN, respectively). Concentrations (pg/ml) of estradiol for the day of the peak were less (P<.05) preceding the short cycle (2.3 ± .3) of SN cows and both (2.7 ± .3 and 1.8 ± .1) cycles of NN cows than the concentration preceding the second (4.8 ± 1.2) cycle of the SN cows. The same result was obtained for areas under the curve of estradiol for the day preceding, the day of, and the day after the peak (Figure 4).

The association of luteal function in the cycle prior to AI with conception was assessed by averaging concentrations of progesterone in milk 7 to 4 d prior to first insemination and classification of cows as having high (>11 ng/ml) or low (<11 ng/ml) concentrations. Conception rate tended to be greater in cows with high (n=10) than with low (n=13) progesterone (50 vs. 15.4%, \( \chi^2 = 3.2, P<.10 \)). Results were similar when all services were considered for cows with high (n=17) and low (n=14) progesterone (53.1 vs. 14.3%, \( \chi^2 = 5.0, P<.05 \)). Although intervals to first service were similar (68.6 ± 6.7 and 71.5 ± 5.2 d), average number of estrous cycles prior to first service was fewer in cows with high than with low progesterone (1.8 ± .2 and 2.5 ± .2 cycles, respectively; P<.05).

**DISCUSSION**

Concentrations of progesterone were higher in milk than in serum and were similar to concentrations reported for serum (7) and milk (2). Conversely, concentration of estradiol was less in milk than in serum. Concentrations of estradiol in milk have been higher (9, 12), equivalent to (10), or less than (1) serum values, indicating variability among assays.

Initial postpartum rises in estradiol and progesterone occurred at intervals similar to those reported for the occurrence of ovulation as detected by rectal palpation of the ovaries (3) or by hormonal determinations (2). The high incidence of short luteal phases during the first estrous cycle supports previous findings (17). However, an initial short estrous cycle occurred longer after parturition than a first luteal phase of normal length. This is not in agreement with previous results (17).

Reduced follicular development may contribute to decreased function of short-lived corpora lutea (17). Concentrations of estradiol in plasma prior to the first (short) and second (normal) luteal phases did not differ in early weaned postpartum beef cows (11). In the present study, concentrations of estradiol in milk collected prior to formation of first short-lived luteal structures were not less than concentrations prior to first luteal structures that had a normal life span. This supports the conclusion of (14) that variations in follicular function, as reflected by follicular size and concentration of estradiol in serum, did not determine differences in subsequent luteal function within treatment groups. Higher estradiol after a short luteal phase raises the possibility that follicular function, in terms of secretion of estradiol, is more a product of luteal function, because estradiol rises immediately when progesterone falls (4).

The concentration of progesterone in milk collected prior to insemination influenced fertility. Retrospective studies have shown that
Figure 3. Concentration of estradiol-17β in whole milk and serum in cows synchronized for estrus (n=7). Peak corresponds to peak estradiol.
conception rates were related to the concentration of progesterone in blood (5) collected before breeding, but this was not true for milk (2, 13). Conception rate increased as the number of estrous cycles prior to breeding increased (18). In the present study, cows with high milk progesterone prior to breeding and inseminated after completing an average of two estrous cycles had a high conception rate, whereas cows with low progesterone and inseminated after 2.5 estrous cycles had a lower conception rate. Thus, concentration of progesterone in the latter days of the luteal phase prior to insemination would seem to be more important than the number of ovarian cycles completed in determining fertility.

Figure 4. Mean concentrations of estradiol-17β in milk prior to the first two luteal phases in cows (n=7) having a short followed by a normal-lengthed luteal phase (SN) and cows (n=7) having two luteal phases normal in duration (NN).
CONCLUSIONS

The short-lived corpus luteum is the object of much current research. Reduced luteal function in first cycles of dairy cows apparently was not a result of reduced follicular development or function as indicated by the concentration of estradiol in milk prior to ovulation. The concentration of estradiol in milk in the preovulatory period after the short luteal phase was higher than in the preovulatory period before the short luteal phase. Concentration and duration of progesterone achieved during a luteal phase may have an effect on subsequent follicular secretion of estradiol. In addition, fertility was affected by the concentration of progesterone in milk 7 to 4 d prior to insemination.

REFERENCES


