New Technologies for Detection of Estrus

P. M. Fricke, A. Valenza, G. Lopes Jr., M. C. Amundson, and J. O. Giordano
Department of Dairy Science, University of Wisconsin-Madison

Introduction

Despite the widespread adoption of hormonal synchronization protocols that allow for timed artificial insemination (**TAI**), detection of behavioral estrus continues to play an important role in the overall reproductive management program on most dairies in the U.S. (Caraviello et al., 2006; Miller et al., 2007). Several challenges for estrus detection on farms include attenuation of the duration of estrous behavior associated with increased milk production near the time of estrus resulting in shorter periods of time in which to visually detect estrous behavior (Lopez et al., 2004), low number of cows expresing standing estrus (Lyimo et al., 2000; Roelofs et al., 2005; Palmer et al., 2010), silent ovulations (Thatcher and Wilcox, 1973; Palmer et al., 2010; Ranasinghe et al., 2010), and reduced expression of estrous behavior due to confinement (Palmer et al., 2010). Whatever the cause, the low efficiency of estrus detection not only increases time from calving to first AI but increases the average interval between AI services (Stevenson and Call, 1983) thereby limiting the rate at which cows become pregnant.

Because of the impact of AI service rate on reproductive performance and the problems associated with visual estrus detection on farms, many technologies have been developed to enhance estrus detection by providing continuous surveillance of behavior including rump-mounted devices and androgenized females (Gwazdauskas et al., 1990), pedometry (Peralta et al., 2005; Roelofs et al., 2005), and radiotelemetry (Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998). New electronic systems that incorporate accelerometers as a means to associate increased physical activity with estrous behavior in cattle (Holman et al., 2011; Jónsson et al., 2011) have been developed and marketed to the dairy industry. Whereas a large body of literature exists on the accuracy and efficacy of using various technologies to predict ovulation and timing of AI in relation to ovulation in lactating dairy cows, no other studies have investigated accelerometers for such purposes.

We recently conducted a series of experiments in collaboration with a commercial dairy farm in Wisconsin to assess the accuracy of an activity monitoring system for timing of AI in relation to ovulation (Valenza et al., 2012) and to incorporate an activity monitoring system into a reproductive management program for first AI (Fricke et al., 2012). The collaborating dairy was typical of a large confinement-based dairy in the upper Midwest region of the U.S. The farm was milking approximately 1,000 cows which were housed in free-stall barns with ad-libitum access to feed and water. Cows were fed a total mixed ration once daily that was formulated to meet or exceed NRC requirements for high producing lactating dairy cows (NRC, 2001). Cows were milked three times daily and averaged ~41 kg of milk per day throughout the course of the two experiments.

At approximately 14 d after calving, all cows were fitted with an activity tag (Heatime, SCR Engineers, Ltd., Netanya, Israel) attached to a neck collar. After each milking, data collected by the activity monitoring system was read by a transceiver unit placed in an archway at the milking parlor exit and transferred to the activity monitoring system herd management software (DataFlow I™ version 4.7; SCR Engineers, Ltd., Netanya, Israel) installed on an on-farm computer. All settings of the activity monitoring system software were those being used by the farm at the time of the experiments, and software settings were not changed throughout the course of the experiments. The activity monitoring system continuously monitored individual cow activity and recorded average activity for 2 h time periods. The raw activity of individual cows was plotted as a bar graph where each bar represented a 2 h block of time. Using a mathematical algorithm, a weighted activity index was calculated by the software that expressed

the momentary deviation of the activity from the average activity in the same time period during the past 7 d, and weighted activity was represented on the activity report by a solid line. Pregnancy outcomes are presented as pregnancies per AI (**P/AI**) evaluated a pregnancy diagnosis at a given day post insemination.

Experiment 1 - Assessment of an accelerometer system for detection of estrus and for treatment with GnRH at the time of insemination in lactating dairy cows.

To assess the use of an accelerometer system for reproductive management, lactating Holstein cows from a commercial dairy farm located in southwestern Wisconsin milking approximately 1,000 cows were used in a field trial, which was performed from August, 2010 to June, 2011 (Valenza et al., 2012). At 14 d after calving, all cows were fitted with an accelerometer (Heatime®, SCR Engineers, Ltd., Netanya, Israel) attached to a neck collar and an electronic identification tag. After each milking, data collected by the accelerometer was read by a transceiver unit placed in an archway at the milking parlor exit and then transferred to the accelerometer herd management software (Data Flow™; Micro Dairy Logic, Amarillo, TX) installed on the on-farm computer. The accelerometer system continuously monitored individual cow activity and recorded average activity for 2 h time periods. The raw activity of individual cows was plotted as a bar graph where each bar represented a 2 h block of time. The onset of activity was defined as the time at which the first bar of raw activity of an estrus event was identified. Duration of activity was defined has the time interval between the beginning and end of activity for an estrus event. Twice daily (a.m. and p.m.), a list of cows determined by the accelerometer system to be eligible for insemination was generated, and cows appearing on the list generated by the accelerometer system were inseminated. Thus, inseminations were conducted twice daily (a.m. and p.m.) by two herd personnel with each cow receiving a single insemination based on activity.

Each week, cohorts of 10 to 15 cows from 46 to 52 DIM were evaluated by transrectal ultrasonography to determine uterine health and record ovarian structures. Cows without signs of uterine disease and at least one follicle ≥ 10 mm in diameter received an i.m. injection of GnRH followed by an i.m. injection of $PGF_{2\alpha}$ 7 d later to synchronize estrus (Figure 1). Transrectal ultrasonography was performed at the time of the $PGF_{2\alpha}$ injection for subsequent determination of ovulatory response to GnRH treatment. A total of 112 cows were enrolled, but only 89 cows that were considered properly synchronized were included in the analyses. Transrectal ultrasonography was performed at the time of the $PGF_{2\alpha}$ injection for subsequent determination of ovulatory response to GnRH treatment. Diameter of ovarian structures was estimated and recorded using on-screen background gridlines comprising squares with 10 mm sides in the portable scanner. Ovulation was defined as the presence of a follicle ≥ 10 mm at the initial ultrasound examination at the time of the GnRH injection and the presence of a new corpus luteum in the same location at the subsequent ultrasound examination at the time of the $PGF_{2\alpha}$ injection. Thereafter, ovarian ultrasonography was performed 48 h after the $PGF_{2\alpha}$ injection and then every 8 h until ovulation occurred or until 96 h, whichever occurred first. Cows failing to ovulate within 96 h of the $PGF_{2\alpha}$ injection were re-examined 3 d later (i.e., 7 d after the $PGF_{2\alpha}$ injection) to determine whether ovulation had occurred.

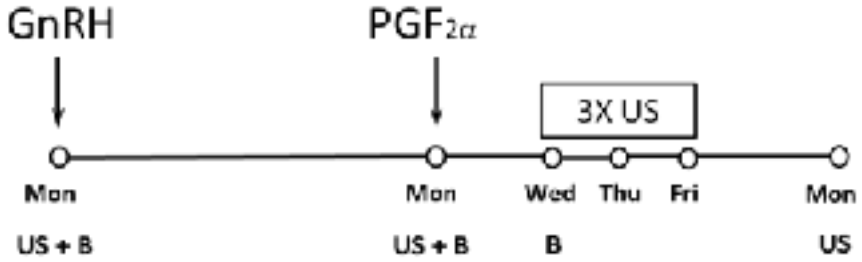

Figure 1. Diagram of experimental activities. Cows from 46 to 52 d postpartum received a G-P protocol to synchronize estrus using i.m. injections of GnRH (100 µg) and $PGF_{2\alpha}$ (25 mg). Transrectal ultrasonography (US) was used to assess ovarian structures during the protocol and time of ovulation after induction of luteolysis, and blood samples (B) were collected to assess serum progesterone (from Valenza et al., 2012).

Results

The percentage of cows with estrus events detected by the accelerometer system and the distribution of cows by occurrence of estrus and ovulation are presented in Table 1. Throughout the study period, 78% of cows ovulated within 7 d after induction of luteolysis. Of the cows that ovulated, 59% ovulated within 96 h, whereas 41% ovulated from 96 to 168 h (4 to 7 d) after induction of luteolysis. Overall, 71% of cows were detected in estrus by the accelerometer system, and 95% of cows showing estrus ovulated whereas 5% did not ovulate within 7 d of induction of luteolysis. Of the cows not detected in estrus by the accelerometer system, 35% ovulated whereas 65% did not ovulate within 7 d of induction of luteolysis.

Table 1. Percentage of cows determined to be in estrus, and distribution of cows by estrous activity and ovulation after induction of luteolysis based on use of an accelerometer system[1] (adapted from Valenza et al., 2012).

Activity and ovulation responses of cows after induction of luteolysis	% (n)
Cows with estrous activity	71 (89)
Cows that ovulated	95 (63)
Cows with no ovulation	5 (63)
Cows with no estrous activity	29 (89)
Cows that ovulated	35 (26)
Cows with no ovulation	65 (26)

[1]Heatime®, SCR Engineers, Ltd., Netanya, Israel.

The duration of estrus activity for cows detected in estrus by the accelerometer system (16.1 ± 4.7 h, range = 4.0 to 28.0; Figure 2) was not affected ($P = 0.74$) by parity (16.4 vs. 17.2 h for primiparous and multiparous, respectively) or milk production near the time of estrus ($P = 0.51$). The duration of estrus observed in this experiment is comparable to the mean duration (13.4 h) reported for cows monitored for estrus by visual observation of both primary (standing to be mounted) and multiple secondary signs of estrous behavior (Roelofs et al., 2004). Conversely, duration of estrus activity observed in the present experiment is considerably longer than the

mean duration of estrus based on the interval between the first and last standing event of estrus detected using an electronic pressure-sensing system (Dransfield et al., 1998; Xu et al., 1998). Discrepancies between the duration of estrus based on activity or visual observation with that recorded based on standing events are possibly due to the uncoupling of expression of secondary signs of estrus behavior and standing estrus. Indeed, Sveberg et al., (2011) reported that secondary signs of estrous behavior, which can certainly be detected by visual observation or increased activity, increased significantly within 1 to 3 h before the initiation of standing estrus in lactating dairy cows.

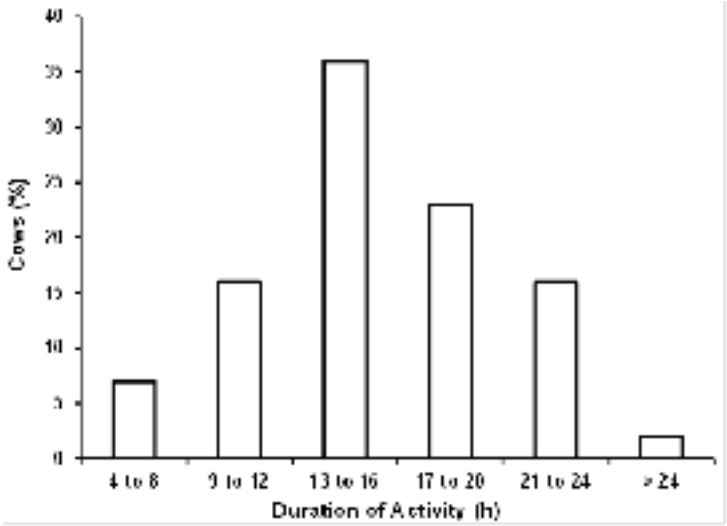

Figure 2. Distribution of cows based on duration of activity associated with estrus for cows detected in estrus by an accelerometer system (Heatime®) within 7 d after synchronization of estrus (from Valenza et al., 2012).

We did not expect that ~30% of cows would fail to show estrus within 7 d after the $PGF_{2\alpha}$ injection because a follicle >10 mm was present in all cows at the time of the $PGF_{2\alpha}$ injection, and all cows included in the analysis underwent luteal regression within 48 h after $PGF_{2\alpha}$ treatment. In another study in which cows received two sequential $PGF_{2\alpha}$ injections at 35 and 49 DIM, only 67.9% of cows determined to be cycling by 49 DIM were detected in estrus and inseminated after the second $PGF_{2\alpha}$ injection leading the authors to conclude that issues other than cyclicity status affected efficiency and accuracy of estrus detection (Chebel and Santos, 2010). The percentage of cows that failed to ovulate within the group of cows not detected in estrus was 65% for the accelerometer system suggesting that estrus did not occur in these cows. The remaining 35% of ovulations in cows not detected in estrus may have been silent ovulations (ovulation without estrus), a phenomena described in lactating dairy cows especially during the early postpartum period (Thatcher and Wilcox, 1973; Palmer et al., 2010; Ranasinghe et al., 2010). In addition, 5% of cows detected in estrus failed to ovulate within 7 d after induction of luteolysis. The overall rate of ovulation failure in lactating dairy cows that showed estrus behavior was 6.5% and was greater during the warm than during the cool season (López-Gatius et al., 2005). This rate of ovulation failure represents a small percentage of the population of cows in this experiment and could occur due to failure in the mechanism triggering ovulation (i.e. no LH surge or insufficient LH secretion) or a lack of response by the dominant follicle to the LH surge.

Due to the short lifespan of the oocyte in cattle (Hunter, 2003), the interval from AI to ovulation is critical for optimizing fertility in lactating dairy cows inseminated after estrus. In the present study, the mean interval from AI to ovulation was 7.9 h (Figure 3). This mean interval may seem appropriate because it allows for the 6 to 8 h required for the sustained phase of sperm transport to the site of fertilization and sperm capacitation (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984; Hawk, 1987); however, the degree of variation in the AI to ovulation interval (Figure 3) is a major concern. Overall, 21% of cows received AI between 0 to 12 h after ovulation, a timing associated with low fertilization rates and embryo quality in lactating dairy

cows (Roelofs et al., 2006) possibly due to aging of the oocyte during the period required for sperm transport and capacitation. By contrast, only 1 cow was inseminated more than 24 h before ovulation, a period that results in high fertilization rates but low embryo quality possibly due to aging of the spermatozoa (Roelofs et al., 2006). Based on these data, it may be helpful to either reduce the variation in the AI to ovulation interval so that more cows are inseminated at the optimal time in relation to ovulation or alternatively to inseminate cows a few hours earlier to reduce the probability of inseminating cows after or around the time of ovulation when detected in estrus using the accelerometer system.

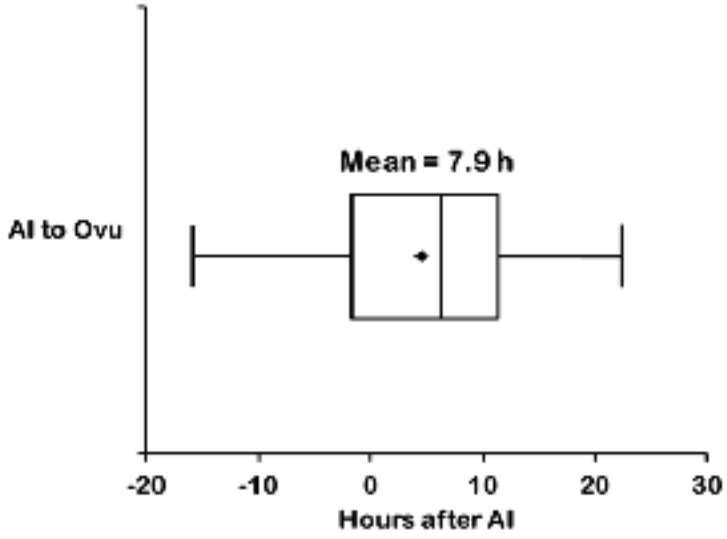

Figure 3. Whisker graph of the interval from AI to ovulation (n = 38 cows). AI was conducted twice daily based on cows detected in estrus by an accelerometer system (Heatime®). Ovulation was determined using transrectal ultrasonography conducted every 8 h from 48 to 96 h after induction of luteolysis. Cows were synchronized using an i.m. injection of GnRH (100 µg) followed 7 d later by induction of luteolysis using $PGF_{2\alpha}$ (25 mg).

Experiment 2 - Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity monitoring system.

A combined approach in which AI is based both on activity detected by an activity monitoring system followed by submission of cows not detected with activity to timed AI after synchronization of ovulation may be an effective and economical strategy to submit lactating dairy cows for first AI. A field trial was conducted to compare reproductive performance of lactating dairy cows managed for first AI using timed AI with or without detection of estrus using an activity monitoring system (Fricke et al., 2012). All cows were administered 2 i.m. injections of $PGF_{2\alpha}$ 14 days apart (Presynch) at 39 ± 3 and 53 ± 3 DIM to presynchronize their estrous cycles 12 days before submission to an Ovsynch protocol, and activity was monitored in all cows using an activity monitoring system (Heatime, SCR Engineers Ltd., Netanya, Israel) beginning at 24 ± 3 DIM. Cows in treatment 1 (n=333) with increased activity after the second $PGF_{2\alpha}$ injection were inseminated based on activity, whereas cows without increased activity were submitted to an Ovsynch protocol beginning 12 d after the second PGF2α injection of the presynchronization protocol and received a timed AI at 75 ± 3 days in milk. Cows in treatment 2 (n=331) with increased activity after the second $PGF_{2\alpha}$ injection were recorded by the activity monitoring system software but were not inseminated so that all cows in treatment 2 completed the Presynch-Ovsynch protocol and received a timed AI at 75 ± 3 days in milk regardless of whether or not they were detected with increased activity after the second $PGF_{2\alpha}$ injection.

Table 2. Effect of treatment on percentage of lactating Holstein cows with activity based on an activity monitoring system, and pregnancies per AI (P/AI) for cows with or without activity and

inseminated to activity (AI) or inseminated after synchronization of ovulation (TAI). Adapted from Fricke et al., 2012.

	Treatment	
Item	1	2
Cows with detected activity, % (n/n)	69 (230/335)	70 (232/331)
P/AI 35 d after AI, % (n/n)		
Cows with activity receiving AI	30[a] (68/230)	-
Cows with activity receiving TAI	-	41[b] (96/232)
Cows with no activity receiving TAI	36 (37/104)	35 (35/99)
Overall P/AI 35 d after AI, % (n/n)	32[c] (105/333)	40[d] (131/331)
P/AI 67 d after AI, % (n/n)		
Cows with activity receiving AI	27 (62/230)	-
Cows with activity receiving TAI	-	40 (92/232)
Cows with no activity receiving TAI	33 (34/104)	35 (35/99)
Overall P/AI 67 d after AI, % (n/n)	29[c] (96/333)	38[d] (127/331)

[a,b]Within a treatment by activity subgroup, statistical contrast differed (P = 0.004).
[c,d]Within a row, percentages with different superscripts differed (P = 0.0454).
[1]Treatments were: 1) cows inseminated based on an activity monitoring system after a presynchronization protocol with cows not detected with activity receiving TAI after synchronization of ovulation using an Ovsynch protocol; 2) cows receiving TAI after a Presynch-Ovsynch protocol.

The activity monitoring system detected increased activity in 69% and 70% of cows after the second $PGF_{2\alpha}$ injection in treatments 1 and 2, respectively (Table 2) which is about 10 to 15 percentage points greater than that reported in studies using tail chalk after the second $PGF_{2\alpha}$ injection of a Presynch-Ovsynch protocol (Stevenson and Phatak, 2005; Chebel and Santos, 2010). Overall, cows in treatment 1 in which inseminations occurred as a combination between AI to activity and timed AI had fewer P/AI compared to cows in treatment 2 in which all cows received timed AI after completing the Presynch-Ovsynch protocol (Table 2). The reduction in P/AI due to inseminating cows with increased activity after the second $PGF_{2\alpha}$ injection was expected because the increase in P/AI due to presynchronization with $PGF_{2\alpha}$ likely results from synchronizing estrus after the second $PGF_{2\alpha}$ injection (Navanukraw et al., 2004) so most cows initiate the Ovsynch protocol on days 5 to 9 of the ensuing estrous cycle thereby improving P/AI to timed AI (Vasconcelos et al., 1999). Inseminating 70% of cows based on activity after the second $PGF_{2\alpha}$ injection removed the presynchronized cows from the protocol thereby negating the increase in P/AI due to presynchronization. Cows without increased activity after the second $PGF_{2\alpha}$ injection and submitted to an Ovsynch protocol had P/AI of 33% and 35% for treatments 1 and 2, respectively (Table 2). Pregnancy outcomes of anovular cows subjected to an Ovsynch protocol is generally about 20% compared to about 35% for cycling cows starting an Ovsynch protocol at a random stage of the cycle (Gümen et al., 2003; Stevenson et al., 2008). Thus, cows without activity that received an Ovsynch protocol had a P/AI similar to that of cycling cows starting an Ovsynch protocol at a random stage of the cycle. Thus, aggressive submission of cows to an Ovsynch protocol after failing to be detected with increased activity is an effective management strategy to establish pregnancy in this subgroup of cows.

Overall, 31% of cows in treatment 1, and 100% of cows in treatment 2 were submitted to the Ovsynch portion of the synchronization protocol, and blood samples were collected from a subgroup (~85%) of cows in each treatment at the first GnRH injection of the Ovsynch protocol to determine progesterone concentration at the onset of the protocol (Fricke et al., 2012). Surprisingly, over 50% of these cows had progesterone concentrations ≥ 1 ng/mL at the first GnRH injection of the protocol, and similar results were observed for cows in treatment 2 that were not detected with activity after presynchronization. Thus, many cows without activity after presynchronization likely ovulated in the absence of detectable activity resulting in high progesterone at the first GnRH injection of the Ovsynch protocol. These results agree with the 10% of cows that ovulated but failed to be detected with activity by the activity monitoring system (Valenza et al., 2012; Table 1). Results from Fricke et al. (2012) support a management strategy in which the 30% of cows not detected with activity are aggressively submitted to an Ovsynch protocol rather than continuing to detect activity using an activity monitoring system.

Conclusion

A practical implication of these data is that only two thirds of the cows that were considered properly synchronized would have been inseminated based on the accelerometer system and would go on to ovulate after AI. The remaining cows either would not be inseminated because they were not detected in estrus or would not have a chance to conceive to AI because they would fail to ovulate after estrus. These data underscore the importance of implementing a comprehensive reproductive management program for identification and treatment of cows that would otherwise not be inseminated and to identify those cows failing to ovulate when cycling spontaneously. Based on data from the present experiment using this accelerometer system, the mean time of AI in relation to ovulation was acceptable for most of the cows detected in estrus; however, variability in the duration of estrus and timing of AI in relation to ovulation could lead to poor fertility in some cows.

References

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J. Dairy Sci. 89:4723-4735.

Chebel, R. C. and J. E. Santos. 2010. Effect of inseminating cows in estrus following a presynchronization protocol on reproductive and lactation performances. J. Dairy Sci. 93:4632-4643.

Dransfield, M. B., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

Fricke, P. M., A. Valenza, J. O. Giordano, M. C. Amundson, and G. Lopes Jr. 2012. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an accelerometer system. J. Dairy Sci. 95(Suppl. 2):74.

Gumen, A. J. N. Guenther, and M. C.Wiltbank. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J. Dairy Sci. 86:3184-3194.

Gwazdauskas, F. C., R. L. Nebel, D. J. Sprecher, W. D. Whittier, and M. L. McGilliard. 1990. Effectiveness of rump-mounted devices and androgenized females for detection of estrus in dairy cattle. J. Dairy Sci. 73:2965-2970.

Hawk, H. W. 1987. Transport and fate of spermatozoa after insemination of cattle. J. Dairy Sci. 70:1487-1503.

Holman, A., J. Thompson, J. E. Routly, J. Cameron, D. N. Jones, D. Grove-White, R. F. Smith, and H. Dobson. 2011. Comparison of estrus detection methods in dairy cattle. Vet. Rec. 169:47.
Hunter, R. H. F. 2003. The process of ovulation and sheeding of an oocyte. Pages 262-286 in Physiology of the graafian follicle and ovulation. Cambridge University Press.
Jónsson, R., M. Blanke, N. K. Poulsen, F. Caponetti, and S. Højsgaard. 2011. Estrus detection in dairy cows from activity and lying data using on-line individual models. Computers and Electronics in Agriculture 76:6-15.
López-Gatius, F., M. López-Béjar, M. Fenech, and R. H. F. Hunter. 2005. Ovulation failure and double ovulation in dairy cattle: risk factors and effects. Theriogenology 63:1298-1307.
Lyimo, Z. C., M. Nielen, W. Ouweltjes, T. A. Kruip, and F. J. van Eerdenburg. 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology 53:1783-1795.
Miller, R. H., H. D. Norman, M. T. Kuhn, J. S. Clay, and J. L. Hutchison. 2007. Voluntary waiting period and adoption of synchronized breeding in dairy herd improvement herds. J. Dairy Sci. 90:1594-1606.
Navanukraw, C., L. P. Reynolds, J. D. Kirsch, A. T. Grazul-Bilska, D. A. Redmer, and P. M. Fricke. 2004. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J. Dairy Sci. 87:1551-1557.
Palmer, M. A., G. Olmos, L. A. Boyle, and J. F. Mee. 2010. Estrus detection and estrus characteristics in housed and pastured Holstein-Friesian cows. Theriogenology 74:255-264.
Peralta, O. A., R. E. Pearson, and R. L. Nebel. 2005. Comparison of three estrus detection systems during summer in a large commercial dairy herd. Anim. Reprod. Sci. 87:59-72.
Ranasinghe, R. M., T. Nakao, K. Yamada, and K. Koike. 2010. Silent ovulation, based on walking activity and milk progesterone concentrations, in Holstein cows housed in a free-stall barn. Theriogenology 73:942-949.
Roelofs, J. B., E. G. Bouwman, S. J. Dieleman, F. J. Van Eerdenburg, L. M. Kaal-Lansbergen, N. M. Soede, and B. Kemp. 2004. Influence of repeated rectal ultrasound examinations on hormone profiles and behaviour around estrus and ovulation in dairy cattle. Theriogenology 62:1337-1352.
Roelofs, J. B., E. A. Graat, E. Mullaart, N. M. Soede, W. Voskamp-Harkema, and B. Kemp. 2006. Effects of insemination-ovulation interval on fertilization rates and embryo characteristics in dairy cattle. Theriogenology 66:2173-2181.
Roelofs, J. B., F. J. van Eerdenburg, N. M. Soede, and B. Kemp. 2005. Pedometer readings for estrous detection and as predictor for time of ovulation in dairy cattle. Theriogenology 64:1690-1703.
Stevenson, J. S., D. E. Tenhouse, R. L. Krisher, G. C. Lamb, C. R. Dahlen, J. R. Pursley, N. M. Bello, P. M. Fricke, M. C. Wiltbank, D. J. Brusveen, M. Burkhart, R. S. Youngquist, and H. A. Garverick. 2008. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901-2915.
Stevenson, J. S. and E. P. Call. 1983. Influence of early estrus, ovulation, and insemination on fertility in postpartum Holstein cows. Theriogenology 19:367-375.
Stevenson, J. S. and A. P. Phatak. 2005. Inseminations at estrus induced by presynchronization before application of synchronized estrus and ovulation. J. Dairy Sci. 88:399-405.
Sveberg, G., A. O. Refsdal, H. W. Erhard, E. Kommisrud, M. Aldrin, I. F. Tvete, F. Buckley, A. Waldmann, and E. Ropstad. 2011. Behavior of lactating Holstein-Friesian cows during spontaneous cycles of estrus. J. Dairy Sci. 94:1289-1301.
Thatcher, W. W. and C. J. Wilcox. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56:608-610.
Valenza, A., J. O. Giordano, G. Lopes Jr., L. Vincenti, M. C. Amundson, and P. M. Fricke. 2012. Assessment of an accelerometer system for detection of estrus and for treatment with GnRH at the time of insemination in lactating dairy cows. J. Dairy Sci. 95:7115-7127.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Walker, W. L., R. L. Nebel, and M. L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci. 79:1555-1561.

Wilmut, I. and R. H. F. Hunter. 1984. Sperm transport into the oviducts of heifers mated early in estrus. Reprod. Nut. and Dev. 24:461-468.

Xu, Z. Z., D. J. McKnight, R. Vishwanath, C. J. Pitt, and L. J. Burton. 1998. Estrus detection using radiotelemetry or visual observation and tail painting for dairy cows on pasture. J. Dairy Sci. 81:2890-2896.