Time of Ovulation Relative to Mounting Activity in Dairy Cattle

W. L. WALKER, R. L. NEBEL,' and M. L. McGILLIARD
Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg 24061-0315

ABSTRACT

Time of ovulation was determined for Holstein cows (n = 51) for estruses occurring spontaneously (n = 33) or those induced by PGF2α (n = 86). Ultrasound examination of ovaries was conducted 42 to 49 d postpartum, followed by administration of 25 mg of PGF2α if a corpus luteum was observed. In the absence of a corpus luteum, ovaries were reexamined weekly, and PGF2α was administered upon observation of the presence of a corpus luteum. Onset of estrus was determined by HeatWatch®, an electronic pressure-sensing system that recorded each mount associated with estrus. To determine ovulation in relation to first detected mount, ultrasound examinations were conducted at 12, 20, and 24 h after the initial mount and then every 2 h until 40 h. Cows were assigned randomly to receive one of two treatments: 1) the cow received 25 mg of PGF2α, 8 to 13 d later or 2) the cow was allowed to cycle spontaneously and then was switched to alternate treatment at a third cycle. The mean estrus period, determined from mounting activity recorded by HeatWatch®, consisted of 10.1 mounts over 9.5 h (6.0 mounts were ≥2 s) for a total 24.1 s of mounting activity. Estrus characteristics were extremely variable and were not different for estruses induced by PGF2α or for those occurring spontaneously. Mean ovulation time relative to first mount was 27.6 ± 5.4 h and was not different between spontaneous and induced estruses. Knowing the time of ovulation in reference to the first mount of estrus and being able to identify the first mount consistently and accurately with the HeatWatch® system allows for accurate timing of AI.

(Key words: artificial insemination, estrous behavior, ovulation, radiotelemetry)

Abbreviation key: CL = corpus luteum, HW = HeatWatch®, STP = sensor-transmitter patch.

INTRODUCTION

A successful AI program must incorporate efficient and accurate detection of estrus, proper semen handling techniques, and timely AI relative to ovulation. Estrus detection is cited many times as the most common and costly failure of AI programs. Inefficient detection of estrus results in lost lifetime milk yield, decreased number of calves born per lifetime, excessive days open, and increased reproductive culling.

Synchronization of ovulation in cows has been investigated for over 50 yr (1). Various methods of estrus detection have been used to investigate time of ovulation. Some investigators have used frequent visual observation (4, 10, 14), frequent exposure to teaser animals or other cows (1, 15, 16, 19, 25), or a combination of frequent visual observation with estrus detection aids (21). Physical verification of ovulation in most studies has been by palpation of the ovaries per rectum at frequent intervals (1, 10, 15, 16, 19, 25) or by frequent ultrasonography (14, 21). Ovulation has been timed from the cessation of estrus (1, 10, 16, 25), from onset of estrus (14, 15, 21), and from PGF2α administration (19). However, additional knowledge can be gained by an adequate number of observations, continuous observation for behavioral estrus with an electronic device, and visual confirmation of ovulation time by reproductive ultrasonography.

A novel pressure-sensing system for the detection of estrus, HeatWatch® (HW; DDx Incorporated, Boulder, CO), has been developed to alleviate the need for estrus detection by visual observation and to provide an accurate determination of the initiation of estrus. Results from an earlier, more sensitive design of this system (17) reported that the mean estrus period was composed of 14.1 mounts (4.9 of which were ≥2 s) over 12.1 h. The initial system was also compared with twice daily visual observation and reproductive performance measured by days to first estrus, days open, and AI services per conception. No differences were detected between the two methods (5). Smith et al. (24) previously reported that the accuracy of this system was 100%, and efficiency was 27% greater.
than that for twice daily visual observation by herders.

Senger (23) listed the following requirements for an ideal system for estrus detection: 1) 24-h continuous surveillance, 2) accurate and automatic identification of cows in estrus, 3) operational for the productive life of the cow, 4) reduced or eliminated labor requirements, and 5) highly accurate identification of behavioral estrus events that correlate with ovulation. The HW system matches those desired requirements of the ideal system for estrus detection in all points except that the HW system is not operational for the productive life of the cow. The sensor-transmitter patch (STP) is not implantable; thus, it requires attachment and removal when breeding eligibility and pregnancy occur. Batteries must be replaced yearly, and the transmitter life is estimated at 5 yr.

Traditional AI has followed the a.m.-p.m. guideline established in 1948 (25), which recommends that cows observed in estrus during the a.m. should be submitted for AI during the a.m., and cows in estrus during the p.m. should be submitted for AI the following a.m. (25). Although ovulation time was reported from the end of estrus, the realization that initiation of estrus was important for AI was noted. Timing of AI from the onset of estrus is important and is quite evident when results of recent research on the a.m.-p.m. guideline are examined. Nebel et al. (18) found no differences in nonreturn rate between first AI performed either once daily or following the a.m.-p.m. guideline. Fewer observations for estrus allowed cows to be submitted for AI shortly after estrus was observed; timing of AI was nearly optimal (18).

The objective of this study was to use HW to detect the first mount of standing estrus, which, by definition, is the true onset of estrus, and from that reference point to determine and compare the time of ovulation with ovarian ultrasonography in estrus periods, both those occurring spontaneously as well as those induced by PGF2α. Knowing the time of ovulation in reference to the first mount of estrus and being able to identify the first mount consistently and accurately with the HW system allows for accurate timing of AI.

MATERIALS AND METHODS

Study Design

Fifty-one lactating Holstein cows ranging from 42 to 49 DIM were submitted for ultrasound evaluation of the reproductive tract. Upon identification of a corpus luteum (CL), 25 mg of PGF2α (Lutalyse®; The Upjohn Company, Kalamazoo, MI) were administered i.m., and an STP was attached to the tailhead of the cow. Cows not having a CL at that time were examined weekly until the presence of a CL was confirmed, PGF2α was administered, and an STP was applied. To determine time of ovulation, ultrasound evaluation of the ovaries was conducted frequently. After this initial PGF2α treatment was completed, cows were randomly assigned to receive either a subsequent injection of PGF2α 8 to 13 d later or were allowed to cycle spontaneously. Ultrasound examinations were similar for all cycles. Once cows completed a second cycle, they were given the alternate treatment for a third and final cycle. Daily minimum and maximum temperatures and rainfall data were obtained from the Virginia Tech Airport, located approximately 800 m from the farm.

Ultrasound Evaluation

Status of the reproductive tract and ovarian structures were monitored with a real-time B-mode ultrasound scanner equipped with a 5-MHz transrectal probe (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). All evaluations of cows entering the study were conducted at a site approximately 75 m from where cattle were housed. Initial evaluations included a careful overview of the vagina, uterus, and uterine horns for presence of any nonechogenic areas (fluid) and views of both ovaries in several planes of section to identify follicular populations and the presence or absence of a CL. Ultrasonographic images of the reproductive tract and ovaries were evaluated for normal and pathological structures as previously described (6, 7, 20). Following the first mount recorded by HW, ultrasonographic exams of ovaries were performed at 12, 20, and 24 h and then every 2 h until ovulation occurred or until 40 h, at which time cows were designated as anovulatory. This evaluation focused on ovarian structures, and a cursory examination was made of the remaining structures. Ovulation time was defined as the number of hours from the first recorded mount to the midpoint of two examinations between which the ovulatory follicle had disappeared. All ovarian evaluations were recorded via video cassette recorder for further evaluation. Ultrasound examinations were performed by an operator who was trained in proper techniques for ultrasound examination. The time required for cow restraint, transport to and from the evaluation area, and the ultrasound procedure was approximately 10 min.
System for Identifying Estrus

The HW pressure-sensing system for detection of mounting activity was installed at the Virginia Tech Dairy Cattle Center in November 1991. Components of the system were battery-powered, reusable pressure-sensing transmitters, a signal receiver with a 0.4-km range, a buffer that received and stored mounting activity data, and software that sorted information by cow, date, and time and generated management lists using an IBM AT personal computer (International Business Machines Corp., Boca Raton, FL) with a 80286 microprocessor. Transmitters were enclosed in a Whirlpack™ bag (Nasco, Fort Atkinson, WI) and securely contained in a durable, tightly woven, nylon envelope that formed a pouch on a 25- x 20-cm disposable burlap patch. This STP was glued to the sacrum region with contact-type adhesive (Kamar®, Kamar Marketing Group, Steamboat Springs, CO). The remote receiver was placed approximately 5 m above the ground and within 30 m of the free-stall barn that housed the cows. All areas of cow traffic were within detection range of the transmitter signal. The HW software was reviewed a minimum of every 8 h to determine time of first mount for commencement of ultrasound evaluation. Data received by HW software included cow identification, transmitter number, time and duration of mounts, and signal strength.

Blood Sampling and Determination of Progesterone

Blood samples were taken at the initial administration of PGF2α and every Thursday, Sunday, and Tuesday thereafter until approximately 115 DIM. Samples from coccygeal venipuncture were drawn into evacuated tubes (Vacutainer®; Becton-Dickinson, Rutherford, NJ) with no added anticoagulants. After transport from the farm, samples were placed on ice in a refrigerated cooler at 15°C. Refrigerated whole blood was allowed to clot for a minimum of 18 h and then was centrifuged at 3000 x g for 30 min to obtain serum. Serum was stored at -20°C until assayed for progesterone. Serum progesterone was determined using a standard human radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA) that had been previously validated in our laboratory for bovine progesterone. A progesterone concentration of ≤1 ng/ml was used to declare a cow in estrus. Interassay and intraassay coefficients of variation were 7.1 and 6.2%, respectively.

Statistical Analysis

Variables were analyzed using the GLM procedure of SAS (22). Independent effects included cycle, cow, season, and parity. Dependent measurements included time from administration of PGF2α to estrus and ovulation, progesterone concentrations and slopes, and characteristics of estrus (total mounts, mounts ≥2s, mounted duration, and duration of estrus). Duration of estrus was omitted when STP were displaced during mounting. To ensure statistical validity of tests, variables normally distributed were compared with a conservative F test (8).

RESULTS AND DISCUSSION

Mean time of ovulation (27.6 ± 5.4 h) did not differ between estruses induced by PGF2α, and those occurring spontaneously (Table 1). This similarity indicated that time of ovulation was not affected by luteolysis induced by PGF2α. Similar results were reported for heifers (14) and cows (21) when ovulation was determined from onset of estrus. In addition, treatment sequence following initial administration of PGF2α did not affect ovulation time from first mount, thus revealing no detrimental effects of two sequential administrations of PGF2α. Trimberger (25) also

<table>
<thead>
<tr>
<th>Estrus classification</th>
<th>Estrus DIM</th>
<th>Ovulation time</th>
<th>Anovulatory²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no.)</td>
<td>(d)</td>
<td>(h)</td>
</tr>
<tr>
<td>Initial cycle induced by PGF2α³</td>
<td>26</td>
<td>61</td>
<td>27.0</td>
</tr>
<tr>
<td>Second cycle induced by PGF2α³</td>
<td>31</td>
<td>92</td>
<td>28.1</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>26</td>
<td>93</td>
<td>27.9</td>
</tr>
<tr>
<td>Overall</td>
<td>93</td>
<td>...</td>
<td>27.6</td>
</tr>
</tbody>
</table>

¹DDx Incorporated (Boulder, CO).
²Cows not ovulating by 40 h after first mount.
³Estrus was induced by administration of 25 mg of PGF2α.
suggested that time of ovulation was a relatively fixed reproductive event. Over the entire study (n = 119), 78% of cows ovulated within 40 h of onset of estrus. Of the 22% not ovulating by 40 h, a large proportion (73%) were from those cycles induced by PGF$_{2\alpha}$. However, only 7 cows became cystic during the study. Five cows became cystic following administration of PGF$_{2\alpha}$, and the remaining 2 were cystic prior to receiving any treatment. Cystic condition was defined as the maintenance of a dominant, anovulatory follicle ≥20 mm in diameter for a minimum of 10 d. Cycles subsequent to cystic condition were included in the analysis if a normal ovulation resulted. For those cows with estrus periods induced by PGF$_{2\alpha}$, the mean number of DIM at first estrus was 60 d. Subsequent estruses induced by PGF$_{2\alpha}$ and those occurring spontaneously had mean DIM of 92 and 93 d, respectively, thus avoiding a potential confounding of treatment with DIM.

A significant, positive relationship between duration of estrus and time of ovulation is shown in Figure 1. Duration of estrus was defined as time from first to last mount of an estrus, thus excluding cows having only one mount. A prolonged duration of mounting activity was associated with an extended interval from first mount to ovulation. This relationship might indicate that, during the early stages of estrus, the concentration of estradiol-17β produced was sufficient to exceed threshold necessity for behavioral estrus to occur, but insufficient to stimulate the preovulatory surge of LH and thus ovulation. Day of the cycle on which PGF$_{2\alpha}$ was administered might explain this phenomenon. However, this relationship existed over a relatively brief time range (24.7 to 33.8 h), and adjustment in timing of AI to achieve optimal results might be neither necessary nor practical.

The time from administration of PGF$_{2\alpha}$ to first recorded mount or ovulation was not different for the initial cycles or for the second cycles induced by PGF$_{2\alpha}$ (Table 2). The overall time from administration of PGF$_{2\alpha}$ to estrus (73.1 h) was longer than the previously reported times of 62.8 to 57.6 h for administration of PGF$_{2\alpha}$ at 0600 and 1800 h, respectively (19). This discrepancy was likely due to differences in the method of detection of estrus. The present study used continuous electronic surveillance, but Nkuuhe and Manns (19) used the introduction of androgenized cows at frequent intervals (0600, 1200, 1800, and 2300 h). However, the results substantiate the work of Britt et al. (2), who found that Holstein heifers expressed behavioral estrus at 71.2 h after administration of PGF$_{2\alpha}$. When PGF$_{2\alpha}$ was administered to Hereford heifers and cows at either 0600 or 1800 h, no differences were noted in the interval from PGF$_{2\alpha}$ administration to ovulation (19). However, heifers had a significantly shorter time from PGF$_{2\alpha}$ administration to ovulation than did cows (83.9 vs. 99.2 h). Rajamahendran et al. (21)

![Figure 1. Relationship between duration of estrus and time of ovulation (r² = 0.68).](image)

Table 2. Time from administration of PGF$_{2\alpha}$ to initiation of mounting activity and ovulation by cycle classification.$^1$

<table>
<thead>
<tr>
<th>Cycle classification</th>
<th>Initiation of estrus</th>
<th>Ovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no.)</td>
<td>(h)</td>
</tr>
<tr>
<td>Initial cycle induced by PGF$_{2\alpha}^3$</td>
<td>46</td>
<td>75.8</td>
</tr>
<tr>
<td>Second cycle induced by PGF$_{2\alpha}^3$</td>
<td>40</td>
<td>70.7</td>
</tr>
<tr>
<td>Overall</td>
<td>86</td>
<td>73.1</td>
</tr>
</tbody>
</table>

$^1$Mounting activity was detected by the HeatWatch® (DDx Incorporated, Boulder, CO) system, and ovulation was determined by frequent ultrasonographic examination.

$^2$Least squares means.

$^3$Estrus was induced by administration of 25 mg of PGF$_{2\alpha}$. 

TABLE 3. Effect of temperature on time from administration of PGF$_{2}$ to initiation of mounting activity and ovulation.

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Initiation of estrus (h)</th>
<th>Ovulation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSM$^1$</td>
<td>SE</td>
</tr>
<tr>
<td>&lt;13</td>
<td>86.6$^a$</td>
<td>6.1</td>
</tr>
<tr>
<td>$\geq$13</td>
<td>59.9$^b$</td>
<td>5.5</td>
</tr>
</tbody>
</table>

$^a,b$Means within a column with different superscripts differ ($P < 0.05$).

$^1$Least squares means.

determined that parity (biparous vs. pluriparous cows) tended to affect ovulation time from the onset of estrus (24 vs. 30 h). However, our results revealed no effect of parity on time of ovulation. When timed AI was used with estruses induced by PGF$_{2}$, no adjustments needed to be made for either parity or PGF$_{2}$ use per se.

Temperature affected time from administration of PGF$_{2}$ to first recorded mount (Table 3). Cooler temperatures ($\leq$13$^\circ$C) ranged from -10.3 to 10.3$^\circ$C ($\bar{X}$ = 3.9$^\circ$C). The mean precipitation was 0.2 cm/d and ranged from 0 to 1.46 cm/d. Warmer temperatures ($\geq$13$^\circ$C) ranged from 5.6 to 24.4$^\circ$C ($\bar{X}$ = 13.4$^\circ$C); mean precipitation ranged from 0 to 1.1 cm/d ($\bar{X}$ = 0.1 cm/d). Extremes in temperature were not experienced during the study, which might explain why intensity of estrus increased as temperature increased. Temperature has been shown (9) to have a positive, curvilinear relationship with intensity of estrus up to 25$^\circ$C and a decline after 30$^\circ$C. The intensity of estrus was calculated by dividing total mounts by duration of estrus. Gwazdauskas et al. (9) defined intensity as the number of mounts per 0.5 h. In ovariectomized cows in lactation induced by estradiol-17$\beta$, a higher intensity of mounting activity occurred during the initial 10 min of a 30-min observation period (3). The present study provided a more accurate estimate of intensity of estrus by recording all mounts throughout estrus. Another possibility would be that higher temperatures caused increased vessel dilation and blood flow to dissipate internal heat. Concurrently, PGF$_{2}$ reached the CL quickly, thereby causing luteolysis and rapid onset of estrus.

Characteristics of estruses monitored by HW are presented in Table 4. There were no differences based on category or order of cycle. Cow within parity was a major ($P < 0.001$) source of variation for total mounts, mounts $\geq$2 s, and mounted duration. Duration of estrus was affected by parity ($P < 0.05$). Duration of estruses was nearly 50% shorter for primiparous cows (7.4 ± 1.4 h) than for multiparous cows (13.6 ± 2.0 h). Maximum temperature on day of estrus did not affect duration of estrus. Britt et al. (3) noted similar findings when season was defined as the mean environmental temperature. These results indicated that willingness to mount or to exhibit estrus might contribute to secondary behavioral characteristics, and environmental temperature might not have been high enough to affect duration of estrus.

The mean number of mounts per estrus was 10.1 and did not differ between those cycles induced by

![TABLE 4. Arithmetic and least squares means (LSM) for characteristics of estrus monitored by HeatWatch$^a$.1](image)
PGF$_{2\alpha}$ and those occurring spontaneously. For Zebu cows, the mean number of attempts to be mounted per estrus was 28.8 when bulls with a deviated penis were used and when visual observation to identify estrus was continuous (13). Discrepancies in the numbers of mounts might have been due to inherent differences in libido between bulls and cows. Helmer and Britt (11) reported that, for heifers, there were 6.1 total mounts with 2.3 total stands when 1 estrual cow and no preestrual cows were present. When 1 estrual cow and 1 preestrual cow were present, total mounts were higher than when no preestrual cows were present (15.3 vs. 5.0). In a comparison of estruses induced by cloprostenol and those occurring spontaneously in lactating Holstein cows, Walton et al. (26) recorded 8.8 and 5.8 standing mounts, respectively, using continuous observation by video camera. Hurnik et al. (12), using continuous observation, determined that increasing the number of cows simultaneously in estrus from 1 to 3 increased mounts per cow from 11.2 to 36.6 and 52.6. Reports on total mounting activity revealed extensive variation in observed behavior, according to multiple factors, including footing surface, temperature, and number of herdmates simultaneously in estrus.

For all cycle classifications, the mean number of mounts $\leq 2$ s was 6.0. Mounts $\leq 2$ s that were due to false activity, such as chin resting directly on the STP, were eliminated. The mean mounting time was 24.1 s and did not differ between cycle classifications. One previous study (24) with HW addressed mean mounting activity in heifers. Smith et al. (24) reported that the mean mounted duration was $8.5 \pm 3.5$ s per heifer. Duration of mount was probably of no practical use except to demonstrate the limited activity during estrus.

CONCLUSIONS

Ovulation times that were determined in this study substantiated several previous reports. Spontaneous estruses and estruses induced by PGF$_{2\alpha}$ did not significantly differ in time from first mount to ovulation. Temperature had an effect on time of initiation of estrus from administration of PGF$_{2\alpha}$. This effect was presumably due to the influence of temperature on initiation of estrus, as defined solely by mounting activity. Estrus characteristics were highly variable and similar for estruses induced by PGF$_{2\alpha}$ and for those estruses occurring spontaneously. Primiparous cows exhibited mounting activity for a shorter time than did multiparous cows. The HW system can reliably and consistently identify the onset of estrus and permit more accurate timing of AI relative to visual observation of estrus. Studies must now be conducted to determine the effect of timing of AI for estruses identified by HW.

ACKNOWLEDGMENTS

This research has been made possible by the partial financial assistance of Virginia Agriculture Council and DDx Incorporated and with the assistance of the dairy farm personnel at Virginia Polytechnic Institute and State University. The authors gratefully thank F. C. Gwazdauskas, J. H. Bame, A. A. Ahmadzadeh, G. L. Bethard, J. R. Gibbons, and C. Cassady for their technical contributions to this study.

REFERENCES