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(Article begins on next page)

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Assessment of an accelerometer system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows

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ABSTRACT

Two experiments were conducted to evaluate an accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) to manage reproduction in lactating dairy cows. In experiment 1, lactating Holstein cows (n = 112) were fitted with an accelerometer system and were treated with GnRH followed 7 d later by PGF2αto synchronize estrus. A total of 89 cows that had a follicle >10 mm in diameter and a functional corpus luteum at the PGF2αinjection that regressed by 48 h after induction of luteolysis were included in the analysis. Overall, 71% of cows were detected in estrus by the accelerom-eter system and 95% of cows showing estrus ovulated within 7 d after induction of luteolysis. Of the cows not detected in estrus by the accelerometer system, 35% ovulated within 7 d after induction of luteolysis. Dura-tion of estrus activity (mean ± SD) was 16.1 ± 4.7 h and was neither affected by parity nor milk production. Intervals (means ± SD) from induction of luteolysis, onset of activity, peak raw activity, and peak weighted activity to ovulation was 82.2 ± 9.5, 28.7 ± 8.1, 20.4 ± 7.8, and 16.4 ± 7.4 h, respectively, and the interval from AI to ovulation was 7.9 ± 8.7 h, but ranged from −12 to 26 h. In experiment 2, cows were assigned ran-domly to receive an intramuscular injection of GnRH at artificial insemination (AI) after detection of estrus by the accelerometer system or receive no treatment (control). Nine hundred seventy-nine AI services from 461 cows were analyzed. Treatment with GnRH at AI did not affect fertility at 35 or 65 d after AI, and no interaction was detected between treatment and season or treatment and AI number. Overall, two-thirds of the cows that were considered properly synchronized were inseminated based on the accelerometer system and ovulated after AI. The remaining cows either were not inseminated because they were not detected in estrus or would not have had a chance to conceive to AI be-cause they failed to ovulate after estrus. Furthermore, mean time of AI in relation to ovulation determined by the accelerometer system was acceptable for most of the cows that displayed estrus; however, variability in the duration of estrus and timing of AI in relation to ovulation could lead to poor fertility in some cows. For lactating dairy cows detected in estrus by the ac-celerometer system, treatment with GnRH at the time of AI without reference to the onset of estrus did not increase fertility.

Key words:estrus detection , accelerometer , timing of ovulation , gonadotropin-releasing hormone at artificial insemination

INTRODUCTION

Despite the widespread adoption of hormonal syn-chronization protocols that allow for timed AI (TAI), detection of behavioral estrus continues to play an important role in the overall reproductive management program on most dairies in the United States (Cara-viello et al., 2006; Miller et al., 2007). Estrus detec-tion rates decreased from 50.9 and 59.6% in 1985 to 41.5 and 49.5% in 1999 for Southeastern US Holstein and Jersey dairy herds, respectively (Washburn et al., 2002), and are likely less than 50% on most US dairy farms (Senger, 1994). Some of the decrease in estrus-detection rates may be explained by attenuation of the duration of estrous behavior associated with increased milk production near the time of estrus (Lopez et al., 2004), few cows expressing standing

estrus (Lyimo et al., 2000; Roelofs et al., 2005; Palmer et al., 2010), si-lent ovulations (Thatcher and Wilcox, 1973; Palmer et al., 2010; Ranasinghe et al., 2010), and reduced expres-sion of estrous behavior of cows housed in confinement systems (Palmer et al., 2010). Whatever the cause, the poor efficiency of estrus detection not only increases time from calving to first AI but increases the aver-age interval between AI services (Stevenson and Call, 1983), thereby limiting the rate at which cows may become pregnant.Because of the effect 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 con-stant surveillance of behavior, including rump-mounted devices and androgenized females (Gwazdauskas et al., 1990), pedometry (Kiddy, 1977; Maatje et al., 1997; Peralta et al., 2005; Roelofs et al., 2005), and radiote-lemetry (Walker et al., 1996; Dransfield et al., 1998; Xu et al., 1998). New electronic systems that incorporate accelerometers as a means to associate increased physi-cal activity with estrous behavior in cattle (Holman et al., 2011; Jónsson et al., 2011) have been developed and marketed to the dairy industry. Although 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 studies have investigated accelerometers for such purposes.Treatment with GnRH at insemination continues to be used as a strategy to increase conception rates in dairy cattle after AI despite the large body of equivocal literature that exists on the efficacy of this treatment. Morgan and Lean (1993) conducted a meta-analysis of 40 experiments described in 27 published papers and concluded that although treatment at insemination with GnRH or GnRH analogs increased conception rates in dairy cattle, some variation in results were attributable to AI number at administration. One mechanism by which GnRH treatment at AI could potentially increase conception rate is by inducing ovulation in cows that would otherwise fail to ovulate after a spontaneous estrus. Ovulation failure after expression of estrus has been reported in dairy cattle (Sartori et al., 2004; López-Gatius et al., 2005a; Bloch et al., 2006) and con-tributes to poor fertility. Variability in the interval from the onset of estrus to ovulation resulting in delayed ovulation for some cows may also contribute to poor conception rates to AI (Bloch et al., 2006), and treat-ment with GnRH may reduce this variability, thereby increasing conception rate (Kaim et al., 2003). Despite the large body of literature published in this area, dairy farmers and veterinarians continue to inquire about the efficacy of GnRH treatment at the time of AI as a strategy to improve fertility.Two experiments were conducted to assess the use of accelerometers for reproductive management of lactat-ing dairy cows. The objectives of experiment 1 were to characterize (1) the percentage of cows detected in es-trus based on activity determined by an accelerometer system and the percentage of cows ovulating after syn-chronization of the estrous cycle and (2) time intervals between the induction of luteal regression and estrus, AI, and ovulation in lactating dairy cows. Experiment 2 was conducted to evaluate the effect of GnRH treat-ment at the time of AI after detection of estrus based on an accelerometer system on fertility. Our hypothesis for experiment 2 was that cows treated with GnRH at the time of AI would have increased fertility compared with untreated cows.

MATERIALS AND METHODS
Cows and Management for Experiments 1 and 2

Lactating Holstein cows from a commercial dairy farm located in southwestern Wisconsin milking ap-proximately 1,000 cows averaging approximately 41 kg of milk/d were used for these experiments. Experi-ment 1 was performed from August 2010 to June 2011, whereas experiment 2 was performed from June 2010 to January 2011. Cows were housed in freestall barns with ad libitum access to feed and water and were fed a TMR diet once daily that was formulated to meet or exceed NRC requirements (NRC, 2001) for high-producing lactating dairy cows. Cows were milked 3 times daily at approximately 8-h intervals, and milk weights were recorded at each milking and stored in the on-farm dairy management software (DairyComp 305; Valley Agricultural Software, Tulare, CA). All cows received injections of bovine somatotropin (500 mg, sometribove zinc; Elanco Animal Health Co., Indianapolis, IN) beginning 57 to 70 d postpartum and continuing every

14 d until dry-off. All procedures were approved by the Animal Care and Use Committee for the College of Agriculture and Life Sciences of the Uni-versity of Wisconsin-Madison.

Experiment 1: Synchronization of Estrus and Evaluation of Physiologic Parameters

One hundred twelve cows (47 primiparous and 65 multiparous) were enrolled in experiment 1. Each week, cohorts of 10 to 15 cows from 46 to 52 DIM were evalu-ated by transrectal ultrasonography using a portable scanner fitted with a 7.5-MHz linear-array transducer (Easi-Scan; BCF Technology Ltd., Livingston, UK) to determine uterine health and record ovarian structures. Cows without signs of uterine disease and at least 1 follicle ≥10 mm in diameter received an intramuscular injection of GnRH (100 µg of gonadorelin diacetate tet-rahydrate, Fertagyl; Intervet Animal Health, Millsboro, DE) followed by an intramuscular injection of PGF2α(25 mg of dinoprost tromethamine, Lutalyse; Pfizer Animal Health, New York, NY) 7 d later to synchro-nize estrus (Figure 1). Transrectal ultrasonography was performed at the time of the PGF2αinjection for sub-sequent determination of ovulatory response to GnRH treatment. Diameter of ovarian structures was estimat-ed 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 (CL) in the same location at the subsequent ultrasound examination at the time of the PGF2αinjection. Thereafter, ovarian ultrasonography was performed 48 h after the PGF2αinjection and then every 8 h until ovulation occurred or until 96 h, which-ever occurred first. Cows failing to ovulate by 96 h of the PGF2αinjection were reexamined 3 d later (i.e., 7 d after the PGF2αinjection) to determine whether ovulation had occurred.

Detection of Estrus and AI

At approximately 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 (ID) tag. After each milk-ing, 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 com-puter. All settings of the herd management software were based on those being used by the farm at the time of the experiment, and these settings were not changed throughout the course of the 2 experiments.The accelerometer system continuously monitored individual cow activity and recorded average activity for 2-h time periods throughout experiment 1. The raw activity of individual cows was plotted as a bar graph where each bar represented a 2-h block of time (Figure 2). Using a mathematical algorithm, a weighted activ-ity index was calculated by the software that expressed the momentary deviation of the activity from the aver-age activity in the same time period during the past 7 d (Bar, 2010), and weighted activity was represented on the activity report by a solid line (Figure 2). Graphs were generated for individual cows, and data regard-ing the timing of the onset of activity, peak raw and weighted activity, intensity of raw and weighted activ-ity, and the duration of activity were recorded for each estrus event. Onset of activity was defined as the time at which the first bar of raw activity of an estrus event was identified. Time to peak raw activity was defined as the time at which the maximum value for raw activ-ity was recorded. Time of peak weighted activity was defined as the time at which the maximum height of the weighted activity was observed. Raw and weighted peak intensity was recorded as the activity value re-corded for the time of maximum raw and weighted activity. Finally, duration of activity was defined as the time interval between the beginning and end of activity for an estrus event.Pressure-activated Heatmount detectors (Kamar Heatmount Detectors; Kamar Inc., Steamboat Springs, CO) were affixed midline to the rump between the tail head and the tuber coxae (hook bones) for all cows in experiment 1 as an additional means for detecting estrus. Heatmount detectors were affixed at the time of the PGF2αinjection of the synchronization protocol and were assessed 48 h after the PGF2αinjection, at each milking (i.e., every 8 h) from 48 h after the PGF2αinjection until activation or until 96 h (whichever oc-curred

first) and 7 d after the PGF2αinjection. The condition of the Heatmount detector was recorded as either activated (red) or nonactivated (white). No cows lost Heatmount detectors during the course of the ex-periment.Twice daily (a.m. and p.m.), a list of cows deter-mined 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 2 herd personnel with each cow receiving a single insemination based on activity.

Blood Collection and Progesterone Analysis
Blood samples for analysis of serum progesterone (P4) concentrations were collected via puncture of the median caudal vein or artery into evacuated tubes (Va-cutainer; BD, Franklin Lakes, NJ). Blood samples (~8 mL) were centrifuged (1,935 × gfor 20 min), and serum was collected and stored at −20°C until assayed for P4 concentrations using a solid-phase, no-extraction RIA (Coat-a-count; Diagnostic Products Corp., Los Ange-les, CA). Intra- and interassay coefficients of variation were 1.6 and 7.6%, respectively.Blood samples were collected at the time of the GnRH injection, the PGF2αinjection, and 48 h after the PGF2αinjection of the synchronization protocol (Figure 1). Blood samples collected at the time of the GnRH and PGF2αinjections were used in addition to transrectal ultrasonography to determine the ovulatory response to GnRH. Samples collected at the time of the PGF2αinjection also were used to determine the pres-ence of a functional CL. Blood samples collected 48 h after the PGF2αinjection were used to determine luteal regression in response to PGF2αtreatment. Cows were considered to have undergone luteal regression when P4 concentration in the sample collected at the time of the PGF2αinjection was ≥1 ng/mL and ≤1 ng/mL in the sample collected 48 h after the PGF2αinjection.

Experiment 2
Cows were eligible for AI after detection of estrus based on the accelerometer system when more than 50 DIM (voluntary waiting period). Cows were random-ized to 2 treatments based on their ear tag ID numbers. Cows with even ID numbers received an intramuscular injection of GnRH (100 μg of gonadorelin diacetate tetrahydrate, Fertagyl; Intervet Animal Health) at the time of AI, whereas cows with odd ID numbers re-mained untreated and served as controls. Nine hundred seventy-nine AI services from 461 cows (177 primipa-rous and 284 multiparous cows) were included in the analysis. Of the 979 AI services, 445 AI services were included in the GnRH treatment and 534 AI services were included in the control. Artificial insemination was performed twice daily based on activity detected by the accelerometer system as described for experiment 1.Official temperature data (Midwestern Climate Center, Champaign, IL) reported at a research station located 5 km from the farm (Lancaster, WI; latitude: 42.8475°N, longitude: 90.7106°W) was collected retro-spectively. The high temperature on the day of AI was used for the analysis of season on pregnancies per AI (P/AI). A daily high temperature >20°C on the day of AI was classified as having occurred during the warm season, whereas a daily high temperature ≤20°C on the day of AI was classified as having occurred during the cool season.Pregnancy DiagnosisPregnancy diagnosis for the evaluation of P/AI and pregnancy loss in experiment 2 was performed by the herd veterinarian 35 ± 3 and 65 ± 3 d after AI using transrectal ultrasonography (Aloka 500V with a 5.0-MHz linear array transducer; Corometrics Medical Sys-tems Inc., Wallingford, CT). Cows diagnosed pregnant 35 ± 3 d after AI and subsequently diagnosed nonpreg-nant at 65 ± 3 d were considered to have undergone pregnancy loss.

Data Management and Statistical AnalysesExperiment 1.
Data from 23 of the 112 cows en-rolled in the experiment were excluded from the data set for the following reasons: 2 cows were anovular (no CL detected and P4 concentrations <1.0 ng/mL at the time of the GnRH and PGF2αtreatment of the synchronization protocol), 9 cows with CL had luteal regression before the PGF2αinjection, 4 cows were detected in estrus by the accelerometer system and were inseminated during the synchronization protocol (i.e., between the GnRH and

PGF2αinjections), and 8 cows did not undergo complete luteal regression by 48 h after the PGF2αinjection. After elimination of data from these 23 cows, data from a total of 89 cows were included in the analysis. Analysis of the percentage of cows in specific groups (estrus vs. no estrus, and estrus-ovulation, estrus-no ovulation, no estrus-ovulation, and no estrus-no ovula-tion) was determined using PROC FREQ of SAS 9.2 (version 9.2; SAS Institute Inc., Cary, NC), whereas differences in the percentage of cows detected in estrus by the accelerometer system and the Heatmount detec-tors were determined by logistic regression using the events over trials option of PROC LOGISTIC of SAS. Analysis of the time intervals for the specific events under consideration (induction of luteolysis to onset of estrus activity, peak raw activity, peak weighted activ-ity, and ovulation, onset of activity, peak raw activity, and peak weighted activity to ovulation, induction of luteolysis to AI, onset of estrus activity to ovula-tion, and AI to ovulation) was performed using PROC MEANS of SAS. Effect of parity on duration of estrus activity as determined by the accelerometer system was analyzed by ANOVA using PROC GLM of SAS with a model that contained parity (primiparous vs. multiparous) as a categorical explanatory variable and milk production as a covariate. The relationship between duration of estrus and the onset of activity to ovulation interval and between milk production and peak intensity for weighted activity was determined by simple linear re-gression using PROC GLM of SAS. Mean milk produc-tion during the 7 consecutive days immediately preced-ing estrus but not including the day of estrus was used for these analyses. Experiment 2. The experimental design was a com-plete randomized block design with parity (primiparous vs. multiparous) as the blocking factor. Analyses of the binary response variables P/AI and pregnancy loss were performed by logistic regression using the GLIM-MIX procedure of SAS. For P/AI at 35 ± 3 and 65 ± 3 d after TAI, the initial model contained as fixed effects the following categorical explanatory variables: treatment (GnRH vs. control), parity (primiparous vs. multiparous), AI number (2, 3, 4, 5, or ≥ 6), and AI technician (1 vs. 2) as well as the interaction between all these variables. Given that the experimental unit was the AI service, all initial models contained both fixed and random effects. Fixed effects included in the initial models were the classification variables: treat-ment, parity, AI service number, AI technician, and their interactions, whereas cow was included as a ran-dom effect to account for any potential variation due to cows receiving multiple AI services. Based on a covariance parameter estimate test, the random effect of cow was removed from the model so that all final models contained only fixed effects. The covariance parameter test using the ZeroG option for the GLIMMIX procedure was used to evaluate if the matrix containing random effects could be reduced to zero. A covariance parameter test based on the residual pseudo-likelihood was run and a nonsignificant chi-squared P-value indicated that random effects could be eliminated from the model (SAS documentation for GLIMMIX, SAS 9.2). After removing random effects, selection of the fixed effects model that best fit the data for each variable of interest was performed by finding the model with the lowest value for the Akaike informa-tion criterion using a backward elimination procedure that removed all variables with $P > 0.10$ from the mod-el. The final model for analyzing P/AI 35 ± 3 and 65 ± 3 d after AI contained the fixed effects of treatment, parity, season, and the parity by season interaction. Treatment was forced to remain in all models. For analysis of pregnancy loss, the same categorical variables (except AI technician) and interactions used for P/AI were used to obtain the models for pregnancy loss from 35 ± 3 to 65 ± 3 d after AI. Procedures and criteria used for model selection were similar to those used for P/AI. The final model included treatment and parity. Both variables were forced in the model. A significant difference between the levels of a clas-sification variable was considered when $P < 0.05$, whereas differences between $P \geq 0.05$ and $P \leq 0.10$ were considered a statistical tendency. Data included in the text are presented as arithmetic means ($\pm$SD) and range when appropriate obtained using PROC MEANS of SAS or percentages obtained with PROC FREQ of SAS.

RESULTS AND DISCUSSION

Experiment 1 Estrus Activity and Ovulation.

The hormonal protocol consisting of GnRH followed 7 d later by PGF2α used to synchronize estrus in experiment 1 effec-tively synchronized the estrous cycles of 79% (89/112) of the cows enrolled that had a functional CL capable of responding to the PGF2α injection and a follicle ≥10 mm at the time of induction of luteolysis. Mean P4 concentration at the time of GnRH treatment was 2.4 ± 2.3 ng/mL (range 0.0 to 8.5), 4.4 ± 2.4 ng/mL (range 1.0 to 11.2) at the time of PGF2α treatment, and 0.2 ± 0.1 ng/mL (range 0.0 to 0.7) 48 h after PGF2α treat-ment. Ovulatory response to GnRH treatment was 74% (66/89). Because only synchronized cows were included in the analyses, we expected the majority of cows to be detected in estrus and ovulate within 7 d after the PGF2α injection. We chose to synchronize the estrous cycle of cows for experiment 1 so that groups of cows would be available for data collection on specific days of the week at the collaborating dairy. No physiological or behavioral differences have been reported between spontaneous and hormonally induced estruses in lactat-ing dairy cows (Walker et al., 1996; Roelofs et al., 2005; Bloch et al., 2006). The percentage of cows with estrus events detected by the accelerometer system and Heatmount detectors and the distribution of cows by occurrence of estrus and ovulation are presented in Table 1. Overall, the percentage of cows detected in estrus did not differ statistically (P= 0.52) between the accelerometer system and the Heatmount detectors (71 vs. 66%, re-spectively). Throughout the study period, 78% (69/89) of cows ovulated by 7 d after induction of luteolysis. Of the cows that ovulated, 59% (41/69) ovulated within 96 h, whereas 41% (28/69) 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 within 7 d of induction of luteolysis. Of the cows not detected in es-trus by the accelerometer system, 35% ovulated within 7 d of induction of luteolysis. The overall percentage of cows detected in estrus in this experiment is within the range reported for lactating dairy cows synchronized using a G-P protocol followed by visual observation of estrus (Stevenson et al., 1999). 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 3) 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). Duration of estrus observed in the present 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 (Ro-elofs et al., 2004). Conversely, duration of estrus activ-ity 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). Dis-crepancies between duration of estrus based on activ-ity 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. Because activity was only monitored during the 7-d period after the PGF2α injection of the synchroniza-tion protocol, it is possible that some cows would have showed estrus after data collection concluded. It was not possible to determine when cows failing to ovulate during the 7-d period after the PGF2α injection ovu-lated because these cows received an Ovsynch protocol and TAI at the end of the 7-d period. Nevertheless, we did not expect that approximately 30% of cows would fail to show estrus within 7 d after the PGF2α injection because a follicle >10 mm was present in all cows at the time of the PGF2α injection, and all cows had luteal regression in 48 h after PGF2α treatment. In another study in which cows received 2 sequential PGF2α 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 PGF2α injec-tion, leading the authors to conclude that issues other than cyclicity status affected efficiency and accuracy of estrus detection (Chebel and Santos, 2010). The per-centage of cows that failed to ovulate within the group of cows not detected in estrus was 65% (17/26) for the accelerometer system and 53% (16/30) for

Heatmount detectors, suggesting that estrus did not occur in these cows. The remaining 35 and 47% of ovulations in cows not detected in estrus may have been silent ovulations (ovulation without estrus), a phenomenon described in lactating dairy cows especially during the early post-partum period (Thatcher and Wilcox, 1973; Palmer et al., 2010; Ranasinghe et al., 2010).In the present experiment, 5% (3/63 for the acceler-ometer system) or 7% (4/59 for Heatmount detectors) of cows detected in estrus failed to ovulate within 7 d after induction of luteolysis. The overall rate of ovula-tion failure in lactating dairy cows that showed estrus behavior was 6.5% and was greater during the warm (12.4%) than during the cool (3.4%) season (López-Gatius et al., 2005a). This rate of ovulation failure represents a small percentage of the population of cows in this experiment and may have occurred because of failure in the mechanism triggering ovulation (i.e., no LH surge or insufficient LH secretion) or a lack of re-sponse by the dominant follicle to the LH surge.

Timing of Events After Induction of Lute-olysis for Cows Detected in Estrus with the Ac-celerometer System.

Time intervals for the events under consideration for all cows that ovulated by 7 d after induction of luteolysis with $PGF2\alpha$and those cows ovulating by 96 h after induction of luteolysis for cows detected in estrus by the accelerometer system are presented in Tables 2 and 3. Interval between induc-tion of luteolysis with $PGF2\alpha$and the onset of activity observed in the present experiment is within the range reported for cows synchronized using the same G-P protocol and twice daily visual observation of estrus after synchronization (Stevenson et al., 1999).In the present experiment in which ovarian ultraso-nography was performed every 8 h, the mean interval from onset of activity to ovulation of 28.7 h agrees with the reported mean interval of 27.6 h from the onset of standing activity to ovulation in lactating dairy cows detected with an electronic pressure-sensing system (Walker et al., 1996) or visual observation of estrus (Kaim et al., 2003; Roelofs et al., 2004; Bloch et al., 2006). Furthermore, the interval between the onset of activity to ovulation based on an accelerometer system in the present experiment is similar to the reported interval between an increase in walking activity mea-sured with pedometers and ovulation (~29 to 30 h) in lactating dairy cows (Roelofs et al., 2005; Roelofs et al., 2006). The wide degree of variation among cows observed for the interval from onset of activity to ovu-lation in this experiment (Figure 4) also agrees with that reported by others (Walker et al., 1996; Roelofs et al., 2005). This variability would contribute to the variation observed in the AI to ovulation interval ob-served in this experiment (Figure 5) and is a cause for concern because of potential errors in the timing of AI in relation to ovulation.The shorter mean time interval from peak raw activ-ity (20.4 h) and peak weighted activity (16.5 h) to ovu-lation suggests that both of these events occur after the onset of standing activity is initiated. Considering an average duration of estrus activity in this experiment of 16.8 h for cows that ovulated within 96 h of the $PGF2\alpha$injection, both peak raw and weighted activity as deter-mined by the accelerometer system are events close to the end of estrus in lactating dairy cows. In agreement with results from Experiment 1, Roelofs et al. (2004) reported that the time interval from the end of estrus to ovulation was 16.7 h when estrus was determined by visual observation whereas in another experiment in which estrus was determined by pedometer activity the interval from the end of estrus to ovulation was 19.4 h (Roelofs et al., 2005). Further research is needed to determine the exact relationship between the various measures of activity and standing estrus in lactating dairy cows.As a result of 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 detection of estrus. Mean interval from AI to ovulation of 7.9 h observed in the present ex-periment in which inseminations were conducted twice daily (a.m. and p.m.) after detection of estrus based on the accelerometer system is 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). In the present experiment, however, the degree of variation in the AI to ovulation interval (Figure 5) 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 fertil-ization 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 reduce the variation in the AI to ovulation interval so that more cows are inseminated at the optimal time in relation to ovula-tion. Alternatively, cows could be inseminated a few hours earlier to reduce the probability of inseminating cows after or near the time of ovulation.

Relationship Between Measures of Activ-ity with Ovulation Time and Milk Production.

A positive linear association ($P< 0.01$; $R^2= 33\%$) between duration of activity and the interval between the onset of activity and ovulation was observed in the present experiment. As duration of activity increased, the interval from the onset of activity to ovulation increased (Figure 6). Walker et al. (1996) reported a similar relationship between the duration of estrus defined as the time elapsed from the first to the last standing event of estrus and the interval from the onset of estrus to ovulation in lactating dairy cows. Based on these observations, Walker et al. (1996) suggested that during the early stages of estrus, the concentra-tion of circulating estradiol may have been sufficient to induce estrous behavior but inadequate to trigger the LH surge from the pituitary gland. In agreement with this idea, Bloch et al. (2006) observed that most of the variation in the estrus-to-ovulation interval in lactating dairy cows could be explained by the variation in the onset of estrus-to-LH-surge interval. Indeed, in cows with a very long estrus-to-ovulation interval (>36 h), the time from the onset of estrus to the LH surge was 9 h compared with <4 h for cows with normal estrus-to-ovulation intervals (Bloch et al., 2006). Interestingly, Bloch et al. (2006) also reported that cows with a very long estrus-to-ovulation interval had less estradiol dur-ing the follicular phase despite a similar size of the dominant follicle than cows with normal estrus-to-ovu-lation intervals. The low estradiol concentration could result from increased hepatic metabolism of steroids associated with high feed intake of lactating dairy cows (Sangsritavong et al., 2002) and could explain the un-coupling between the onset of estrus and the LH surge.A negative linear association between milk produc-tion during the 7 d preceding estrus activity and peak intensity for weighted activity ($P= 0.01$; $R^2= 16\%$) was observed. As milk production near the time of estrus increased, peak intensity of weighted activity decreased (Figure 7). In agreement with these data, López-Gatius et al. (2005b) reported a negative linear association between milk production and walking ac-tivity during estrus. Although estradiol concentrations were not measured in our experiment, we speculate that this negative association between milk produc-tion and estrus intensity could be explained, at least in part, by decreased estradiol during the follicular phase (Wiltbank et al., 2006) due to hepatic steroid metabolism (Sangsritavong et al., 2002). Indeed, posi-tive correlations between estrous behavior scores and estradiol concentrations near the time of estrus have been reported for lactating dairy cows (Lyimo et al., 2000; Roelofs et al., 2004). Further research is needed to clarify the relationship between estrus expression and milk production in dairy cows.

Experiment 2—Fertility of Cows Receiving GnRH at AI After Detection of Estrus Based on the Accelerometer System

Our hypothesis for experiment 2 was that treatment with GnRH at the time of AI would increase fertil-ity compared with untreated cows. In the present ex-periment, however, P/AI did not differ ($P= 0.40$) for GnRH (26.2%, 140/534) compared with control (22.7%, 101/445) cows at 35 ± 3 or 65 ± 3 d [21.8%, (97/445) vs. 24.9%, (133/354) respectively; $P= 0.48$] after AI. Our hypothesis was partially based on results from Morgan and Lean (1993) who conducted a meta-analysis of 40 experiments described in 27 published papers and con-cluded that treatment with GnRH or GnRH analogs at insemination increased the overall risk of pregnancy by 12.5% in treated cows. Treatment of cows and heifers 72 h after the second of 2 sequential injections of PGF2αincreased conception rate compared with saline-treated controls and induced a preovulatory LH surge in some of the cattle, but also decreased serum P4 concentrations in cattle receiving GnRH (Lucy and

Stevenson, 1986). One physiologic mechanism by which GnRH treatment at AI could increase fertility is by inducing cows to ovulate that would otherwise fail to ovulate after a spontaneous estrus. Indeed, 5% of synchronized cows in experiment 1 failed to ovulate after estrus (detected with the accelerometer system), a similar rate of ovula-tion failure reported by López-Gatius et al. (2005a). If the mechanism by which GnRH increases fertility is by reducing the percentage of cows with ovulation failure, perhaps there were too few cows experiencing ovulation failure to produce a treatment effect in experiment 2. In addition, Morgan and Lean (1993) reported that the risk of pregnancy tended to be greater when cows were treated with ≥250 μg of GnRH, whereas only 100 μg of GnRH was administered at AI in the present experi-ment. Despite the increase observed in GnRH-induced LH secretion in lactating dairy cows after doubling the dose of GnRH from 100 to 200 μg (Giordano et al., 2012), we speculate that insufficient LH secretion may not have been the limitation in experiment 2 because GnRH was given in a low progesterone environment (during estrus) when the LH response to a 100-μg dose of GnRH should be adequate to induce ovulation in most cows (Giordano et al., 2012).Another potential mechanism by which treatment with GnRH at the time of AI could improve fertil-ity of repeat breeder cows is an increase in circulat-ing progesterone after ovulation (Mee et al., 1993). In the present study, we did not attempt to measure progesterone concentrations after AI; however, it seems unlikely that treatment with GnRH may have affected fertility through increased progesterone, as fertility was not improved by treatment with GnRH at any service number.Morgan and Lean (1993) also reported that some variation in results among the studies in their meta-analysis were attributable to AI number at GnRH treatment with the increased risk of pregnancy be-ing greatest (22.5%) for repeat breeders, whereas the risk of pregnancy was similar for GnRH-treated cows to that of control cows at first service. In experiment 2, however, we detected no treatment by AI service number interaction ($P= 0.40$) on P/AI (Figure 8). Treatment of cows with GnRH at the time of AI is widely used in the dairy industry partly because it is easy and relatively inexpensive to administer GnRH to cows when they are identified and restrained for AI. It is likely, however, that treating cows with GnRH at AI is too late to have a physiologic effect on fertility. Treatment of cows and heifers 72 h after the second of 2 sequential injections of PGF2αincreased conception rate compared with saline-treated controls and induced a preovulatory LH surge in some but not all cattle (Lucy and Stevenson, 1986). Treatment with a GnRH analog within 3 h of the onset of estrus increased con-ception rates by more than 14 percentage points, and the effect of treatment was greatest during the summer, in cows with low body condition at AI, and in primipa-rous cows (Kaim et al., 2003). Furthermore, treatment with GnRH eliminated differences in conception rate for cows inseminated early or later relative to the onset of estrus and increased conception rate in cows with postpartum reproductive disorders (Kaim et al., 2003).Although no effect of GnRH treatment was observed in experiment 2, a season by parity interaction ($P< 0.01$) on P/AI was observed. This interaction indicated no difference in P/AI between parities during the cool season, but multiparous cows had fewer P/AI com-pared with primiparous cows during the warm season (Figure 9). We are not aware of other data that have reported such a differential effect between parities due to heat stress, and we cannot determine whether this is a physiologic effect or an effect related to this particu-lar farm. Finally, pregnancy loss from 35 ± 3 to 65 ± 3 d after TAI did not differ ($P= 0.70$) between GnRH and untreated control cows (4.0 vs. 5.0%, respectively), or between primiparous and multiparous cows (3.8 vs. 5.3%, respectively; $P= 0.59$).

CONCLUSIONS

A practical implication of the data from experiment 1 is that for lactating dairy cows under similar condi-tions, two-thirds of the cows that would be considered properly synchronized would be inseminated based on the accelerometer system and would ovulate after AI. The remaining cows either would not be inseminated because they are 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 reproduc-tive management program for identification

and treat-ment 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 the accelerometer system, the mean time of AI in relation to ovulation was acceptable for most of the cows detected in estrus; however, vari-ability in the duration of estrus and timing of AI in relation to ovulation could lead to poor fertility in some cows. In experiment 2, treatment with GnRH at the time of AI after estrus determined by the accelerometer system did not affect fertility in lactating dairy cows, and we detected no treatment by AI service number interaction. Contrary to some previous experiments, these data do not support the use of GnRH at the time of AI after detection of estrus using an accelerometer system as a strategy to improve fertility in lactating dairy cows. Future experiments should investigate the effect of GnRH administration relative to the onset of activity and as determined by accelerometer systems as a potential means to improve fertility in dairy cattle.

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Figure 1. Schematic diagram of activities for experiment 1. Cows (n = 112) from 46 to 52 d postpartum received a hormonal protocol to synchronize estrus using intramuscular 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 concentration. After eliminating 23 cows that failed to synchronize to the protocol, 89 cows were included in the analysis.
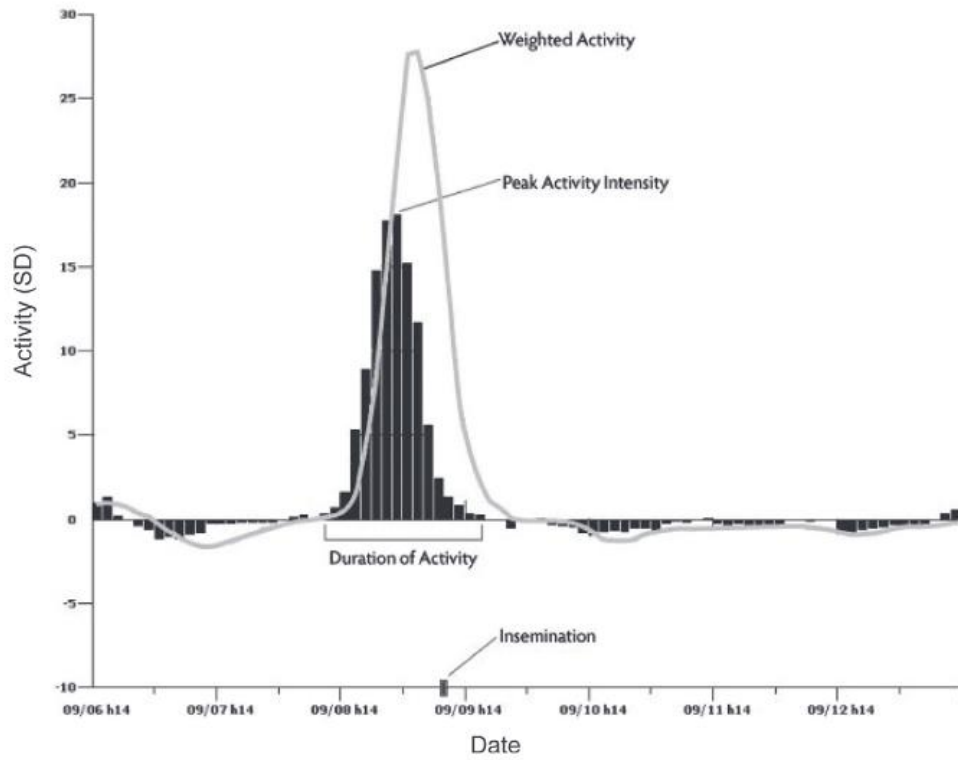
Figure 2. A representative graph of an activity report for a cow in experiment 1 generated by the Data Flow software of the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel). The accelerometer system continuously monitored individual cow activity and recorded average activity for 2-h time periods (bars) throughout the experiment. Activity data were analyzed by the system software using an algorithm that created a weighted activity value represented by a line from which timing of insemination was determined.
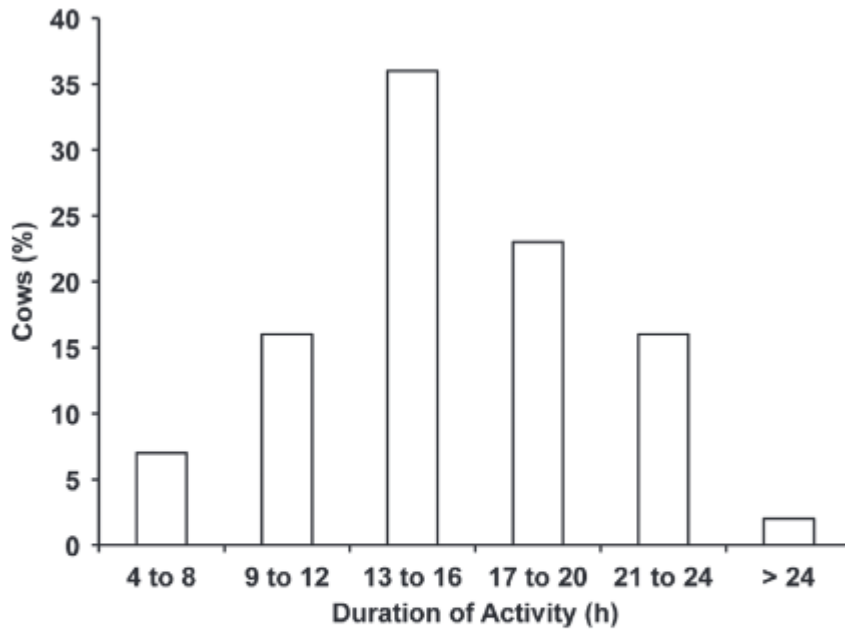
Figure 3. Percentage distribution of cows based on duration of activity associated with estrus for cows detected in estrus (n = 61; no duration data was available for 2 cows of the 63 cows detected in estrus) by the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) within 7 d after synchronization of estrus (experiment 1). Estrus was synchronized using an intramuscular injection of GnRH (100 μg) and induction of luteolysis using $PGF_{2\alpha}$ (25 mg) 7 d later.

Table 1. Percentage of cows defined to be in estrus, and distribution of cows by estrous activity and ovulation based on use of an accelerometer system or Heatmount detectors

Item	Accelerometer system[1] [% (no. of cows detected/total no. of cows)]	Heatmount detectors[2] [% (no. of cows detected/total no. of cows)]
Estrus[3]	71 (63/89)	66 (59/89)
Ovulation	95 (60/63)	93 (55/59)
No ovulation	5 (3/63)	7 (4/59)
No estrus	29 (26/89)	34 (30/89)
Ovulation	35 (9/26)	47 (14/30)
No ovulation	65 (17/26)	53 (16/30)

[1]Heatime (SCR Engineers Ltd., Netanya, Israel).

[2]Kamar Heatmount detectors (Kamar Inc., Steamboat Springs, CO).

[3]The percentage of cows detected in estrus within 7 d after treatment with GnRH followed 7 d later by $PGF_{2\alpha}$ to synchronize estrus in this experiment did not differ ($P = 0.52$) between the accelerometer system and the Heatmount detectors.

Table 2. Interval from induction of luteolysis with $PGF_{2\alpha}$ to onset of activity, peak of raw and weighted activity, and AI, and from the onset of activity to AI for cows[1] that ovulated within 7 d after induction of luteolysis and were detected in estrus based on activity determined by an accelerometer system[2]

Interval	n	Mean ± SD	Minimum	Maximum
Induction of luteolysis to onset of activity (h)	59	62.7 ± 18.0	30.0	128.0
Induction of luteolysis to peak raw activity (h)	59	71.2 ± 17.2	42.0	130.0
Induction of luteolysis to peak weighted activity (h)	60	74.0 ± 17.1	40.0	136.0
Induction of luteolysis to AI (h)	60	83.7 ± 18.0	49.0	146.0
Onset of activity to AI (h)	59	20.8 ± 7.0	11.0	47.0

[1]One cow was not included in these intervals because no data were available from the software system for the calculation of specific intervals.
[2]Heatime (SCR Engineers Ltd., Netanya, Israel).

Table 3. Interval from induction of luteolysis with $PGF_{2\alpha}$ to ovulation, onset of activity to ovulation, peak of raw and weighted activity to ovulation, and AI to ovulation for cows (n = 38) that ovulated within 96 h after induction of luteolysis and were detected in estrus based on activity determined by an accelerometer system[1]

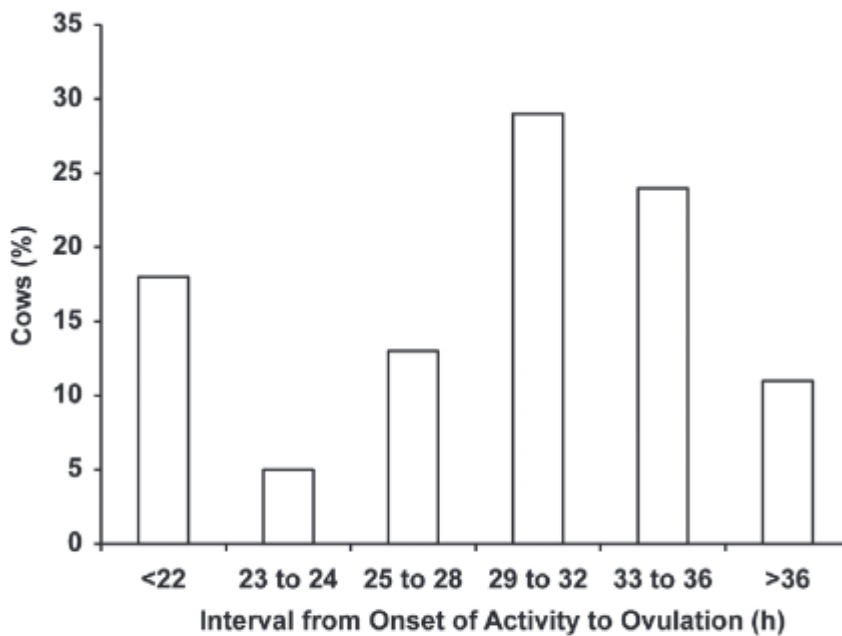| Interval	Mean ± SD	Minimum	Maximum
Induction of luteolysis to ovulation (h)	82.2 ± 9.5	60.0	92.0
Onset of activity to ovulation (h)	28.7 ± 8.1	6.0	42.0
Peak raw activity to ovulation (h)	20.4 ± 7.8	0.0	32.0
Peak weighted activity to ovulation (h)	16.4 ± 7.4	0.0	34.0
AI to ovulation (h)	7.9 ± 8.7	−12.0	26.0

[1]Heatime (SCR Engineers Ltd., Netanya, Israel).

Figure 4. Percentage distribution of cows (n = 38) based on interval from onset of activity detected using the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) to ovulation (experiment 1). Estrus was synchronized using an intramuscular injection of GnRH (100 µg) and induction of luteolysis using $PGF_{2\alpha}$ (25 mg) 7 d later.
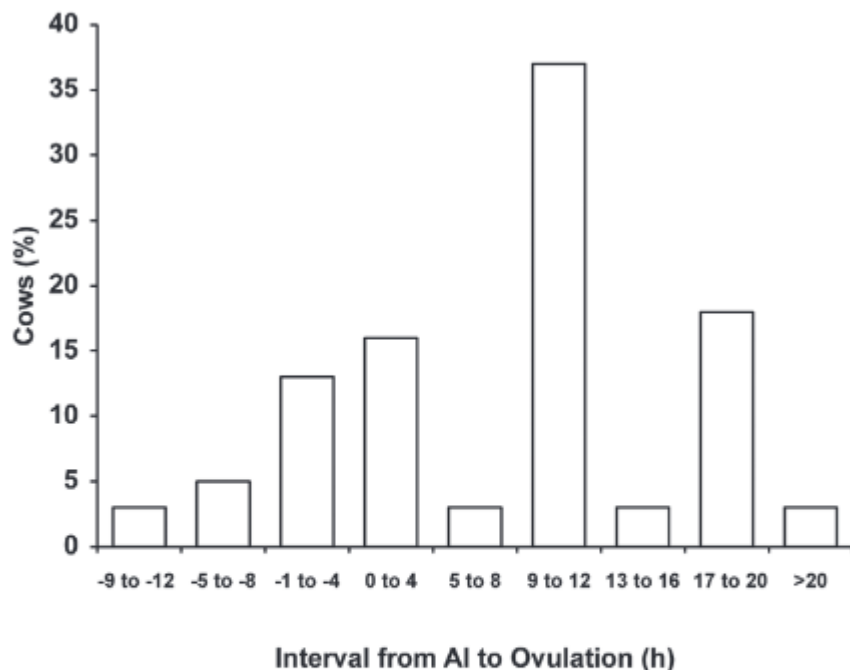
**Figure 5.** Percentage distribution of cows (n = 38) based on the interval from AI to ovulation. Artificial insemination was conducted twice daily based on detected estrus defined by the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel; experiment 1). Ovulation was determined using transrectal ultrasonography conducted every 8 h from 48 to 96 h after induction of luteolysis. Estrus was synchronized using an intramuscular injection of GnRH (100 μg) and induction of luteolysis using $PGF_{2\alpha}$ (25 mg) 7 d later.
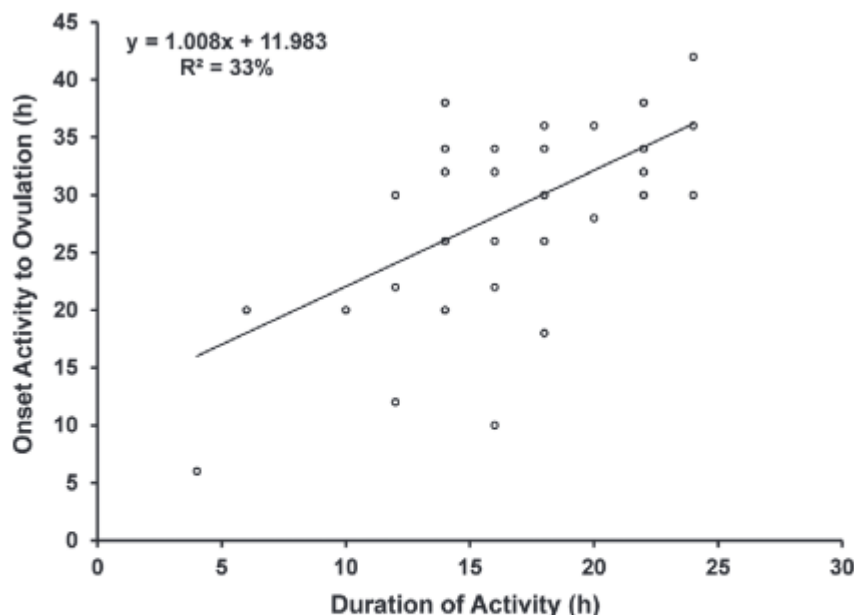
Figure 6. Relationship between duration of activity and the interval from onset of activity to ovulation for cows (n = 38) detected in estrus by the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) within 96 h after induction of luteolysis (experiment 1). Estrus was synchronized using an intramuscular injection of GnRH (100 μg) and induction of luteolysis using $PGF_{2\alpha}$ (25 mg) 7 d later.
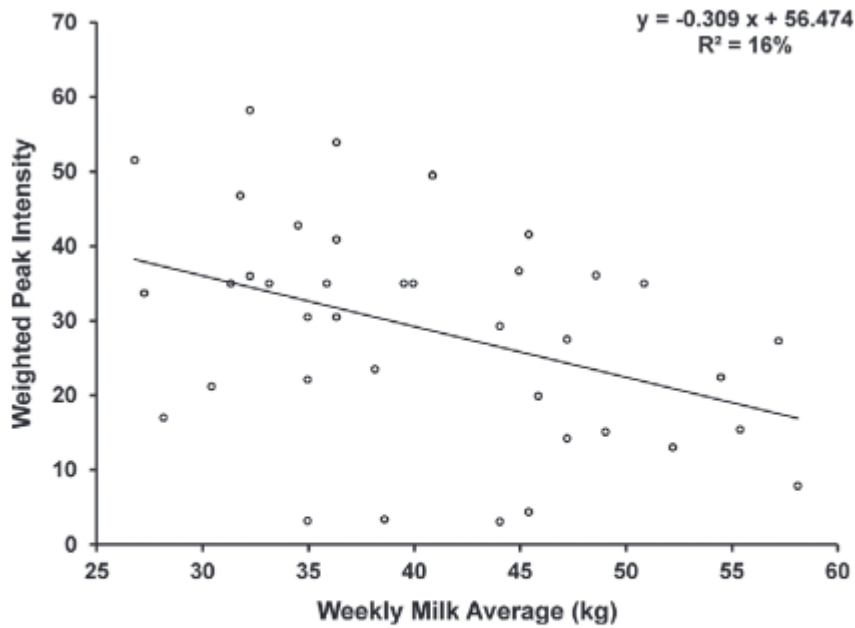
Figure 7. Relationship between milk production and weighted peak intensity recorded by the accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel; experiment 1). Milk production was calculated as the mean daily production during the 7-d period preceding estrus activity but not including the day of estrus. Estrus was synchronized using an intramuscular injection of GnRH (100 µg) and induction of luteolysis using $PGF_{2\alpha}$ (25 mg) 7 d later.
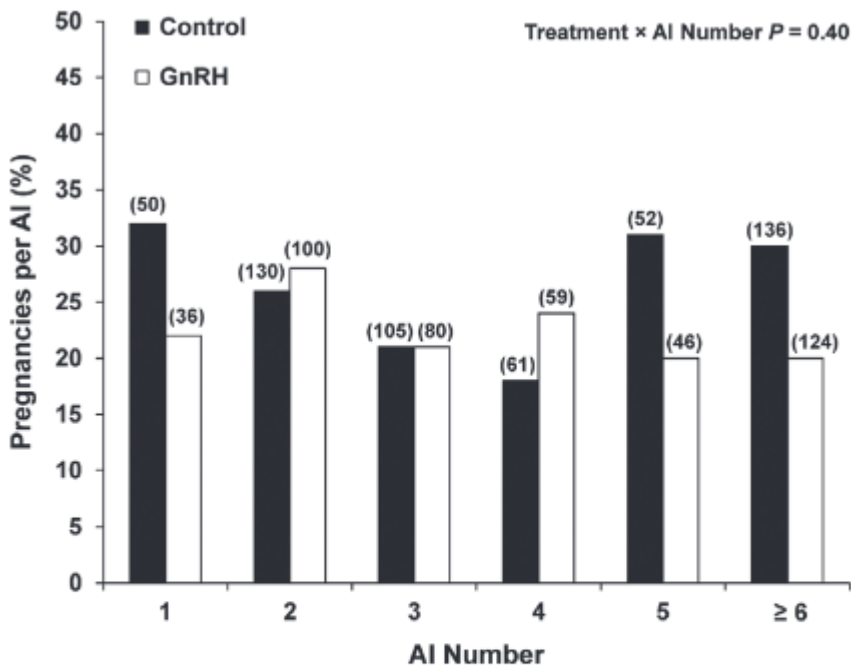

Figure 8. Effect of AI service number on pregnancies per AI for cows receiving GnRH (100 µg) or no treatment (control) at the time of AI after detection of estrus using an accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) in experiment 2. The numbers in parentheses above bars denote the number of cows in the control and GnRH treatments for each AI service number.
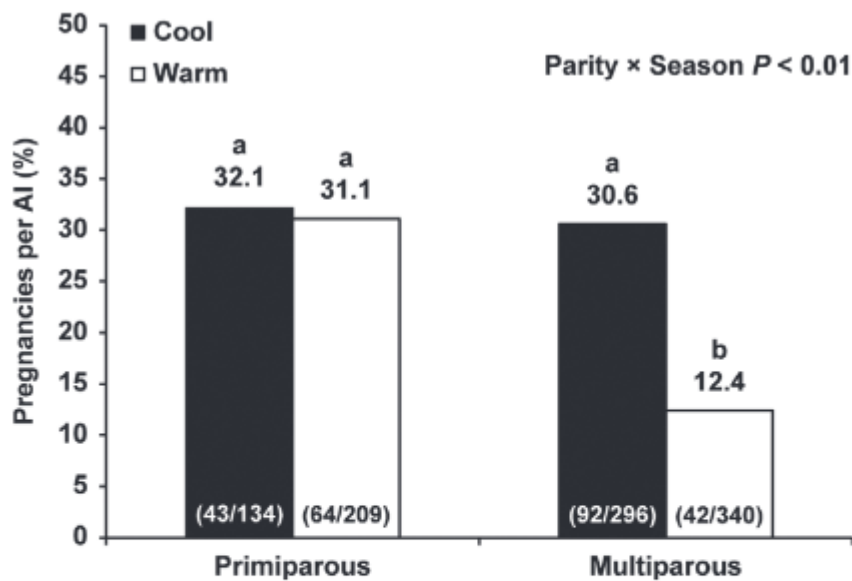
Figure 9. Pregnancies per AI for primiparous and multiparous cows inseminated after detection of estrus using an accelerometer system (Heatime; SCR Engineers Ltd., Netanya, Israel) during the cool versus the warm season of the year in experiment 2. A daily high temperature >20°C on the day of AI was classified as having occurred during the warm season, whereas a daily high temperature ≤20°C on the day of AI was classified as having occurred during the cool season. [a,b]Parity by season interaction ($P < 0.01$). Numbers above bars represent pregnancies per AI (%).