

Determinants of Estrous Behavior in Lactating Holstein Cows¹

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ABSTRACT

The objective was to determine factors that affect the expression of estrus. Thirteen lactating Holstein cows were ovariectomized about 4 to 6 wk postpartum and then challenged repeatedly with progesterone and estradiol benzoate to induce estrus six times during the postpartum period. Each challenge included 5 d when the cow was primed with progesterone through insertion of a progesterone-impregnated, foam rubber pessary. Estradiol benzoate (1 mg) was injected intramuscularly 36 h after removal of the pessary. Groups of two to three cows each began the experiment at 3-mo intervals to avoid confounding treated simultaneously. Observations for estrous behavior were at 8-h intervals following each challenge. A minimum of three sexually active cows were always observed together to avoid differences in estrous behavior caused by having too few sexually active animals in the group. Observations for estrous behavior were at 8-h intervals following each challenge. During each observation, cows were observed for 30 min on dirt and for 30 min on concrete. Standing behavior was not influenced by postpartum interval, season of year, or milk yield. Mounting behavior increased from the first to the sixth postpartum challenge, but it was not affected by season of year or milk

yield. Duration of estrus, mounting activity, and standing activity were greater on dirt than on concrete. These results indicate that the surface on which cows were observed had a profound effect on sexual behavior; however, postpartum interval, season of year, and milk yield were of minor importance.

INTRODUCTION

Reproductive performance of dairy herds is influenced by estrous detection, because failure to detect estrus leads to increased days open, which results in significant economic losses to producers (2, 3, 8). Lactating dairy cows normally ovulate about 3 wk after parturition, but estrus is not usually detected until the second or third postpartum estrous cycle, because cows show less sexual activity prior to the first two postpartum ovulations (9, 14). Detection of estrus is affected by factors such as time of day of observations (11, 13), frequency of observation (8, 13), type of housing (11), and environmental temperature (13). Rate of detection increases in proportion to the number of cows in a group (up to three cows per group) in proestrus or estrus, because mounting activity is highest during these stages of an estrous cycle (12, 13).

Manifestation of estrous behavior is due primarily to the action of estrogens, but expression of estrus is more normal when cows are primed with progesterone before being challenged with estrogen (1, 4, 7). In intact cattle, expression of estrus is dependent on sufficient secretion of endogenous estrogen to provoke the standing response (10), but there is no direct correlation between concentration of estrogen in blood and intensity of estrus as long as sufficient estrogen is secreted (10). It is unclear whether factors that affect expression of estrus do so directly by action on the central nervous system or whether these factors have

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indirect effects through their influence on secretion of progesterone and estrogen.

The objective of this study was to determine whether factors such as milk yield, season of year, and type of surface on which cows were observed affected expression of estrus. We sought to eliminate differences among cows in secretion of endogenous ovarian steroids, so that differences in expression of estrus would only be affected by the factors under study. Thus, we ovariectomized cows after parturition and then challenged these cows repeatedly with progesterone and estradiol benzoate to determine factors that affected estrous behavior.

MATERIALS AND METHODS

Thirteen Holstein cows (two primiparous and 11 multiparous) that calved without complications were ovariectomized about 4 to 6 wk postpartum. Ovariectomies were performed under local anesthesia through an incision in the left lumbar fossa. Cows were allowed to recover from surgery at least 1 wk before being challenged with hormones to induce estrus. Two to three cows that calved near the same date formed a group and were ovariectomized on the same date. Successive groups were ovariectomized at about 3-mo intervals to avoid confounding postpartum interval with season of year. Five groups of cows were used during the experimental period from November 1983 until March 1985.

Cows were challenged with progesterone and estradiol benzoate six times during the postpartum period. Challenges were separated by approximately 4 wk. At each challenge, cows in one or two groups were treated simultaneously so that a minimum of three sexually active cows were always tested together. In some cases, a testosterone-treated detector cow was included in the group. This approach was used to avoid situations in which the number of sexually active cows could limit the amount of mounting activity. Previous research had shown that mounting activity was near maximum when at least three cows were in estrus at the same observation period (12, 13). At each challenge period, cows were primed for 5 d with a foam rubber pessary impregnated with progesterone. Pessaries were formed by cutting a foam rubber sheet (2.5-cm thick) into a 31 × 10-cm rectangle. The rectangle was then rolled

into a tight cylinder and sutured with synthetic suture to form a pessary 7 cm in diameter and 10 cm in length. The surface of the pessary was then saturated with a solution of 2 g crystalline progesterone dissolved in 40 ml of ethanol. The pessaries were air dried and stored in a sealed plastic bag until used. Pessaries were prepared within 1 wk of use. Pessaries were inserted manually into the anterior vagina with a gloved hand. A 20-cm length of suture was attached to each pessary to assist in its removal. At 36 h after removal of the pessary, cows were injected intramuscularly with estradiol benzoate to induce estrus. We utilized three cows in preliminary trials to determine the dose of estradiol benzoate necessary to induce an estrus of normal duration. This dose (1 mg) was used in all subsequent challenges. Blood samples for analysis of progesterone and estradiol were collected at 0, 24, 48, 120, and 156 h after insertion of the pessary and at 0, 12, 20, 28, 36, and 44 h after treatment with estradiol benzoate. Samples were allowed to clot and serum was harvested after centrifugation. Serum was stored at -20°C until assay of hormones by radioimmunoassays validated previously (6, 16). Interassay and intraassay coefficients of variation were 12.5 and 10.3% for progesterone assays and 18.5 and 15.3% for estradiol assays.

Cows were observed for estrus at 8-h intervals beginning 13 h after injection of estradiol benzoate because preliminary trials showed that estrus began subsequent to this time. Observations were at 0700, 1500, and 2300 h until all estrous activity had ceased for cows being tested together. Onset and end of estrus were estimated to have occurred midway between observations encompassing standing heat. At each observation, challenged cows were moved as a group from the freestall area where they were housed routinely to each of two test sites. Only challenged cows and the testosterone-treated detector animal (when used) were allowed to interact, because no cows from the regular herd were at the test sites. Cows were observed for 30 min at each test site, and the site of first 30-min observation was chosen at random. One site was a dirt lot adjacent to the freestall barn and the other was a clean, grooved concrete alley between two barns. Both the dirt lot and the concrete alley were sloped (about 2 to 3%) for proper drainage, and the concrete

alley was not used routinely for movement of other cows.

During each 30-min observation, all mounting and standing activity was recorded and included identification of the mounting cow and the cow that was mounted. Exact times of each standing or mounting event were recorded through the aid of a stopwatch. Front mounts were noted. All observations were made by individuals experienced in detection of estrus. A cow was considered to stand when she was immobile or staggered to maintain her balance while being mounted by another cow. A mount was recorded when the front feet of the mounting cow left the ground, even if the mounted cow did not stand. Butting, licking, and headresting were not recorded. Environmental temperature at the test site was recorded at each observation.

Mounting and standing activity was defined by two methods. Total stands and total mounts (both successful and unsuccessful) were the sum of those activities observed during all 30-min observation periods after each hormonal challenge. Average mounts and average stands were the average of those activities only for those 30-min observation periods during which a cow showed the specified activity at least once.

Estrous behavior data were subjected to least-squares analyses of variance by the general linear models procedure in SAS (15). Initial statistical models included group, cow within group, postpartum challenge (six periods), location (dirt or concrete), season of year (four seasons based on environmental temperature: <4 , 4 to 13, 13 to 21, and $>21^{\circ}\text{C}$), days postpartum, daily milk yield for the 5 d before each challenge period, and appropriate two-way interactions. In subsequent models, sources of variation that were not significant were incorporated into the residual error. Enumeration data were tested by chi-square analysis (15). Changes in concentrations of progesterone and estradiol were subjected to analyses of variance with only time from insertion of the pessary or injection of estradiol benzoate as the independent variable.

RESULTS

Preliminary tests with three cows revealed that a single injection of 1 mg of estradiol benzoate, given 36 h after removal of the

pessary, induced an estrus 13 h in duration (range 0 to 23 h). Higher doses of estradiol benzoate induced prolonged estrus. For example, when cows were given 3 mg estradiol benzoate at 36 h after removal of the pessary and 2 mg, 12 h later, duration of estrus was 40 h (range 8 to 64). Similarly, when 2 mg estradiol benzoate was given at 36 h after removal of the pessary and 1 mg given 12 h later, duration of estrus was 35 h (range 24 to 40). Thus, during the remainder of the experiment, 1 mg estradiol benzoate was given 36 h after removal of the pessary, because we considered this to be an appropriate dose to induce an estrus of normal duration with minimal and maximal values within the normally accepted range. Data from cows that received higher doses of estradiol benzoate were excluded from subsequent analyses.

Concentrations of progesterone in blood increased ($P<.05$) from $.6 \pm .1$ ng/ml ($\bar{X} \pm \text{SE}$) prior to insertion of the pessary to $3.1 \pm .1$ ng/ml 24 h after the pessary was inserted. Progesterone in serum then gradually declined to $1.2 \pm .1$ ng/ml when the pessary was removed. Progesterone averaged $.3 \pm .1$ ng/ml 36 h after pessary removal, at which time estradiol benzoate was given. Concentrations of estradiol-17 β increased ($P<.05$) from 4.2 ± 1.0 pg/ml prior to estradiol benzoate to a peak of 17.2 ± 1.4 pg/ml 12 h after injection of estrogen. Estradiol then declined to $2.6 \pm .4$ pg/ml at 44 h after estradiol benzoate.

Of the 13 cows assigned to the study, 8 provided data from six complete challenges, 2 provided data from five challenges, 2 from four, and 1 from three. Cows that did not complete all six challenges were culled for a variety of reasons unrelated to the experiment. Thus, the final data set included 69 observations from repeated tests of the 13 cows on dirt and concrete, and the observations were distributed across the experimental period. Overall, estrus was detected following 65 of the 69 challenges (94.2%). Detection rate was higher ($P<.05$) on dirt than on concrete (91.3 vs. 76.8%) but did not differ among the six postpartum challenge periods or among seasons of the year. Estrus began an average of 22.4 ± 3.5 h after injection of estradiol benzoate.

Means for the six postpartum challenge periods, averaged over location, are in Table 1. Total mounts differed ($P<.01$) among the six

TABLE 1. Means for days postpartum, milk yield, and estrual activities at each postpartum challenge period.¹

Trait	Postpartum challenge period											
	1	2	3	4	5	6						
No. observations	10	10	13	13	13	10	SE	SE	SE	SE	SE	SE
Postpartum interval, d	54	78	113	143	168	194	2	3	5	4	4	4
Daily milk yield, kg ²	31	31	32	30	29	26	2	2	1	1	1	1
Duration of estrus, h	9.8	11.4	12.3	10.4	10.7	11.0	1.1	1.2	1.3	1.3	1.2	1.2
Total mounts ^{3,4}	2.6	3.5	5.6	5.7	5.9	6.9	.6	.9	1.0	.9	1.0	1.0
Average mounts ³	2.3	2.5	3.3	3.5	3.2	3.6	.4	.4	.5	.4	.3	.3
Total stands ³	5.3	4.8	4.8	3.9	3.9	5.3	1.1	.9	.6	.7	.9	.9
Average stands ³	3.9	3.1	3.1	3.2	2.9	3.8	.5	.4	.4	.4	.7	.7

¹ Means are averaged over dirt and concrete locations.

² Average for 5 d preceding each challenge, rounded to nearest kilogram.

³ Total mounts or stands are the sum of those activities for all observations after each challenge; average mounts or stands are the average for those traits during observations when cows actually showed the activity at least once.

⁴ Differs ($P<.01$) among postpartum challenge periods.

challenge periods, increasing from $2.6 \pm .6$ at the first challenge to 6.9 ± 1.0 at the last challenge. No other estrous behavior trait differed significantly among the six challenge periods.

Means by location, averaged over the six postpartum challenge periods, are in Table 2. Location affected ($P<.01$) duration of estrus, mounting activity, and standing activity. When cows were observed on dirt, estrus was 4.4 h longer than when cows were observed on concrete. Means for duration of estrus (Table 2) include all cows that showed estrus at either location, regardless of whether or not they were detected at the alternate location. Means and standard errors for duration of estrus for cows that specifically showed estrus on dirt or concrete were $14.2 \pm .5$ and $11.5 \pm .6$ h, respectively ($P<.01$). Overall, standing and mounting activities were 40 to 119% greater on dirt than on concrete. Difference was larger between locations for total mounts and stands than for average mounts and stands. This larger difference in total mounts and stands was caused by longer estrus on dirt. Thus, when cows were observed on dirt they were detected during more of the 8-h observation periods after each challenge than when they were observed on concrete.

There were no significant effects of season (defined by average environmental temperature) on duration of estrus or standing or mounting activity. Average temperature ranged from 3.9°C for the coldest season to 21.6°C for the warmest season.

TABLE 2. Means for estrual activities on dirt and concrete.¹

Trait	Location ²			
	Dirt		Concrete	
	$\bar{X}$	SE	$\bar{X}$	SE
No. observations	69		69	
Duration of estrus, h	13.8	.6	9.4	.8
Total mounts	7.0	.6	3.2	.3
Average mounts	3.7	.3	2.5	.2
Total stands	6.3	.5	2.9	.3
Average stands	3.8	.3	2.7	.2

¹ Means are averaged over postpartum challenge period.

² All means differ ($P<.01$) between locations.

Expression of standing or mounting activity differed ($P<.01$) between locations (Table 3). The main difference between the two locations was in the number of cows that exhibited activity on dirt but not on concrete. Thus, 12 of 69 challenged cows (17.4%) stood on dirt but not on concrete, and seven of 69 (10.1%) mounted on dirt but not on concrete. Of the 69 observations, 74% were represented by stands and 84% by mounts on both dirt and concrete.

Both standing and mounting activity were affected ($P<.01$) by cow within group, but there were no significant differences among groups. To examine differences among cows, durations of estrus after the repeated challenges were averaged for each cow. Average duration of estrus was >12 h for 11 of 13 cows on dirt compared with two of 13 cows on concrete (Table 4). Duration of estrus was longer on concrete than on dirt for 6 of 65 detected heats (9.2%), was equal on dirt and concrete for 23 heats (35.4%), and was longer on dirt than concrete for 36 heats (55.4%).

There were 750 attempted mounts recorded during the study. Of these, 26 (3.4%) were front mounts, where the mounting cow attempted to mount another cow from the front. Front mounting cows were in estrus 88.5% of the times that they attempted to mount other cows from the front.

Standing activity during consecutive 5-min increments decreased ($P<.05$) over the 30-min observation period on both dirt and concrete (Table 5). If standing and mounting activity were distributed uniformly over 30-min, one would expect 16.7% of the stands or mounts to occur every 5 min. During the first 10 min of each 30-min period, activity was more than

expected, and during the last 20 min, it was generally less than expected.

Repeatability of standing activity was estimated by determining the within-cow correlation for number of stands over all challenge periods. Within-cow correlations were also estimated by degree of adjacency where the pairs of periods were consecutive or separated by one to three intervening periods. Overall repeatability of number of stands was .308; the correlation between adjacent periods was .328 and this declined nonsignificantly to .300 for periods separated by two to three intervening periods.

Daily milk yield during the 5 d preceding a challenge did not affect standing or mounting activity. However, milk yields at the a.m. and p.m. milkings after challenge with estrogen were apparently influenced by estrus. In this study, estradiol benzoate was always injected at 1800 h and milking was at 0200 and 1400 h. Thus, estrogen was always given at a fixed time in relation to the next milking. Estrus was first detected in 79% of the cows during the period from 7 h before until 2 h after the p.m. milking on the day following injection of estrogen. Thus, most cows in the study were near onset of estrus at this p.m. milking and their yield was 2 kg lower ($P<.05$) than the average for the five previous p.m. milkings ($10.2 \pm .5$ vs. $12.2 \pm .3$ kg). At the next a.m. milking, there was a slight increase ($P>.05$) in yield compared with values for the six previous a.m. milkings ($15.5 \pm .5$ vs. $14.8 \pm .4$ kg).

DISCUSSION

These results provide new insights into factors that affect expression of estrus in

TABLE 3. Expression of standing and mounting activities on dirt and concrete.¹

Activity	Was the activity observed at this location?			
	Dirt: Concrete:	No No	No Yes	Yes No Yes
Standing ²		4	2	12 51
Mounting ²		5	1	7 56

¹ Number of times observed following 69 challenges.
² Differs ($P<.05$) between dirt and concrete.

TABLE 4. Number of challenges, standing activity, and duration of estrus of individual cows on dirt and concrete.

Cow no.	n	Location			
		Dirt		Concrete	
		Times stood	Duration	Times stood	Duration
			(h)		(h)
239	4	4	15.3	3	9.5
460	3	3	13.0	1	5.0
544	6	6	15.6	6	17.2
607	6	5	15.5	2	3.2
614	6	6	15.2	6	11.3
679	6	6	15.8	5	9.2
699	6	6	11.8	5	7.8
701	6	6	14.2	5	7.5
716	6	2	7.5	4	9.8
757	5	5	14.4	5	9.4
759	5	5	16.0	4	9.6
780	4	3	12.7	3	12.7
835	6	6	12.0	4	10.5

lactating Holstein cows. Our approach was unique because it eliminated the possibility that differences among cows in expression of estrous behavior during the postpartum period were due to differences in secretion of endogenous ovarian steroids. We utilized a steroid treatment regimen designed to mimic normal changes in progesterone and estradiol that precede estrus (5, 10). We also eliminated differences in expression of estrus caused by having cows at different stages of an estrous cycle in the group (12, 13). This was done by restricting the number of sexually active cows in the group to a minimum of three. Thus, we were able to determine unequivocally effects of postpartum interval, milk yield, season of year, and surface on which cows were observed on duration of estrus and manifestation of mounting and standing activity.

The results showed that the standing response of cattle was not affected by postpartum interval. We cannot rule out the possibility that standing activity following treatment earlier during the postpartum period would have been affected by days postpartum, but the experimental period (54 to 194 d postpartum) encompassed the typical breeding period in most dairy herds (2, 3, 8, 11). It appears that cows will stand to be mounted to similar degrees throughout the normal breeding period

if they receive adequate steroid challenge. Thus, one can infer from these data that differences among intact cows in their willingness to stand during the postpartum period must be associated with differences in secretion of endogenous steroids.

In contrast to standing activity, mounting activity, defined as total mounts after a challenge, increased during the postpartum period. We have no explanation as to why mounting activity increased. The largest increase in mounting activity in this study occurred between 78 and 113 d postpartum, the period

TABLE 5. Percentage of stands made during successive 5-min increments of the 30-min observations.¹

Time	Location	
	Dirt	Concrete
	(%)	
(min)		
0–5	22.03	20.33
6–10	19.93	22.33
11–15	15.40	13.69
16–20	17.23	14.84
21–25	12.73	13.69
26–30	12.68	15.12

¹ Percentages differ ($P<.05$) from expected.

when feed intake normally peaks in lactating cows. Milk yield during the 5 d preceding a challenge was not related to mounting activity, so increased mounting activity during the later challenges was apparently not associated with decreased milk yield.

The factor that had the most dramatic effect on duration of estrus and on both standing and mounting activity was the location where cows were observed for estrus. When cows were observed on dirt, they were in estrus longer and showed more total mounts and stands than when they were observed on concrete. Average number of mounts and stands during 30-min observations when that specific activity occurred at least once were also higher on dirt than on concrete. This is of particular interest, because observations on both dirt and concrete occurred sequentially within 1 h, and the site of the first 30-min sequence was chosen at random. This finding has important practical significance in terms of routine estrous detection in dairy herds. One would predict that cows moved to dirt lots for estrous detection would be detected in estrus with higher efficiency than if they remained on concrete. In fact, when we averaged the duration of estrus for cows, 11 of 13 cows showed estrus for more than 12 h on dirt but only 2 of these showed estrus for more than 12 h when they were observed on concrete. Thus, with a typical estrous detection program of two observations scheduled at 12-h intervals, rate of detection should be much higher on dirt than on concrete. We also observed that more cows were willing to stand or mount if they were on dirt than if they were on concrete. Thus, it appears that some cows express little estrous activity on concrete, whereas they express mounting and standing behavior on dirt.

The finding that standing and mounting activities were higher during the early part of each 30-min observation period on both dirt and concrete suggests that movement of cows to a different location arouses sexual activity. In practice, efficiency of detection of estrus might be enhanced by movement of cows to a different location than where they are routinely housed, regardless of whether the new location has a dirt or concrete surface.

Other observations made during this study also have practical implications. For example, cows that mounted other cows from the front

were nearly always (88.5%) in heat. This secondary sign of estrus has a high degree of accuracy. Milk yield at the milking nearest onset of estrus was characterized by lower production while the yield at the next milking was slightly higher. It seems reasonable to use this information to identify cows that need closer observation. For example, if electronic recording of milk weights is practiced in a herd, it would be appropriate to program the system to flag cows that show a drop in yield of approximately 2 kg from that expected at a given milking. These cows could be observed more closely for signs of estrus or progesterone could be monitored in milk samples collected for 2 to 4 d to verify that the cow was in or near estrus.

In summary, we determined that the most important factor affecting sexual activity in lactating Holstein cows was the surface on which they were observed. Cows showed more standing and mounting activity and were in estrus longer if they were observed for estrus in a dirt lot compared with a concrete alley. Other factors that affected sexual activity were of minor importance.

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