Estrus, Ovulation, Luteinizing Hormone, and Suckling-Induced Hormones in Mastectomized Cows With and Without Unrestricted Presence of the Calf

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ABSTRACT: Angus × Hereford multiparous cows were assigned to four treatments: 1) mastectomized + calf weaned at birth (MCW; n = 7); 2) mastectomized + calf presence restricted to noninguinal contact (MCR; n = 7); 3) mastectomized + unrestricted calf presence (MCP; n = 7); and 4) udder-intact cows + unrestricted calf presence (UICP; n = 8). Except for MCW cows, cow-calf pairs were penned together individually from parturition (d 0) until d 35 when calves were weaned. On d 7, calves in MCP and UICP treatments were separated overnight from their dams, and before and upon reunion, blood samples were collected from the cows to assess changes in oxytocin, cortisol, and prolactin. Calves in the MCP and UICP treatments attempted to or suckled their dams for a similar duration upon reunion, respectively. Concentrations of cortisol and percentage of change in oxytocin and prolactin were increased (P <.05) for up to 12 min in MCP cows after reunion with their calves. Average concentrations of serum LH in samples collected on d 14, 21, 28, and 35 did not differ in noncyclic cows among treatments within day postpartum (except for greater [P <.05] LH in MCW cows on d 21). However, MCP cows had more (P <.05) LH pulses (d 21), greater (P <.05) variability in LH concentrations, and greater (P <.05) average maximum concentrations of LH than UICP cows after d 14. Intervals to first ovulation were similar in MCW and MCR cows but shorter (P <.01) than those in MCP and UICP cows. Attempted suckling of mastectomized dams by their calves was associated with increased serum cortisol and percentage of increase in serum oxytocin and prolactin. Despite increased LH in MCP cows, intervals to first ovulation did not differ from those of UICP cows.

Key Words: Beef Cows, LH, Mastectomy, Anestrus, Suckling, Hormones

Introduction

Suckling in cattle prolongs postpartum anovulation longer than milking primarily because of suckling frequency and presence of the calf (Short et al., 1990; Williams, 1990). Earlier studies demonstrated that cows suckled ad libitum had longer postpartum intervals to estrus and (or) ovulation than cows without their calves (Oxenreider, 1968; Wettemann et al., 1978). Suckled cows with denervated udders, maintained with their calves, had postpartum intervals to estrus similar to those of udder-intact suckled cows but longer than those of nonsuckled cows (Short et al., 1976).

Mastectomized cows had shorter periods of anestrus than udder-intact suckled cows (Short et al., 1972), unless the calf was present and attempted to suckle (Viker et al., 1989, 1993). Suckling inhibits secretion of LH (Williams, 1990), but no information is available concerning the effect of calf presence on LH and various suckling-induced hormonal secretions in mastectomized cows. This information is essential to provide insight into the mechanism by which anestrus is maintained in mastectomized and udder-intact cows, when provided unrestricted contact with their own calves. The objectives of our study were to determine whether changes in LH, oxytocin, prolactin, and cortisol, and interval to first postpartum ovulation differed among udder-intact suckled cows and mastectomized cows from which calves were weaned, or allowed restricted or unrestricted contact with their calves.

1Contribution no. 93-452-5 from Kansas Agric. Exp. Sta. We acknowledge the generous gift of assay reagents from Douglas Bolt, USDA Animal Hormone Program.
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Received June 25, 1993.
Accepted October 23, 1993.
**Materials and Methods**

**Cows and Diets**

Twenty-one mastectomized and eight udder-intact, Angus × Hereford multiparous cows were used. Mastectomized cows ranged from 4 to 7 yr of age and were mastectomized at least 26 mo before the onset of this study with a procedure described previously (Viker et al., 1989). Mastectomies were performed under general anesthesia during the first or second pregnancy in all cows. Udder-intact cows ranged from 3 to 6 yr of age.

Prepartum diets were similar for all cows and designed to meet or exceed NRC (1984) requirements for nonlactating mature pregnant cows (last third of pregnancy). At parturition, body condition scores ranged from 5 to 7 (X = 6), on a scale from 1 to 9 (Whitman, 1975). Mastectomized (6.2 ± .3) and udder-intact cows (5.7 ± .3) had similar body condition scores. Calves were left with their dams for approximately 24 h after birth for development of cow-calf recognition before treatments were imposed.

Following parturition, all cows were maintained in individual pens (1.5 m x 7.3 m) and fed a diet consisting of prairie hay (4.5 kg/d for mastectomized cows and 6.8 kg/d for udder-intact cows) and concentrate (.39 Mcal/kg, 14.5% crude protein,.85% Ca, and .69% P), which was fed according to weekly changes in BW throughout the study. Postpartum energy, protein, Ca, and P intakes for mastectomized cows met or exceeded NRC (1984) requirements for nonlactating pregnant mature cows (middle third of pregnancy), whereas udder-intact cows were fed to meet or exceed NRC (1984) requirements for cows with superior milking ability. Nonlactating (mastectomized cows) and lactating (udder-intact) cows were fed accordingly to prevent resulting availability and need for nutrients to be confounded with potential effects of treatments.

**Experimental Design**

Mastectomized cows were assigned randomly to three treatments as they calved: 1) calves were weaned from their mastectomized dams and had no further association with their dams (MCW; n = 7); 2) calves were restricted individually to a small pen (1.5 m²) within the individual pen housing each mastectomized dam in which contact between calf and cow was limited to noninguinal tactile contact (i.e., head and neck region of its dam; MCR; n = 7); and 3) calves had unrestricted access to their mastectomized dams, with unlimited tactile, olfactory, visual, and auditory stimuli (MCP; n = 7). Udder-intact cows (control) had unrestricted access to their calves (UICP; n = 8). Except for the MCW treatment, cow-calf pairs remained together in their respective treatments until approximately d 35 (33 to 37 d) postpartum when remaining calves were weaned from their dams.

The calf pen in the MCR treatment was triangular-shaped, open on top, and surrounded on two sides by the pen containing the cow. Notched openings (approximately .9 m high) on both side panels allowed the dam to place her head into the pen and lick or nuzzle the calf in any manner. All calves from mastectomized cows were bottle-fed twice daily a whole-milk replacer. Cows were turned out of pens once daily for exercise and detection of estrus during the 5-wk treatment period. Following permanent calf removal, cows were group-fed the same diet, housed together in a large (15 m x 45 m) dirt lot with fence-line feed-bunk space, and observed thrice daily (0630, 1630, and 2100) for estrus. All estrual activity was recorded, but only cows that stood when mounted or were mounted repeatedly and/or displayed significant secondary signs of estrus were considered to be in estrus.

**Blood Collection**

Blood samples were collected daily from all cows via jugular venipuncture, and serum was harvested and frozen until progesterone was quantified with a RIA (Skaggs et al., 1986) in six assays; intra- and interassay CV were 7.2 and 7.0%, respectively. Sensitivity of the assays was 20 pg/tube or 100 pg/mL. Daily blood collection continued until observation of the second postpartum estrus. Day of ovulation was considered to be the day following estrus, if a subsequent rise in progesterone occurred. Day of first ovulation, if not accompanied by behavioral estrus, was estimated to have occurred 3 d before serum progesterone exceeded .5 ng/mL and remained at or above that concentration for at least two consecutive days.

On d 6 (d 4 to 8) postpartum, calves in the MCP and UICP treatments were separated (10 to 20 m apart) from their dams overnight and then returned to their dams the next morning (time of separation was 9 to 10 h). Cows were unable to see their calves during separation but were within auditory range. When calves were returned to their dams, calves were replaced with their own dams within a few seconds of renewed visual contact. Calves in the MCR treatment were not separated from their dams. Catheters were inserted into a jugular vein of each cow on the afternoon before cow-calf separation (d 6) or the day preceding each intensive sampling period. Blood samples were collected from each cow at −24, −12, and 0 min (reunion of cow-calf pairs); at 2-min intervals for 12 min; then at 12-min intervals for 1 h. During sampling periods, cows were haltered and restrained in their individual pens. Duration of suckling was recorded when calves were returned to their dams. Concentrations of oxytocin (0 to 12 min at 2-min intervals and at 24 min after cow-calf reunion)
were measured in serum with a RIA (Lutz et al., 1991) in three assays; intra- and interassay CV were 10.5 and 10.7%, respectively. Sensitivity of oxytocin assays was 1 pg/tube or 4 pg/mL. Concentrations of cortisol were measured in serum with a RIA (Skaggs et al., 1986) in samples collected at 12-min intervals; intraassay CV was 11.8%. Sensitivity was 10 pg/tube or 45 pg/mL.

Concentrations of prolactin were measured with a RIA similar to that described for LH (Perry et al., 1991a). Highly purified USDA-bPRL-I-1 was used as the standard and radioligand in the assay. The antiserum was rabbit anti-bovine prolactin (AFP-753180). Crossreactivities of the antiserum were < .2% for USDA-bGH-I-1 and < .1% for NIADDK-bTSH-I-1, USDA-bLH-I-1, and USDA-bFSH-I-1. Dilutions of bovine serum (10 to 300 μL) displaced [125I]bovine prolactin similarly to unlabeled USDA-bPRL-I-1 to produce a binding curve that paralleled the standard curve. When .1, .2, .4, .8, 1.6, and 3.2 ng of prolactin per assay tube were added to serum containing .32 ng of prolactin/tube, 39, 52, 68, 1.14, 1.95, and 4.03 ng of prolactin/tube were measured (average of 100.3% recovery; r = .998). All samples were analyzed in two assays; intra- and interassay CV were 10.2 and 12.3%, respectively. The assay was sensitive to .12 ng/tube or .6 ng/mL.

Blood samples also were collected via jugular catheters during 6 h at 12-min intervals on d 14 (d 12 to 16), 21 (d 19 to 23), 28 (d 26 to 30) for cows in each treatment, and on d 35 (d 33 to 37 for MCP and UICP cows only) after calving to measure concentrations of LH in serum with a RIA (Perry et al., 1991a) in four assays; intra- and interassay CV were 10.3 and 10.1%, respectively. Sensitivity was 40 pg/tube or 130 pg/mL. Assessment of variability of LH patterns on a within-cow basis was accomplished by determining the average minimum and maximum concentrations of LH within each sampling period (30 samples collected at 12-min intervals during 6 h), the standard deviation of 30 samples within sampling period, and the number and amplitude of LH pulses according to our previous definitions (Skaggs et al., 1986).

Statistical Analyses

Gestation intervals and birth weight of calves were analyzed using GLM procedures (SAS, 1988), and comparisons were made between mastectomized and UICP cows before treatments were imposed (model included sex of calf). Intervals to first and second ovulation, duration of first luteal phase and first estrous cycle, and duration of suckling events were analyzed using GLM procedures. Concentrations and characteristics of hormones in serum and percentage change in postpartum BW, oxytocin, and prolactin were analyzed as a split-plot ANOVA that included in the model treatment, cow within treatment, time, and treatment × time interaction. Treatment effects were tested with the error, cow within treatment. Effects of time and treatment × time were tested with the residual error. Percentage data (percentage observed in estrus or percentage with a normal first estrous cycle) were analyzed with logistic regression for categorical data using the same model described previously for continuous variables (procedure CATMOD; SAS, 1988). Each treatment of mastectomized cows was compared separately with the udder-intact cows (control) using orthogonal contrasts (MCW vs UICP, MCR vs UICP, and MCP vs UICP).

Results and Discussion

Changes in Body Weight and Onset of Estrous Cycles

All calves were born without assistance over a 48-d period. Mastectomized cows tended to have longer (P = .07) periods of gestation (284 ± 9 vs 281 ± 1.6 d), but birth weights of calves (35.9 ± 8 vs 37.9 ± 1.5 kg) were slightly less than for udder-intact cows. Changes in BW were similar among cows in the four treatments (Figure 1). Maintaining a uniform pattern of BW was essential to avoid confounding resulting nutrient availability and need with potential effects of treatments. The similarity in BW was achieved by individually feeding cows according to their physiological status until d 35 postpartum, when remaining calves were weaned. Changes in body condition followed those of BW. Because all cows had body condition scores of 5 or more at parturition, we did not expect the effects of BW and body condition to be a factor in altering ovarian activity and onset of estrous cycles (Short et al., 1990).

Occurrence of first ovulation and other characteristics of the first estrous cycles are summarized in Table 1. In UICP cows with unrestricted calf presence, intervals to first ovulation were longer (P < .01) than for those nonsuckled cows without calves (MCW) or with restricted calf presence (MCR). Furthermore, this interval did not differ for cows in the MCP and UICP treatments. These findings are analogous to our earlier report (Viker et al., 1993) and demonstrate that postpartum anovulation is delayed only when the calf is allowed to have inguinal contact and attempt to “suckle” its dam. Because progesterone was monitored daily, an increase in serum progesterone for two or more days indicated ovulation (Figure 2). This methodology was based on earlier work in which ultrasonograms of ovaries of suckled beef cows verified ovulation and subsequent development of a corpus luteum in cows in which serum progesterone sometimes only exceeded .5 ng/mL for 1 or 2 d following first postpartum ovulation (Perry et al., 1991b). Concentrations of progesterone (P4) in 23 of 29 cows exceeded a peak of 1 ng/mL for 2 d or more after first ovulation (first increase > .5 ng/mL), and in the
remaining cows serum P4 reached peaks exceeding .7 ng/mL for 2 d or more. Concentrations of progesterone in serum were similar among treatments during 10 d after first ovulation (Figure 2), although those in UICP cows seemed to decrease more uniformly by d 6 compared with those in mastectomized cows.

Actual duration of the first increase in P4 (> .5 ng/mL) was not affected by treatment but the first estrous cycle was longer (P < .05) in the MCW cows than in the UICP cows (Table 1). A greater (P < .05) proportion of UICP cows (eight of eight) had first estrous cycles with a duration of 10 d or less than that of MCP cows (three of six). Cows in the MCW (four of six) and MCR (six of seven) treatments had proportions of short first cycles not different from cows in the UICP treatment. The longer duration of first estrous cycles in MCW cows is somewhat consistent with observations in milked cows (calves removed at birth), in which first postpartum estrous cycles were generally of normal or slightly less than normal duration (Stevenson and Britt, 1979) and only 50% of first ovulations were preceded by detected estrus, based on retrospective 24-h video surveillance (King et al., 1976).

The percentage of cows detected in estrus before first ovulation was less (P < .05) in all treatments of mastectomized cows than that of UICP cows (Table 1). This difference may have occurred because zero of eight UICP cows ovulated before calves were weaned at 5 wk of age, whereas most of the MCW (six of seven) and MCR (four of seven) cows and two of seven MCP cows ovulated during the treatment period (before weaning) of the experiment. However, because onset of cyclic activity and stage postpartum were confounded due to the nature of the treatments, it is possible that expression of estrus was more related to the postpartum interval to ovulation than to effects of treatment. Interval to second ovulation and percentage of the cows observed in estrus before second ovulation were consistent among treatments with findings associated with first estrus and ovulation (Table 1), except that more MCR and MCP cows were detected in estrus before second ovulation.

In an earlier study (Short et al., 1972), mastectomized cows were not maintained with their calves after calving as in our study, and therefore, returned to estrus sooner than suckled, udder-intact cows.

Table 1. Intervals to first ovulation and estrous cycle traits in mastectomized and udder-intact beef cows with weaned, restricted, or unrestricted presence of calf during 35 d postpartum

<table>
<thead>
<tr>
<th>Item</th>
<th>MCW</th>
<th>MCR</th>
<th>MCP</th>
<th>UICP</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cows</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>Days to first ovulationb</td>
<td>22.6d</td>
<td>29.1d</td>
<td>36.1</td>
<td>41.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Detected first estrus</td>
<td>2/7e</td>
<td>2/7e</td>
<td>2/7e</td>
<td>7/8</td>
<td>—</td>
</tr>
<tr>
<td>Duration of first increase in P4, d</td>
<td>5.3</td>
<td>4.9</td>
<td>5.8</td>
<td>4.3</td>
<td>.9</td>
</tr>
<tr>
<td>Duration of first estrous cycle, d</td>
<td>12.8e</td>
<td>8.8</td>
<td>10.7</td>
<td>8.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Short first estrous cyclec</td>
<td>4/6</td>
<td>6/7</td>
<td>3/6e</td>
<td>8/8</td>
<td>—</td>
</tr>
<tr>
<td>Interval to second ovulation, d</td>
<td>37.3d</td>
<td>38.0d</td>
<td>45.8</td>
<td>49.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Detected second estrus</td>
<td>2/6d</td>
<td>5/7</td>
<td>5/6</td>
<td>8/8</td>
<td>—</td>
</tr>
</tbody>
</table>

aMastectomized cows with calves weaned within 24 h of birth (MCW); mastectomized cows with restricted contact with their calves (MCR); mastectomized cows with unrestricted access to their calves (MCP); and udder-intact cows with unrestricted access to their calves (UICP).

bEstimated day of first postpartum ovulation based on estrus and(or) daily concentrations of progesterone (P4) in serum (at least two consecutive days > .5 ng/mL).

cProportion of cows with a first cycle duration of 10 d or less.

dDifferent (P < .01) from UICP treatment.

eDifferent (P < .05) from UICP treatment.
Figure 2. Average concentrations of progesterone (P_4; pooled SE = .18) in serum in daily samples during 10 d after first ovulation for mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▼); mastectomized cows with restricted contact with their calves (MCR; n = 7; ◀); mastectomized cows with unrestricted access to their calves (MCP; n = 7; □); and udder-intact cows with unrestricted access to their calves (UICP; n = 8; ■). First ovulation was indicated when at least two serum samples on two consecutive days exceeded .5 ng/mL.

Viker et al. (1989) reported that mastectomized cows maintained with their calves remained anovulatory until calves were removed at 46 to 53 d postpartum. In our studies (Table 1; Viker et al., 1993), housing calves with their dams in a restricted treatment format (MCR) maintained cow-calf recognition but did not prolong anovulation as in the MCP or UICP treatments.

**Hormonal Responses to Cow-Calf Reunion**

In previous studies with mastectomized cows (Viker et al., 1989; 1993), we observed calves attempting to suckle their dams following twice-daily bottle feedings. In addition, spontaneously attempted suckling bouts were observed at other periods of the day and night. Calves in the MCP treatment assumed a normal suckling posture and attempted to "suckle" hair or skin folds in the inguinal area of their dams. In the present study, we measured this suckling activity following overnight separation of cow-calf pairs in the MCP and UICP treatments on d 7 postpartum. Upon reunion of cow-calf pairs following overnight separation, the calves in the UICP treatment suckled their dams for 15.3 ± 1.9 min, which was similar to the "suckling" activity of calves in the MCP treatment (13 ± 1.9 min). The first sucking bout was followed after a few minutes by a second sucking bout that did not differ in duration for both groups of calves (5 ± 1.4 vs 7.6 ± 1.4 min, respectively).

The MCP and UICP cows demonstrated similar maternal behavior during these suckling events. The cows stood quietly contented and occasionally turned back their heads to observe their calves during the suckling process. Similar activity occurred after each bottle feeding throughout the experiment for cow-calf pairs in the MCP treatment.

One objective was to determine whether suckling-induced hormones were secreted in mastectomized cows in a fashion similar to that in udder-intact cows after temporary separation of cow and calf. Concentrations of oxytocin in serum of all cows upon reunion of cow-calf pairs in the MCP and UICP treatments and in cows of the MCW and MCR treatments (calves not separated) varied from 12.8 ± 4 (UICP) to 34.8 ± 4 pg/mL (MCP) at 0 min. Therefore, percentage of change in concentrations of oxytocin relative to each individual cow's concentration at 0 min before cow-calf reunion are illustrated by treatment in Figure 3. Oxytocin increased in the MCP and UICP cows, beginning either 2 or 4 min after reunion of cow-calf pairs, respectively, and remained increased for approximately 8 to 10 min before decreasing to basal concentrations. Percentage of change in concentrations of oxytocin was greater (P < .01) in MCP and UICP cows during the 24-min period than in those in nonsuckled cows (MCW + MCR). The increase in

Figure 3. Average percentage of change (∆) (pooled oxytocin relative to one sample collected at 0 min before calf return) collected at 2-min intervals for 12 min and at 24 min after reunion of cow-calf pairs on d 7 postpartum following overnight cow-calf separation for mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▼); mastectomized cows with restricted contact with their calves (MCR; n = 7; ◀); mastectomized cows with restricted contact with their calves (MCP; n = 7; □); and udder-intact cows with unrestricted access to their calves (UICP; n = 8; ■). Calves were not separated from MCR cows, and no calves were present after parturition for MCW cows. Average concentrations of oxytocin ranged from 12.8 ± 4 to 34.8 ± 4 pg/mL at 0 min.
oxytocin in UICP cows seemed to be less marked than that observed in MCP cows, although oxytocin increased (P < .05) in UICP cows at 4 to 8 min after return of their calves compared with 0 min. The rather large variation in concentrations of oxytocin observed among treatments at 0 min is difficult to reconcile; yet it did not seem to be associated specifically with mastectomy or overnight calf separation.

Serum oxytocin increased in response to either suckling or milking among cows maintained with their calves; suckling elicited the greater response (Akers and Lefcourt, 1982). These authors suggested that factors other than generalized tactile stimulation of teats are involved in the release of oxytocin in response to suckling because more release of oxytocin in cows was associated with calves nursing all four teats compared with those nursing only one or two teats. The most intriguing aspect of our oxytocin results is the observation that direct tactile stimulation of the teat or udder is not required for suckling-associated release of oxytocin. A differential release of oxytocin at suckling is possible because release during suckling is greater in cows that nursed their own calves in comparison to cows that nursed unrelated foster calves (Silveira and Williams, 1991). Although changes in oxytocin over time likely reflect the perception of suckling in our mastectomized cows, the possibility cannot be ruled out that some of the observed increase is due simply to anticipation or actual return of the calf to the pen containing its dam (because MCR calves were not separated overnight).

The oxytocin response to milking among cows housed in the absence of their calves was greater than the response to milking among cows maintained with their calves (Akers and Lefcourt, 1982). Furthermore, stimuli other than direct tactile stimuli to the teats initiated milk ejection (Peeters et al., 1960), and possibly elicited release of oxytocin, including presentation of the calf to its dam (Peeters et al., 1973).

Because of great variability in concentrations of prolactin in serum of individual cows, percentage of change in prolactin is illustrated relative to each individual cow's baseline during 24 min (three samples) before cow-calf reunion (Figure 4). No treatment or interactions of treatment × time were detected; however, the effect of time was significant (P < .05). Percentage of change in prolactin within treatment (paired t-tests) was greater (P < .05) in the MCP and UICP cows 12 min after reunion than at 0 min and decreased by 36 min to match average concentrations of prolactin in the MCW and MCR treatments (Figure 4). These data may indicate that contact with the teats or udder is not essential for suckling-associated release in prolactin. However, even in intact cows maintained with their calves, milking- and suckling-associated prolactin release was not marked (Akers and Lefcourt, 1982), but was more frequently associated with suckling events as days postpartum increased from 14 to 42 (Convey et al., 1983).

Cortisol in serum was already greater (P < .05) in MCP and UICP cows upon calf return compared with that in MCW and MCR cows (Figure 5). Increased cortisol at 0 min in the MCP and UICP treatments compared with the MCW and MCR treatments may reflect a maternal response to the overnight physical separation from their calves. A treatment × time interaction (P < .05) was detected. Concentrations of cortisol in MCP cows increased (P < .05) during 12 min following cow-calf reunion and then decreased during the remaining hour, whereas those of UICP cows were greatest upon reunion of cow-calf pairs and decreased in a fashion parallel to that of MCP cows during the remaining hour. Concentrations of cortisol were unchanged in MCW and MCR cows during the 1-h period. Although all cows were haltered and restrained for 45 to 60 min before reunion of cow-calf pairs, the resulting stress associated with this restraint may have increased cortisol differently among cows during that time. Udder-intact cows were used for the first time in these housing conditions, whereas the mastectomized cows had been used in two or three previous experiments and were somewhat more docile than the UICP cows. However, none of the cows had been exposed previously to intensive blood sampling.

Figure 4. Average percentage of change [Δ] (pooled SE = 41.2) in serum prolactin (relative to three samples collected at -24, -12, and 0 min before calf return) collected at 12-min intervals for 1 h after reunion of cow-calf pairs on d 7 postpartum following overnight cow-calf separation for mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▽); mastectomized cows with restricted contact with their calves (MCR; n = 7; ▼); mastectomized cows with unrestricted access to their calves (MCP; n = 7; ▼); and udder-intact cows with unrestricted access to their calves (UICP; n = 7; ■). Calves were not separated from MCR cows, and no calves were present after parturition for MCW cows. Average concentrations of prolactin ranged from 5.9 ± 5 to 33.3 ± 4.6 ng/mL at 0 min.

Figure 5. Cortisol concentrations in serum were measured at 15-min intervals immediately before and following reunion of cow-calf pairs for mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▽); mastectomized cows with restricted contact with their calves (MCR; n = 7; ▼); and udder-intact cows with unrestricted access to their calves (UICP; n = 7; ■). Calves were not separated from MCR cows, and no calves were present after parturition for MCW cows. Average concentrations of cortisol ranged from 0.6 ± 0.2 to 1.4 ± 0.4 ng/mL at 0 min.
Tomized cows with calves weaned within 24 h of birth were present after parturition for MCW cows. Tomized cows with unrestricted access to their calves after reunion of cow-calf pairs on d 7 postpartum have been possible because of their lactation status or UICP treatment may have been related to these conditions. Mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▼); mastectomized cows with restricted contact with their calves (MCR; n = 7; ○); mastectomized cows with unrestricted access to their calves (MCP; n = 7; ▽); and udder-intact cows with unrestricted access to their calves (UICP; n = 7; ■). Calves were not separated from MCR cows, and no calves were present after parturition for MCW cows.

Figure 5. Average concentrations of cortisol (pooled SE = 2.2) in serum collected at 12-min intervals for 1 h after reunion of cow-calf pairs on d 7 postpartum following overnight cow-calf separation for mastectomized cows with calves weaned within 24 h of birth (MCW; n = 7; ▼); mastectomized cows with restricted contact with their calves (MCR; n = 7; ○); mastectomized cows with unrestricted access to their calves (MCP; n = 7; ▽); and udder-intact cows with unrestricted access to their calves (UICP). Consult Table 2 for number of observations per treatment on each sampling day. *Indicates different probability (P < .05) from UICP cows on d 21.

Figure 6. Average concentrations of luteinizing hormone (LH) in serum on d 14, 21, 28, and 35 postpartum for mastectomized cows with calves weaned within 24 h of birth (MCW); mastectomized cows with restricted contact with their calves (MCR); mastectomized cows with unrestricted access to their calves (MCP); and udder-intact cows with unrestricted access to their calves (UICP). Consult Table 2 for number of observations per treatment on each sampling day. *Indicates different probability (P < .05) from UICP cows on d 21.

Average concentrations of cortisol during 60 min before, during, and after a suckling event in cows with unrestricted calf presence were unaltered during 3 d compared with those cows from which calves were weaned 3 d earlier (Faltys et al., 1987). Indeed, at 15 and(or) 30 min after initiation of 73 suckling events in that study, peaks in serum cortisol were detected in only 53% of the suckled events; 45% of the peaks occurred during nonsuckled periods.

Concentrations and Patterns of Luteinizing Hormone in Blood Serum

Average concentrations of LH for cows in each treatment on d 14, 21, 28, and 35 postpartum are illustrated in Figure 6. Only concentrations of LH in cows that had not ovulated (anestrus) were summarized. All cows in the MCW treatment had ovulated before or during wk 4, so no LH values are illustrated for MCW cows on d 28. Only cows in the MCP and UICP treatments were sampled on d 35. Serum LH was greater (P < .05) on d 21 in MCW cows than in UICP cows. Because intervals to first ovulation were similar, it was expected that average concentrations of LH might be similar between MCP and UICP treatments when comparisons were made within week, unless cows in a deeper state of anestrus had less LH secretory activity (i.e., suckled cows in MCP and UICP treatments) than those about to initiate estrus and(or) ovulation (Short et al., 1990).

Other characteristics of the LH patterns are presented in Table 2. Attempts were made to assess the variability in concentrations of LH by analyzing the minimum and maximum values for LH within cow for each weekly sampling period. Although minimum concentrations of LH were similar among treatments, average maximum concentrations of LH tended (P < .10) to be greater in MCW than in UICP cows on d 14. On d 21 and 28, average maximum concentrations of LH were greater (P < .05) in MCP than in UICP cows, and tended (P < .10) to be greater on d 35. Average maximum concentrations of LH also tended (P < .10) to be greater in MCP than in UICP cows and was greater (P < .05) on d 28 for MCP and MCR cows than for UICP cows. The number of LH pulses was (P < .01) or tended (P < .10) to be greater on d 21 in MCP and MCR cows, respectively, than in UICP cows. Amplitude of LH pulses was not affected by treatments except on d 28 when average amplitude was greater (P < .05) in MCP (.56 ± .07 ng/mL) than in UICP cows (.3 ± .08 ng/mL). In a study (Holt et al., 1991) in which mastectomy was performed in primigravid Holsteins,
Table 2. Characteristics of luteinizing hormone patterns on d 14, 21, 28, and 35 postpartum in mastectomized and udder-intact beef cows with weaned, restricted, or unrestricted presence of calf before onset of first estrous cycles.

<table>
<thead>
<tr>
<th>Item and day</th>
<th>Treatmenta</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCW</td>
<td>MCR</td>
</tr>
<tr>
<td>No. of cows</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Average maximum LH, ng/mLb</td>
<td>14</td>
<td>1.4c</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.6c</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Average SD of LH, ng/mLb</td>
<td>14</td>
<td>.2</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>.26</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>No. of LH pulses/6 h b</td>
<td>14</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
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</tr>
</tbody>
</table>

aMastectomized cows with calves weaned within 24 h of birth (MCW); mastectomized cows with restricted contact with their calves (MCR); mastectomized cows with unrestricted access to their calves (MCP); and udder-intact cows with unrestricted access to their calves (UICP).

bAverage maximum concentration, standard deviation (SD), or number of pulses of luteinizing hormone (LH) for 30 blood samples collected at 12-min intervals during 6 h within cow-day.

cDifferent (P < .10) from UICP treatment.
dDifferent (P < .05) from UICP treatment.
eDifferent (P < .01) from UICP treatment.

Postpartum (d 7 and 16) concentrations of prolactin and LH were assessed following injections of GnRH and thyrotropin-releasing hormone (TRH), respectively. Udder removal resulted in similar intervals to increased LH after GnRH, but peak concentrations occurred later in mastectomized cows than in udder-intact, milked cows. Peak concentrations of prolactin after TRH were greater in intact cows than in cows without udders. The authors concluded that udder removal allowed greater pituitary responsiveness to GnRH but diminished the prolactin response to TRH, indicating that presence of the mammary gland differentially affected pituitary secretion of LH and prolactin (Holt et al., 1991). It seems possible that differences observed in basal LH in our MCP and UICP treatments were due to mastectomy, although such differences were not sufficient to produce a difference in their postpartum intervals to first ovulation.

Our results tend to agree with those of Short et al. (1972) in which concentrations of LH in serum were greater in mastectomized cows than in udder-intact suckled cows at various postpartum sampling times (Table 2). However, making this comparison may not be appropriate because calves were not present with the mastectomized dams in their study (Short et al., 1972) as in our study. Unrestricted calf presence was essential for prolonging anovulation in mastectomized cows (Table 1; Viker et al., 1989, 1993). Activity of LH in MCR and MCW cows, which ovulated early postpartum in our study, was similar to that in cows in another study that were suckled until d 18 postpartum (Williams et al., 1987). Continued presence of their nonsuckling calves after weaning on d 18 did not prevent the increase in LH detected in nonsuckled cows without their calves present.

Despite similarity in average concentrations of LH among treatments (except on d 21 for MCW cows), delayed onset of first ovulation in UICP cows was related to less pulsatile LH activity, which did not seem to be true for the MCP cows. The number of LH pulses and pulse amplitude in the MCP cows resembled that in MCW and MCR cows, which ovulated earlier, rather than that in UICP cows (Table 2). Average maximum and standard deviation of LH concentrations generally were greater in MCP than in UICP cows after d 14 (Table 2). The measures of LH activity in UICP cows were similar to what was expected for cows that are suckled after calving (Carruthers et al., 1980; Williams, 1990), whereas the LH activity in MCP cows was somewhat different. If suckling either interferes with the release of
hypothalamic GnRH pulses and(or) the pituitary gland is unable to respond appropriately to GnRH stimulation (Williams, 1990), then suckling in the absence of the udder does not seem to limit LH pulse activity in the same manner as in udder-intact cows. The potential “depth” of anestrus in MCP and UICP cows might account for some of these differences in LH pulse activity (Short et al., 1990) because pulsatile LH secretion is initiated earlier in nonsuckled cows than in suckled cows and LH pulse activity occurs in 30 to 50% of suckled cows (Williams, 1990). Although the difference in LH activity between MCP and UICP cows indicates that the presence of the calf in the MCP treatment might not have been entirely similar to the intact cow-calf relationship, it was sufficient to prolong anovulation. Furthermore, changes in oxytocin, cortisol, and prolactin (hormonal secretions associated with most suckling events) in MCP cows were more similar to those of intact cows with unrestricted calf presence than to MCW or MCR cows. The fact that anovulation continued despite increased concentrations and pulses of LH indicates that additional factors may prolong anovulation besides suckling-suppressed postpartum secretion of LH. These observations warrant further study.

Because MCR cows had increased LH activity and earlier cyclic activity than either of the MCP or UICP treatments, we believe cow-calf self-recognition is prerequisite but not sufficient to maintain anestrus. Recognition of her own calf by the cow must precede normal suckling activity by the calf. When cow-calf pairs were allowed 2 min of head-to-head contact before calves were allowed to suckle either their own dam or a foster dam (4× daily beginning on d 14 to 17 postpartum), fewer cows that nursed their own calves (17%) ovulated within 12 d than those that nursed a foster calf (71%) or were weaned (67%) 12 d earlier (Silveira and Williams, 1991). To maintain anovulation, cow-calf self-recognition must be maintained and coupled with “suckling,” regardless of whether the udder is present.

Implications

Unrestricted presence of the calf prolongs anovulation in udder-intact and mastectomized cows. A cow must receive stimuli resembling a normal suckling event to prolong anovulation, whether or not the udder is normally innervated or present. Suckling events in mastectomized cows are associated with significant increases in oxytocin, cortisol, and prolactin. Therefore, our results demonstrate that various inguinal sensory cues between a cow and calf probably are associated with normal suckling-induced hormones. Despite increased activity of luteinizing hormone in mastectomized cows, maintaining them with their calves in an unrestricted scenario prolongs postpartum anovulation as in udder-intact suckled cows.

Literature Cited


