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Short communication: Chronology of different sexual behaviors and motion activity during estrus in dairy cows

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ABSTRACT

In studying the efficiency of a variety of methods for estrus detection in a large dairy herd, we suspected a definite sequence of estrus signs. Consequently, we observed a subset of animals continuously between 0400 and 2400 h, making a note of the precise timing and frequency of each sexual behavior. Sixteen Holstein-Friesian cows, >20 d postpartum, were equipped with motion activity-sensing neck collars and had milk progesterone profiles monitored simultaneously. The duration between the first and last observed estrus behavior was (mean ± SE) 14.0 ± 1.9 h, with a range 8.5 to 28.75 h. The duration of standing to be mounted (STBM) was 4.68 ± 1.49 h, with a range of 0.25 to 18.25 h. Sniffing the vulva of another cow occurred on average 5.5 ± 1.3 h (range = 0.25–18.25 h) before the first STBM. By ranking the first appearance of each behavior, we established that sniffing was followed by the active behaviors of mounting another cow and not accepting a mount, as well as the passive behaviors of being sniffed and STBM by another cow. Chin resting occurred before not accepting a mount and STBM. All these behaviors were observed in the reverse order after the last STBM. The mean profile of motion activity revealed an increase in motion activity with the onset of exploratory behaviors, and highest values occurred within the period of STBM. Such distinct behavioral sequences may be controlled by changes in peripheral progesterone and estradiol concentrations, as well as by subtle independent mechanisms via pheromones in differing concentrations or divergent composition.
Key words: pheromones, sniffing, mounting, motion

Short Communication

To achieve successful reproduction, it is essential for sperm to fertilize the ovum at a precise time. Consequently, female sexual behavior begins shortly before the LH surge that triggers ovulation (Dobson, 1978; Morris et al., 2011). Several species within the family *Bovidae* also display estrus behaviors in all-female groups. This is thought to be part of a strategy engaged to attract males across large savanna grazing plains (Estes, 1966), although these attracting behaviors are also displayed in domesticated herds. In present-day intensive dairy herds, a need exists to enhance genetic improvement, for which AI is essential. Hence, farm staff need a clear understanding of the timing of all estrus behaviors to inseminate cows with a successful outcome.

In studying the efficiency of a variety of methods for estrus detection in a large dairy herd (Holman et al., 2011), we suspected a definite sequence of estrus signs could be determined. Consequently, during the latter part of that study, we watched a subset of animals continuously between 0400 and 2400 h, making a careful note of the timing and frequency of each estrus event.

We performed this study under a UK Home Office license for work on living animals and with the approval of the University of Liverpool Ethical Review Process. As part of the large study, we monitored a whole herd of approximately 200 Holstein–Friesian cows that calved throughout the year on 1 commercial-housed UK dairy farm. Mean milk sales per cow were around 11,000 L annually, with average peak yields of 54 L/d. At any one time, a group of approximately 80 cows in their first to eleventh lactation were housed in a 100-cubicle shed with grooved concrete passageways that were automatically scraped clean every 2 h. Within on-going herd management, every 2 to 3 d cows entered the shed 2 d after calving and left in rotation 4 mo later. Milking of all cows took place 3 times a day, starting at 0530, 1400, and 2030 h. All animals had continual access to a TMR that was pushed up to a feed-fence 5 times a day. All fertility examinations and treatments required during the study took place as part of the normal activity on the farm.

In the present subset study, every third week from May to August we recruited 4 to 6 cows before their second or third expected estrus, between 40 to 80 d postpartum. Each cow had a large unique number

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freeze-branded on its rump to aid identification. Cows were observed in 3-hour blocks by 2 research staff, who recorded the precise times and frequency of behavioral signs of estrus continuously between 0400 and 2400 h, including the period when the cows were gathered for milking. The layout and lighting (200 lx for 18 h/d with a 6-h period of 20 lx) permitted easy observation, with a freely accessible, open lit outdoor concreted loafing area (20 × 200 m) being a congregating place for cows in estrus. Observations were conducted by walking among the cows while noting the signs such as vulva sniffing/vulva being sniffed, chin resting/being chin rested on, mounting others, not accepting a mount, and standing to be mounted (**STBM**). The herd had been acclimatized to the research staff by frequent visits for 3 mo before the study.

At the time of entry to the study, all cows were fitted with Heatime neck collars (SCR Engineers, Netanya, Israel), with data being downloaded to a dedicated program (Dataflow, SCR Engineers) in 2-h blocks at the milking parlor exit. According to the manufacturer's specification, a value of greater than 4.7 standard deviations above the mean of the previous eight 2-h blocks represented an increase. Data were collated at the end of the study for each cow to produce a motion profile relative to the time of STBM. Because motion activity data were downloaded in 2-h blocks, the value at the time of STBM was recorded as occurring in the middle of the prior 2-h block (i.e., 1 h before, with all other data points calculated 2 h before or after).

Milk samples for progesterone analysis were collected each Tuesday and Friday throughout the study. All samples were taken immediately before milking and promptly stored at −20°C without preservative. Progesterone was analyzed as pregnancy metabolites in 50 μL of whole milk samples using an established enzyme-immunoassay (Walker et al., 2008). For the present study, the minimum detectable amount was 0.02 ng/mL, and the intra- and interassay coefficients of variation were 9.7 and 15.8%, respectively. When values were less than 0.2 ng/mL, luteal tissue was considered to be absent, whereas values greater than 0.3 ng/mL indicated the presence of luteal tissue. Progesterone profiles were produced for each study animal to indicate potential estrus periods identified by the research staff or neck collars. The term "potential event" used in data analysis was defined as each time progesterone decreased below 0.2 ng/mL, preceded and followed by at least 2 values above 0.3 ng/mL.

Every 2 wk, the same member of the research team assessed each cow's BCS using a 1 to 5 system incorporating 0.5 scores (Edmonson et al., 1989), and lameness was assessed using a standardized 1 to 5 system (Sprecher et al., 1997). Treatments for lameness and mastitis followed normal farm practice, with regular or remedial foot trimming and antibiotic treatment for udder infections.

A total of 42 potential estrus periods in 33 cows were identified, out of which data from 14 potential estrus events were discarded because they had fewer than 10 observations of any estrus behavior (insufficient for statistical analysis): 9 silent periods were from 3 individual cows, and 5 from 5 different cows. In none of the 14 excluded periods were cows observed STBM, nor was an increase in activity recorded. Data from another 6 potential estrus events were discarded because evidence from activity monitors showed behaviors had started in the 4-h period when visual observations were not made (0000 to 0400 h). Six cows had more than 1 estrus period monitored; in each case, only data from the second estrus period were retained for analysis.

Data were analyzed using Minitab (Version 14, Minitab Inc., State College, PA). Results were expressed as mean (±SE). Analysis of variance analyses with post hoc Tukey's comparisons were used to examine the frequency and timing of different sexual behaviors. Differences between first and last appearance ranks of behaviors were subjected to nonparametric analysis (Mann-Whitney). All differences were considered significant when $P < 0.05$.

A total of 16 potential events (1 per cow) were analyzed, and these cows had a mean lameness score of 1.41 ± 0.11 and a mean BCS of 2.47 ± 0.10. For 14 out of 16 potential events, another cow was in estrus in the shed at the same time.

The frequency and range of each behavior is given in Table 1; each cow did not display every behavior. Sniffing the vulva of another cow, chin resting on the rear of another cow, and being chin rested upon by another cow were the most frequently observed behaviors with not accepting a mount being the least frequent (indeed, this latter behavior was not observed in 6 cows). These frequency data are similar to those previously noted

Table 1. Frequency (±SE and range) of observed estrus behaviors

Behavior	No. of cows	Frequency	
		Mean ± SE	Range
Sniffing	16	57.3 ± 9.9[a]	4–122
Sniffed	16	27.5 ± 5.8[bcd]	3–74
Chin resting	16	45.6 ± 8.4[ab]	3–102
Chin rested	14	39.8 ± 9.7[abc]	5–135
Mounting	14	12.6 ± 2.5[cd]	1–27
Did not accept mount	10	4.9 ± 1.2[d]	1–13
Standing to be mounted	13	9.69 ± 3.0[cd]	1-30

[a–d]Within a column, values with different superscript letters are different ($P < 0.001$; $r^2 = 29.4$; ANOVA with post hoc Tukey test).