

Presence of the Calf Affects Secretion of Prolactin in Cows (40565)¹

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Stimuli associated with milking or suckling cause acute release of prolactin in cows and rats (1-4). Relative to concentrations of prolactin during diestrus or pregnancy basal concentrations of prolactin are increased in serum of lactating rats (3), whereas basal prolactin in cows is unaffected by lactation (5). A possible explanation for this apparent species difference is that rat pups nurse the teats at frequent intervals throughout the day, and each nursing usually lasts at least 30 min. In contrast, cows are usually milked only twice daily, and each nursing event is completed within approximately 5 min. Peak concentrations of prolactin in serum are greatest at the end of a normal milking in cows and following removal of the milking machine concentrations of prolactin in serum decrease rapidly (2). Thus, the first objective of these experiments was to determine if continuous application of the tactile milking stimulus for 30 min prevents the decrease in concentrations of prolactin that normally occurs following milking in cows.

In addition to tactile stimuli, auditory (6), and olfactory (7) cues emanating from their pups elicit release of prolactin in mother rats. Calves are normally separated from their mothers within 1 to 2 days postpartum, whereas rat pups remain with their mothers throughout lactation. Thus, the lack of exteroceptive stimuli from their calves may also affect the concentrations of prolactin in cattle. Therefore, the second objective of this study was to determine if the presence of their calves affected basal-, milking-, or thyrotropin-releasing hormone (TRH)-induced release of prolactin in cows.

Materials and methods. In a first experiment, milking machines were attached for 30 min to the teats of two nonpregnant pri-

miparous Holstein cows which had been lactating 6 to 7 weeks. Two similar cows who were not milked during the 30-min interval served as controls. The next day treatments were reversed. Cannulas were inserted into a jugular vein 24 hr prior to the start of the experiment, and blood was collected at various intervals prior to, during, and after milking.

In a second experiment, 19 Holstein cows were permitted to nurse their offspring for 24 hr following parturition (Day 1). On Day 2 after parturition 10 of these cows (7 primiparous and 3 multiparous) were placed in an adjacent, similar barn containing other cows, but no calves (calves absent). The other 9 cows (4 primiparous and 5 multiparous) were maintained with their calves (calves present) but a slatted gate was inserted between the cow and her calf. In this second group, mothers and offspring could see, touch, hear, and smell each other, but calves could not nurse. Cows in both groups were routinely milked at approximately 0400 and 1600 hr daily. Blood was collected from a tail vessel in both groups of cows at approximately 0800 and 1700 hr on Days 3, 4, and 5 postpartum.

A third experiment was designed similarly to the second experiment except that a jugular vein was fitted with a cannula on Day 3 postpartum. At 1400 hr on day 4 post-partum the mammary glands and teats of 29 cows (15 with calves absent and 14 with calves present; all but 1 were multiparous) were washed briefly with warm water and a milking machine was attached to the teats for 6 min, then removed. On Day 5 postpartum TRH (33 µg/100 kg body wt) was injected through the cannula of each cow at 1400 hr. Jugular blood was collected at -60, -30, -15, 0, 5, 10, 15, 30, 45, and 60 min relative to the onset of milking or injection of TRH.

In all experiments blood was allowed to clot at room temperature for 4 hr, held at 4°

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for 12 to 15 hr and centrifuged at 3000g for 30 min. Serum was then decanted and stored at -20° until assayed for prolactin as previously described (2). Least-squares split-plot analysis of variance and regression were used to analyse these data (8).

Results. Continuous milking for 30 min increased ($P < 0.01$) the concentration of prolactin in sera from an average of 28 ± 9 ng/ml 0 min before milking to a peak of 290 ± 29 ng/ml at 20 min after milking commenced. Serum prolactin decreased linearly ($P < 0.05$) between 20 and 30 min, to 229 ± 32 ng/ml, and within 30 min after the milking machine was removed prolactin had decreased to 107 ± 11 ng/ml in serum. Prolactin in serum simultaneously collected from the nonmilked cows fluctuated randomly between 21 and 31 ng/ml (Fig. 1).

In the second experiment concentrations of serum prolactin were more than threefold greater ($P < 0.01$) in cows maintained in the absence of their calves than in cows with their calves (Table I). These differences were not affected by parity, days postpartum, nor by time of day when blood samples were collected.

In the third experiment overall mean basal concentrations of prolactin before milking and TRH were 13 ± 1 ng/ml of serum for cows in the presence of their calves, and 38

± 2 ng/ml for cows without their calves ($P < 0.05$; Figs. 2 and 3). Application of the milking stimulus in cows immediately adjacent to their calves increased ($P < 0.01$) prolactin to a maximum of 27 ± 3 ng/ml of serum within 15 min (Fig. 2). But in cows maintained in the absence of their calves, prolactin increased to 96 ± 10 ng/ml within 5 min. The milking-induced increase in serum prolactin was greater ($P < 0.01$) in cows without their calves than in cows maintained adjacent to their calves.

TABLE 1. PROLACTIN IN SERUM OF COWS IN THE PRESENCE OR ABSENCE OF THEIR CALVES ON DAYS 3, 4, OR 5 POSTPARTUM.^a

Days post-partum	Prolactin (ng/ml)	
	Calf present (n = 10)	Calf absent (n = 9)
Day 3		
0800 hr	10.6 ± 5.4^b	38.1 ± 11.2
1700 hr	4.9 ± 1.1	37.8 ± 8.4
Day 4		
0800 hr	7.7 ± 2.4	16.6 ± 2.6
1700 hr	6.3 ± 1.1	29.7 ± 7.1
Day 5		
0800 hr	14.2 ± 6.0	23.7 ± 5.4
1700 hr	12.3 ± 3.9	27.5 ± 5.6
Overall mean	9.3^c	28.9

^a Cows were milked at 0400 and 1600 hr.

^b Values are means $\pm$ SE.

^c Less ($P < 0.01$) than 28.9.

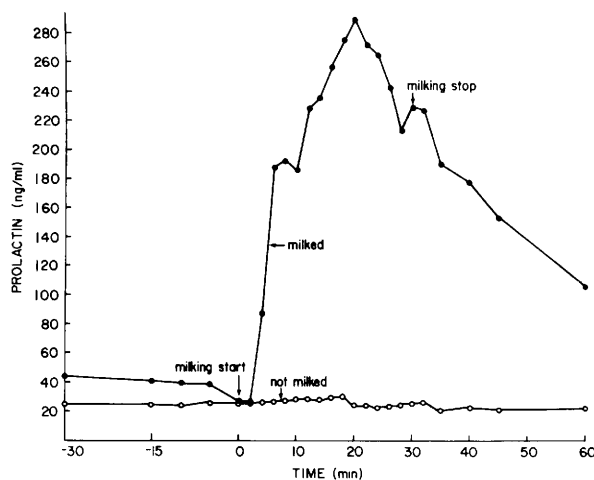


FIG. 1. Mean serum prolactin in cows lactating 6 to 7 weeks and milked for 30 min (●) or not milked (○). In cows scheduled for milking, standard errors ($n = 4$) before milking ranged from 9 to 14 ng/ml; between 6 and 30 min during milking they ranged from 12 to 46 ng/ml. In unmilked cows standard errors ($n = 4$) ranged from 4 to 12 ng/ml.

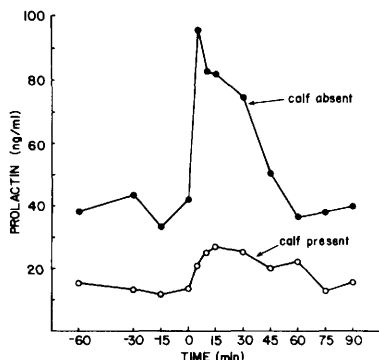


FIG. 2. Mean serum prolactin in lactating cows milked for 6 min on Day 4 postpartum, beginning at time zero, in the absence (●) or presence (○) of a calf. In cows maintained in the absence of their calves standard errors ($n = 15$) ranged from 2 to 7 ng/ml before milking and was 10 ng/ml 5 min after milking commenced. In cows maintained in the presence of their calves standard errors ($n = 14$) ranged from 1 to 2 ng/ml before milking and was 3 ng/ml 15 min after milking commenced.

Within 10 min after injection of TRH, prolactin increased from 13 ± 1 ng/ml to a peak of 236 ± 22 ng/ml of serum in cows maintained adjacent to their calves. In cows maintained in the absence of their calves prolactin increased from 28 ± 5 to 340 ± 34 ng/ml. Thus, TRH-induced release of prolactin into serum was greater ($P < 0.05$) in cows without their calves than in cows maintained adjacent to the calves.

Discussion. Results of our first experiment confirm those of others (1, 2) that stimuli associated with milking cause a rapid increase in concentrations of prolactin in serum of cows. It is interesting to note that maintenance of the stimulus for 30 min resulted in a progressive increase in serum prolactin through 20 min, and at 30 min serum prolactin still remained almost eightfold greater than at the start of milking. The disappearance rate ($T_{1/2}$) of prolactin after removal of the milking machine averaged 23 min which is in excellent agreement with values previously reported following infusion of prolactin (9). Thirty minutes of milking caused much greater releases of prolactin than we previously observed with 5 or 6 min of milking ((2) also Fig. 2). We concluded that application of the milking stimulus will maintain increased secretion of prolactin in cows during early lactation at least through 20 min.

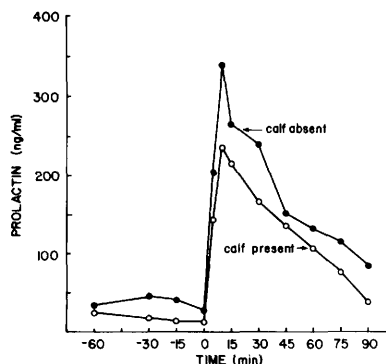


FIG. 3. Mean serum prolactin in lactating cows injected with $33 \mu\text{g}$ TRH/100 kg body wt at time zero on Day 5 postpartum in the absence (●) or presence (○) of a calf. In cows maintained in the absence of their calves standard errors ($n = 15$) ranged from 4 to 10 ng/ml before TRH and was 34 ng/ml 10 min after TRH. In cows maintained in the presence of their calves standard errors ($n = 14$) ranged from 2 to 4 ng/ml before milking and was 22 ng/ml 10 min after TRH.

However, the 21% decrease in concentration of prolactin between the 20- and 30-min period of continuous milking in our cows does not agree completely with data collected in rats which showed that increased prolactin concentrations were maintained throughout 45 to 75 min of suckling (10).

We (2) previously observed that continuous milking would not maintain elevated concentrations of prolactin in cows during advanced lactation. However, milking-induced release of prolactin declines with advancing lactation (11), and it seems probable that cows in late lactation are likely to be refractory to the milking stimulus, regardless of the duration.

According to Grosvenor (12), olfactory stimuli from the pups stimulate release of prolactin in lactating rats, and this exteroceptive mechanism complements the suckling stimulus in promoting secretion of prolactin. The data of the present study clearly show that in comparison with cows housed in the absence of their calves, cows maintained in the presence of their calves, unexpectedly, had decreased basal-, milking-, and TRH-induced release of prolactin. At this time we are unable to determine whether the act of removing the offspring from the immediate environment of the cow creates a "stressful" situation and thus accounts for the increased

secretion of prolactin in the cow. Certainly, acute (pain, noise, and restraint) and chronic (water deprivation) stress increases concentrations of prolactin in cattle (2, 13). It should be noted, however, that cows maintained in the absence of their calves showed no visible signs of distress. Thus, an alternative explanation could be that exteroceptive stimuli emanating from the calf reduce the secretion of prolactin from their lactating mothers. Bruce and Parkes (14) previously showed that exteroceptive stimuli from unfamiliar males introduced to recently mated female mice inhibited prolactin secretion and blocked pregnancy. Thus it seems possible that an analogous mechanism in terms of prolactin secretion may exist between the calf and the cow.

Summary. Prolactin in sera of four cows subjected to 30 min of continuous milking increased from 28 ± 9 ng/ml before milking to a peak of 290 ± 29 ng/ml at 20 min, and averaged 229 ± 32 ng/ml at 30 min. In comparison with 25 cows maintained in the absence of their offspring on Day 2 postpartum, 23 cows maintained immediately adjacent to their calves (suckling not permitted) decreased basal-, milking-, and TRH-induced release of prolactin by 68, 69, and 29%, respectively, on Days 3 to 5 postpartum. We conclude that direct tactile stimulation of teats maintains increased secretion of prolactin, whereas presence of the calf immediately

adjacent to their mothers but without suckling, does not stimulate secretion of prolactin. Indeed, the presence of the calf may be inhibitory to the secretion of prolactin in lactating cows.

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