

# Timing of Insemination for Dairy Cows Identified in Estrus by a Radiotelemetric Estrus Detection System

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## ABSTRACT

The optimal time of artificial insemination (AI) was determined from data for 2661 AI in 17 herds utilizing a radiotelemetric system for estrus detection that has the potential for continuous 24-h surveillance to monitor behavioral events associated with estrus. The system consisted of pressure-sensitive radio frequency transmitters affixed over the sacrum region of cows. The activation of the sensor sent a radiotelemetric signal to a microcomputer via a fixed antenna. Cow identification, date, time, and duration of each standing event were recorded in the software program provided with the system. Each farm selected a 3-h interval to AI for cows that were identified in estrus during the previous 24 h. Pregnancy status was determined from data for return to estrus and palpation of the uterus 35 to 75 d following AI. Standing events during estrus averaged ( $\pm$  SD)  $8.5 \pm 6.6$  per cow, and the number of events per estrus across herds averaged from  $6.2 \pm 5.1$  to  $12.8 \pm 9.9$  per cow. The duration of estrus ranged from  $5.1 \pm 3.8$  to  $10.6 \pm 6.8$  h across herds; the mean was  $7.1 \pm 5.4$  h. The interval from the first standing event to AI affected the probability of pregnancy; the highest conception rates for AI occurred between 4 and 12 h after the onset of standing activity. The probability of pregnancy was higher for cows  $>100$  d in milk, exhibiting  $>2$  standing events during estrus, and inseminated during March, April, or May.

(**Key words:** artificial insemination, telemetry, estrus, detection of estrus)

**Abbreviation key:** CI = confidence interval, HW = HeatWatch<sup>®</sup> (DDx Incorporated, Denver, CO).

## INTRODUCTION

The adoption by dairy producers has made AI one of the most important technologies of this century; AI

has been important in reducing disease transmission, allowing for genetic selection, and ultimately increasing the health, longevity, and yield of dairy cows. However, an estimated annual loss of  $>\$300$  million to the US dairy industry because of the failure to detect estrus or the misdiagnosis of estrus has reduced the positive economic impact of AI (25). Thus, the efficient and accurate detection of estrus and the timing of resulting AI remain major challenges to improving reproductive and economic efficiencies of many dairy farms (7, 8, 20, 25, 28, 29).

The timing of AI relative to the stage of estrus has been investigated for  $>50$  yr. During the early development of the AI industry, several studies (28, 29) were designed to determine the optimal time of AI. Those studies indicate that maximum conception rates were achieved from mid estrus until a few hours after the end of expression of standing behavior, which led to the establishment of the a.m.-p.m. management guideline. This guideline for AI states that cows in estrus during the a.m. should be submitted for AI during the next p.m., and cows in estrus during the p.m. should be submitted for AI during the next a.m. (29). Two recent large field trials (8, 20) using professional AI technicians have shown that pregnancy rates using once daily AI schedules were similar to AI following the a.m.-p.m. guideline. The first trial used fresh semen and inseminated 44,707 cows either the same a.m. of observation, between 1200 and 1800 h on the day of observation, or on the following a.m. for cows identified after 1800 h (8). There was no difference in nonreturn rates at 150 to 180 d for cows that received AI the same a.m. or during the p.m. following a.m. detection. The second study (20), performed with frozen semen and 7240 first service AI, determined that nonreturn rates resulting from once daily AI did not differ from nonreturn rates for AI following the a.m.-p.m. guideline.

The optimal time of AI was predicted using mathematical models based on pedometer readings and rec-

Received December 11, 1997.

Accepted March 12, 1998.

<sup>1</sup>Reprint requests.

tal palpation of 171 cows (18). The chance of pregnancy was highest between 6 and 17 h after increased pedometer activity, and the calculated optimum time of AI was 11.8 h. Unfortunately, studies designed to evaluate the optimal time of AI generally had two technical deficiencies: inadequate numbers of cows for valid statistical comparisons (18, 28, 29) and inaccurate knowledge of the onset of estrus because of low frequency and efficiency of methods used for estrus detection (8, 20).

Biological events that affect the aspect of timing of AI and fertilization are the functional viable life of gametes (sperm and ova), the transport time of viable sperm from the site of AI to fertilization, and the timing of ovulation in association with AI. Using intrarectal ultrasonography to detect ovulation and the HeatWatch® (HW; DDx Incorporated, Denver, CO) estrus detection system to determine the onset of standing activity associated with estrus, the interval from the first standing event of estrus to ovulation was determined to be  $27.6 \pm 5.4$  h (30). The transport of viable spermatozoa to the oviducts requires a minimum of 6 h to obtain a population capable of fertilization, and sperm numbers progressively increase over 8 to 18 h (13, 27, 32). The functional viable life of bovine spermatozoa in the reproductive tract has been estimated at 24 to 30 h (28, 29). Although the

maximum time that the ovum may retain its capacity for fertilization is 20 to 24 h, the optimum period is remarkably transitory and is estimated to be 6 to 10 h (2). Thus, with the availability of a 24-h surveillance system to monitor behavioral events associated with estrus, it seems appropriate to reexamine the timing of AI of dairy cows. The objectives of this study were to evaluate the timing of AI of dairy cows located in 17 herds that utilized the HW system to identify and record precisely the first mount of standing estrus and to characterize estrus periods for cows monitored with the HW estrus detection system.

## MATERIALS AND METHODS

### Herds

A variety of herd sizes (56 to 556 lactating cows) and management styles were represented by the 17 dairy farms that participated in this study (Table 1). Three of the 17 herds employed three times per day milking; 14 of the herds were on a DHI testing program and had rolling herd averages ranging from 7330 to 10,847 kg/yr. This study was conducted between July 1995 and June 1996, and no effort was made to balance data across herds and seasons.

TABLE 1. Profile of herds that participated in trial.

Herd	Cows (no.)	Housing <sup>1</sup>	Conception rate <sup>2</sup> (%)	Daily milking frequency	DHI rolling herd average for milk (kg)
1	393	C	53.0	2	9785
2	477	C	41.9	3	10,227
3	146	F	45.8	2	9324
4	106	P	44.1	2	7579
5	556	F	41.4	3	10,847
6	142	P	49.2	2	7331
7 <sup>3</sup>	97	P	53.4	2	
8	131	F	47.4	2	8399
9	188	C	31.1	3	10,862
10	85	F	54.4	2	9356
11 <sup>3</sup>	99	F	44.9	2	
12	82	P	55.3	2	9655
13 <sup>3</sup>	64	F	44.9	2	
14	225	F	41.8	2	8675
15	56	F	38.2	2	7769
16	145	F	52.4	2	9039
17	228	P	43.5	2	7330

<sup>1</sup>Housing classification: C = total confinement, F = free stall and drylot, P = pasture and free stall.

<sup>2</sup>Conception rate = number of cows diagnosed pregnant divided by total number of cows inseminated.

<sup>3</sup>Herd did not participate in the DHI program.

### System for Identifying Estrus

The HW system was utilized to detect the onset of estrus and to record standing events associated with estrus. Radio frequency data communications is the base technology employed by the HW system. The radiotelemetric device that was attached to each cow consisted of a miniaturized radiowave transmitter, powered by a lithium 3-V battery and linked to a pressure sensor enclosed in a hard plastic case 5.3- × 8.1-cm and 1.8 cm in height. Each device was secured in a water-resistant pouch and was attached to a 35- × 20-cm saddle-shaped nylon mesh patch that was glued with contact-type adhesive (DDx Incorporated) to the hair caudal to the sacral region.

Activation of the pressure sensor by weight of a mounting herdmate for a minimum of 2 s produced a radiowave transmission (0.4-km range). Transmitted data consisted of sensor identification, date (month, day, and year), time (hour and minute), and duration of sensor activation. Transmitted signals were sent to a microcomputer via a fixed radio antenna. The remote signal receiver was centrally located on each farm to minimize transmission interference. Transmitted data from the remote receiver were chronologically stored in a buffer external to the microcomputer and transferred to a microcomputer at the request of the HW software. The HW software generated both fixed management reports and individual cow files that could be viewed or printed. Individual herd files of all recorded events emitted by transmitters were electronically copied, decoded into standard ASCII files, sorted by cow into individual periods of estrus activity, and scrutinized. The HW software classified a standing estrus as occurring when a cow had three standing events in any 4-h period; fewer standing events were noted as a suspected estrus, and visual observation for secondary signs of estrus prior to the decision to AI was recommended. The herd manager or inseminator made the ultimate determination of estrus and the decision to perform AI. Inseminations were performed daily during a 3-h period chosen by each farm manager for cows identified in estrus during the previous 24 h. Because spontaneous estruses and estruses induced by PGF<sub>2α</sub> do not differ in time from the first standing event recorded by the HW system to ovulation (30), spontaneous and hormonally induced estruses were not categorized.

Pregnancy status was determined by return to estrus or palpation of the uterus by the herd veterinarian 35 to 75 d following AI. Data for time of AI, pregnancy status, individual cow standing activity, and AI information (service sire, lactation number,

and calving date) were collected bimonthly from herd personnel and HW software.

### Statistical Analyses

The association between several explanatory variables and the probability of pregnancy was analyzed using the logistic procedure of SAS<sup>®</sup> (24). This procedure uses the maximum likelihood method to fit linear logistic regression models for binary response variables. The statistical significance of each explanatory variable was evaluated using likelihood ratio tests. The change in  $-2 \log$  likelihood between the full model including the factor of interest and a reduced model without the factor was calculated. This value was then compared with the results of a chi-square distribution; the degrees of freedom corresponded to the change in the number of parameters estimated by the two models (16). The cows determined to be in estrus with <3 standing events that were recorded by HW were included in the logistic regression analysis. Explanatory variables included effects of herd, AI interval relative to onset of estrus, number of standing events per estrus, DIM at AI, and season. Intervals from the initial standing event of estrus to AI were divided into seven categories by 4-h increments. The number of standing events per estrus were categorized into three groups (<3, 3 to 15, and >15 standing events). Effects of season were evaluated according to climatic data for mean daily temperature from records provided by the National Oceanic and Atmospheric Administration (Blacksburg, VA). Months of the year were grouped into seasons by similar daily mean temperatures and categorized as follows: season 1 = November, December, January, and February; season 2 = March, April, and May; season 3 = June July, August; and season 4 = September and October. In order to evaluate the potential consequences of having observations from the same cow in the ordinary logistic regression model, the logistic regression results were checked using a random effects logistic regression model with cow included as a random effect (19).

Parameter estimates of the logistic regression model were used to calculate odds ratios, which are a measure of the strength of association between explanatory and response variables (16, 23). Odds ratios were interpreted as the odds of pregnancy occurring for a particular explanatory variable category relative to the baseline category for that variable when the other explanatory factors were controlled for in the model: 1, no effect on pregnancy; >1, increased probability of pregnancy; and <1, a decreased

probability of pregnancy compared with the baseline category. The 95% confidence intervals (CI) were calculated to show the precision of odds ratio estimates. A CI that contained the numerical value of 1.0 suggested no significant difference between the category and the baseline category for that variable. Arithmetic means were calculated for duration of estrus and number of standing events per estrus, and Tukey's Studentized range tests were used to determine the differences among means.

## RESULTS AND DISCUSSION

Characteristics of the 17 dairy farms represented diverse management styles from total confinement feeding and housing systems to a system of primarily grazing (Table 1). The varied herd size (56 to 556 lactating cows) of farms participating in the trial represented much of the range in cow numbers found on Virginia dairy farms. The mean conception rate for the 2661 AI of 1616 cows was 45.3% and ranged across farms from 31.1 to 55.3%, which was similar to the 46% first AI conception rate for all Virginia dairy

farms participating in DHI (n = 549) during the study. Rolling herd averages for milk yield were >10,000 kg in three herds; these herds also were milked three times daily, and two of these herds used a total confinement system of feeding and housing. No relationship was revealed between the conception rate and herd size, housing type, milking frequency, and milk yield using backwards stepwise variable selection.

### Profile of Sexual Behavior

This study is the first quantitative examination of estrus characteristics across many herds using a device designed to monitor continuously the standing activity that indicates estrus. Three herds that removed the transmitters prior to the end of estrus as an attempt to prevent transmitter loss were not included in this analysis. The profile of estrus characteristics monitored by HW is presented in Table 2. The overall mean ( $\pm$  SD) number of standing events per estrus was  $8.5 \pm 6.6$  (n = 2055), which was comparable with that observed ( $9.5 \pm 6.9$ ) for 88

TABLE 2. Profile of estrus characteristics identified by HeatWatch® electronic estrus detection system<sup>1</sup> for cows within herd.

Herd	Characteristics of estrus					
	Estrus period <sup>2</sup>	Standing event		Duration <sup>3</sup>		
		(no.)		(h)		
		$\bar{X}$	SD	$\bar{X}$	SD	
1	362	7.7	7.2	7.3	5.8	
2	351	8.2	6.4	6.9	4.9	
3 <sup>4</sup>	176	6.0	5.0	5.8	5.6	
4 <sup>4</sup>	115	5.5	4.0	5.2	4.6	
5	307	7.4	6.1	6.6	4.8	
6	128	8.2	5.8	8.1	5.6	
7 <sup>4</sup>	55	3.8	2.4	4.5	5.9	
8	202	8.7	7.0	6.4	4.8	
9	125	6.4	4.4	6.5	6.6	
10	68	9.4	6.1	6.9	4.2	
11	76	12.8	9.9	7.8	5.2	
12	36	12.0	10.6	8.0	6.0	
13	80	7.1	5.1	5.5	5.0	
14	63	8.7	6.9	7.9	6.5	
15	32	6.2	5.0	5.0	3.8	
16	20	8.4	4.0	10.6	6.8	
17	205	8.2	8.0	6.3	5.7	
Total <sup>5</sup>	2055	8.5	6.6	7.1	5.4	

<sup>1</sup>HeatWatch® electronic estrus detection system (DDx Inc., Denver, CO).

<sup>2</sup>Estrus periods with only one standing event removed from analysis.

<sup>3</sup>Duration of estrus defined as time interval in hours from first standing event to last standing event as record by HeatWatch® system.

<sup>4</sup>Transmitters were removed voluntarily after cows were identified in estrus.

<sup>5</sup>Herds that removed transmitters prior to end of estrus were not included in totals.

estrous cycles from lactating cows in a research herd using the HW system (30). Standing events per estrus ranged from  $6.2 \pm 5.0$  to  $12.8 \pm 9.9$ . The conception rate was lower ( $P < 0.05$ ) for cows ( $n = 260$ ) that received AI after only one recorded standing event by HW than for cows ( $n = 2401$ ) that received AI after exhibiting  $\geq 2$  standing events (36% vs. 46%). Cows that were identified in estrus and received AI after only one standing event that was recorded by HW were removed from the data set prior to calculation of means for estrus characteristics to account for cows that were possibly not in estrus.

The two herds representing the range in standing events per estrus utilized a system of free stall and drylot housing. However, the herd with the fewest standing events per estrus (herd 15) also had the fewest number of cows. Using continuous observation by video recording, Walton et al. (31) reported 8.8 standing events for cows following treatment with cloprostenol to induce estrus and 5.5 standing events for spontaneously occurring estrus in lactating Holstein cows. Factors related to environment, nutrition, herd mates, and condition of feet and legs dramatically affected the behavioral characteristics of estrus and may explain herd variation of the estrus characteristics that were monitored by the HW system (3, 4, 6, 10, 14, 17). The duration of estrus, defined as the time interval from first to last standing event, ranged from 33 min to 35.8 h, and the overall duration across herds was  $7.1 \pm 5.4$  h. Duration of estrus was not determined for cows ( $n = 260$ ) that were inseminated following only one standing event. Additionally, three herds were excluded from analysis because transmitters were routinely removed once estrus was identified to prevent the loss of the transmitter by mounting activity. As with standing activity per estrus, the duration of estrus across herds did not differ ( $5.0 \pm 3.8$  to  $10.6 \pm 6.8$  h). Duration of estrus that was based on video recording (15) varied with the number of cows in estrus simultaneously, increasing from 7.5 to

TABLE 3. The circadian distribution of first and last standing events of estrus ( $n = 2055$ ).<sup>1</sup>

Item	0001 to 0600 h	0601 to 1200 h	1201 to 1800 h	1801 to 2400 h
Onset of first standing event, %	24.5	28.4	19.8	27.3
Termination of last standing event, %	24.8	27.8	23.4	24.0

<sup>1</sup>Standing activity identified by HeatWatch® system (DDx Inc., Denver, CO). Estrus consisting of only one standing event and when transmitter was removed voluntarily after cows identified in estrus were removed from analysis.

TABLE 4. The distribution of estrus periods categorized by intensity and duration and identified by HeatWatch® electronic estrus detection system.<sup>1</sup>

Estrus category <sup>2</sup>	Period	Distribution	Conception rate <sup>3</sup>
	(no.)	————— (%) —————	—————
Low intensity, short duration	579	24.1	45.6
Low intensity, long duration	798	33.2	45.5
High intensity, short duration	823	34.3	47.0
High intensity, long duration	201	8.4	49.8

<sup>1</sup>DDx Inc., Denver, CO.

<sup>2</sup>Low intensity is defined as an estrus containing  $< 1.5$  standing events/h; short duration lasted  $< 7$  h from first to last standing event. High intensity is defined as an estrus containing  $\geq 1.5$  standing events/h, and long duration lasted for  $\geq 7$  h from first to last standing event.

<sup>3</sup>Number of cows diagnosed pregnant divided by the total number of cows inseminated.

10.1 h for 1 or 3 cows in estrus at one time, respectively. Earlier studies using the HW system reported that estrus averaged 9.5 h for lactating Holstein cows (30) and 14 h for beef heifers that had synchronized estrus (26).

The circadian distribution of the first and last standing events of estrus is summarized in Table 3. There were no differences in the distribution of onset and end of estrus among the 6-h periods. Video recording was used to monitor behavior continuously for 80 d; 70% of the mounting activity occurred between 1900 and 0700 h (15). This observation suggested that cows were more likely to exhibit mounting activity when they were not distracted by other activities, such as feeding, milking, and barn cleaning. The onset of standing activity is hormonally controlled by elevated estradiol concentrations in the presence of low progesterone concentrations (1); the intensity and duration of standing behavior are dramatically influenced by environmental factors (3, 4, 6, 10, 14, 17).

The distribution of estrus periods having  $\geq 2$  standing events ( $n = 2401$ ) by duration and intensity is presented in Table 4. The proportions of total estrus periods were as follow: low intensity and short duration, 24.1%; high intensity and long duration, 8.4%; high intensity and short duration, 34.3%; and low intensity and long duration, 33.2%. The distribution of estrus periods by intensity and duration was similar for cows that conceived ( $n = 1102$ ) and for cows that either returned to estrus or were diagnosed not

TABLE 5. Logistic binomial regression for effects of interval from first standing event of estrus to AI (n = 2661) on the conception rates of dairy cows identified in estrus by the HeatWatch® electronic estrus detection system.<sup>1,2</sup>

Interval from onset of estrus to AI (h)	AI (no.)	Conception rate (%)	Odds ratio <sup>3</sup>	95% Confidence Interval
0 to 4	327	43.1	1	...
>4-8	735	50.9	1.35	1.03-1.77
>8-12	677	51.1	1.33	1.01-1.75
>12-16	459	46.2	1.12	0.83-1.50
>16-20	317	28.1	0.51	0.36-0.71
>20-24	139	31.7	0.57	0.37-0.87
>24-26	7	14.3	0.18	0.02-1.56

<sup>1</sup>DDx Inc., Denver, CO.

<sup>2</sup> $P < 0.01$ .

<sup>3</sup>Odds ratio is the estimated odds of a cow inseminated in a particular interval becoming pregnant relative to cows inseminated from 0 to 4 h following the first standing event, controlling for the effects of herd, season, DIM, and the number of standing events per estrus. Odds ratios: 1, no effect on pregnancy; >1, increased probability of pregnancy, and <1, a decreased probability of pregnancy compared with the baseline category.

pregnant during palpation by the herd veterinarian (n = 1299). Conception rates did not differ in intensity or duration across the four categories of estrus periods. Conception rates ranged from 45.5% for cows that had estrus periods of low intensity and long duration to 49.8% for estrus periods classified as consisting of high intensity and long duration. This distribution reinforces the importance of both the frequency of visual observation and the proficiency of individuals performing visual examination in the detection of estrus. Monitoring of behavior with the HW system increased the efficiency of estrus detection in estrus-synchronized beef heifers that had fewer standing events or shorter duration of standing activity in which estrus was missed by visual observation at specific observation periods (26).

### Optimal Time of AI

Logistic regression analysis for the probability of pregnancy was performed using a model including herd, interval from onset of estrus to AI, standing events per estrus, season, and DIM at AI. The results of the logistic regression model for random effects, with cow included as a random effect, showed the correlation of observations from the same cow to be nonsignificant ( $P = 0.13$ ). Therefore, the results presented are from the ordinary logistic regression model. The interval from onset of estrus to AI influenced ( $P < 0.01$ ) the percentage of cows that were

diagnosed as pregnant between 35 and 70 d post-AI. The odds of a pregnancy resulting from AI after various time intervals following the detection of estrus are presented in Table 5. The odds of pregnancy resulting from AI increased approximately 34% for cows inseminated between 4 and 12 h after the onset of estrus compared with a baseline interval of 0 to 4 h after onset. The intervals from onset of estrus to AI >16 h were related negatively to the probability of conception. The bar graph shown in Figure 1 graphically represents the cows that were diagnosed as pregnant relative to hourly intervals from the first standing event to AI. A curvilinear relationship between the interval and pregnancy is unmistakable; conception rates were highest for cows that were inseminated from 4 to 14 h following the first standing event of estrus. Variation in conception rates by hour is readily apparent, as is the overall trend of lowered conception rates >14 h from onset of estrus. Inseminations performed between 4 and 12 h following the onset of estrus achieved a conception rate of approximately 50%; conception rate was 30% for AI performed after 16 h from onset (Table 5). From previous reports (12, 18, 20, 28, 29), near optimal conception rates would be expected for cows that were submitted for AI 12 to 18 h after the detection of estrus. The frequency of visual observation prevented the accurate determination of estrus onset in previous studies (8, 12, 18, 20, 28, 29); therefore, the ability to identify the first standing event of estrus consistently and accurately with the HW system should allow for accurate timing of AI. Our results agree with those of

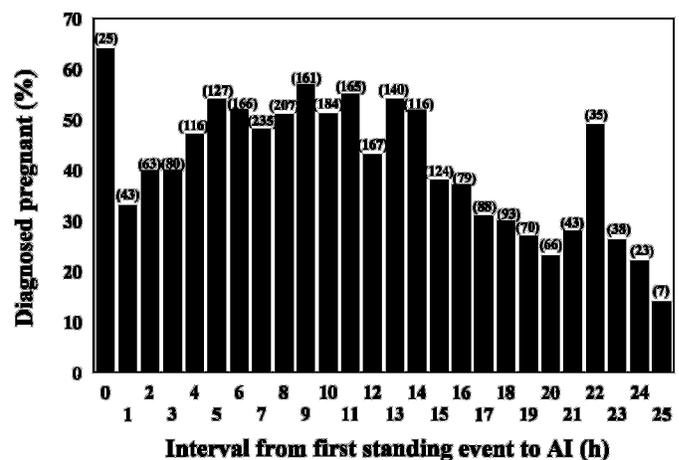


Figure 1. Percentage pregnant by hour relative to timing of AI from first standing event detected by HeatWatch® system (DDx Inc., Denver, CO) across 17 herds. Number of AI per period is within parentheses.

others (11, 21) who reported no difference in conception rates for cows and heifers that were submitted to AI shortly after the detection of estrus or 12 h later. Mathematical modeling to predict the optimal time for AI using activity pedometers and visual signs of estrus, estimated 11.8 h from onset, which coincides with the approximate midpoint of 4 to 16 h as optimum using HW (18).

The logistic binomial regressions for season, DIM at AI, and total standing events per estrus with conception rate are shown in Table 6. Season affected the pregnancy rate; the expected conception rate ( $P < 0.05$ ) was higher for AI performed during March, April, and May. The odds ratio for pregnancy occurring within this period was 1.45, and the 95% CI was 1.12 to 1.86. The odds of pregnancy for cows inseminated during summer months were 12% lower than for AI performed during November to March. This expected drop in conception is not surprising because of the high mean temperatures for the region during the summer months and the additional heat stress experienced then. This result agrees with previous reports of higher rates of embryonic loss and lower conception rates during periods of heat stress (5). Mean daily temperature and humidity have been reported (9) to account for 80% of the variation in conception by month.

The probability of pregnancy increased as DIM at AI increased ( $P < 0.01$ ). Inseminations occurring after 100 DIM had a greater probability ( $P < 0.05$ ) of resulting in pregnancy; the odds of pregnancy were 46% greater than from AI prior to 75 DIM. Adverse effects of negative energy balance during early lactation have been implicated as one cause of reduced conception in early lactation (4). Reimers et al. (22) reported higher conception rates for AI in cows after 120 DIM (60%) for AI prior to 70 DIM (49%). If it is assumed that DIM and service number would follow a similar relationship, these results are similar to those of Stevenson et al. (26) that reported the pregnancy rate of beef heifers for third AI (58%) was higher than first and second AI (40%).

Interestingly, standing events per estrus also affected ( $P < 0.01$ ) the probability of pregnancy. The baseline group of <3 standing events prior to AI, which corresponded to the activity required to activate the suspect classification in HW software, had 41% lower odds of pregnancy than cows inseminated following  $\geq 3$  standing events. Many of the cows exhibiting <3 standing events may have not been in estrus at AI, which may have contributed to a lower conception rate of cows. These findings corroborate reports of others (20, 26) and suggest that increased activity may be associated with higher conception

TABLE 6. Logistic binomial regression for effects of season, DIM, and standing events per estrus (n = 2661 AI) on the conception rates of dairy cows identified in estrus by HeatWatch® electronic estrus detection system.<sup>1</sup>

Category	AI (no.)	Conception rate (%)	Odds ratio <sup>2</sup>	95% Confidence interval
Season <sup>3</sup>				
September and October	477	40.7	1.0	...
November to March	1512	45.0	1.14	0.90–1.47
March to June	546	51.3	1.45	1.12–1.89
June to September	126	41.3	0.88	0.57–1.35
DIM <sup>4</sup>				
≤75	594	39.6	1.0	...
76–100	583	42.2	1.12	0.89–1.42
>100	1484	48.9	1.46	1.20–1.79
Standing events per estrus <sup>4</sup>				
≤2	601	39.1	1.0	...
3–15	1803	47.3	1.41	1.16–1.72
>15	257	46.3	1.41	1.02–1.93

<sup>1</sup>DDx Inc., Denver, CO.

<sup>2</sup>Odds ratio is the estimated odds of becoming pregnant for a cow inseminated in a particular category relative to the baseline category for that variable for the effects of the other two explanatory variables shown and for the effects of herd and interval from first standing event to AI. Odds ratios: 1, no effect on pregnancy; >1 increased probability of pregnancy; and <1, a decreased probability of pregnancy compared with the baseline category.

<sup>3</sup> $P < 0.05$ .

<sup>4</sup> $P < 0.01$ .

rates. Whether the estrus periods with lower activity are actually less fertile or whether many cows with <3 standing events were not in estrus at time of AI was not known.

### CONCLUSIONS

Characteristics of estrus recorded by HW were highly variable and were not significantly different across herds. The onset of estrus was equally distributed during the day, and 24.1% of all estrus periods were classified as having low intensity (<1.5 standing events/h) and short duration (<7 h). These two characteristics strongly contribute to the low efficiency of estrus detection that was experienced by many dairy herds in the US. Guidelines for the timing of AI set forth by Trimberger (28) suggest approximately 12 h after observation of standing estrus as the optimal interval for pregnancy results. Results reported here would suggest that the timing of AI should be performed earlier following observation of estrus. Using the a.m.-p.m. guideline would lower the probability of resulting pregnancy, as many cows that were observed most likely had been in estrus for several hours previous to observation. Previous studies have reported that, when onset of estrus is not known, once daily AI for cows observed in standing estrus can be used as effectively as the a.m.-p.m. guideline and results in no difference in resulting conception rate (8, 20). Our results would suggest that, if onset of estrus is unknown, AI should be performed within 4 to 12 h of observation of estrus.

### ACKNOWLEDGMENTS

This research has been made possible by the partial financial assistance of American Breeders Service Global, Inc. (DeForest, WI) and DDx, Inc. (Denver, CO). The authors gratefully thank Jim Amos, S. M. Pandolfi, T. L. Bailey, C. F. Ferris, C. Cassady, and J. L. Winter for their technical contributions and are grateful to the herd owners and managers who participated in this study.

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