



Automated estrous detection using multiple commercial precision dairy monitoring technologies in synchronized dairy cows

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ABSTRACT

Estrus in dairy cattle varies in duration and intensity, highlighting the need for accurate and continuous monitoring to determine optimal breeding time. The objective of this study was to evaluate precision dairy monitoring technologies (PDMT) for detecting estrus. Estrus was synchronized in lactating Holstein cows ($n = 109$) using a modified G7G-Ovsynch protocol (last GnRH injection withheld to permit expression of estrus) beginning at 45 to 85 d in milk. Resumption of ovarian cyclicity at enrollment was verified by transrectal ultrasonography for presence of a corpus luteum. Cows were observed visually during 30 min (4 times per day) for behavioral estrus on d -1 to 2 (d 0 = day of estrus). Periods peri-estrus were defined by the temporal blood plasma progesterone patterns on d -5 , -4 , -3 , -2 , -1 , 0, 2, 4, 6, and 8. Estrous detection by PDMT, an estrous behavior scoring system, and by visual observation of standing estrus were compared with the reference (gold) standard. Only 56% of cows that ovulated were observed standing by visual observation. Sensitivity and specificity for estrous detection were not different among all PDMT. Devices in this study measuring activity in steps, neck movement, high activity of head movement, or a proprietary motion index increased on the day of estrus 69 to 170% from the baseline before estrus. The change in rumination time on the day of estrus decreased for both neck and ear-based technologies (-2 to -16%). Temperature of the reticulorumen, vagina, and ear skin were not different on the day of estrus than day peri-estrus. Daily lying times decreased on average to 24.6% on the day of estrus for IceQube (IceRobotics Ltd., Edinburgh,

Scotland). In contrast, lying time increased 15.5 and 33.1% for AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel) and Track a Cow (ENG Systems Innovative Dairy Solutions, Rosh Pina, Israel), respectively. All PDMT tested were capable of detecting estrus at least as effectively as visual observation. Four of the 6 PDMT that reported estrous alerts correctly detected 15 to 35% more cows than visual observation 4 times per day. Use of temporal progesterone patterns correctly identified more cows than visual observation alone. Dairy producers considering PDMT should focus on (1) the reference (gold) standard used to test efficacy of a device's alerts and (2) the device that will have the fewest false readings in their operations.

Key words: precision dairy monitoring, estrus, efficacy

INTRODUCTION

Resumption of the estrous cycle after calving is critical for dairy cattle fertility. In dairy cows, estrus is defined as the period of sexual receptivity during which a cow will accept mounting by a bull or another cow (Senger, 2005). Sexual receptivity is associated with behavioral changes that occur during a period of 3 to 16 h with varying intensity (Dransfield et al., 1998). Common methods used to detect these behavioral changes include visual observation without other distractions, tail painting or chalking, androgynous females, rump-based pressure or scratch-off patches, or creating sexually active groups with synchronization programs (Nebel and Jones, 2002). The inability of dairy farm personnel to detect estrus by visual observation is not a new problem (Esslemont, 1974; Barr, 1975; Senger, 1994). Lopez et al. (2004) found cows with greater milk production to have shorter durations of secondary estrous behaviors (6.2 ± 0.5 h vs. 10.9 ± 0.7 h) and standing estrus (6.3 ± 0.4 versus 8.8 ± 0.6) compared with cows with lesser milk production during 380 estrual periods. As milk production per cow continues to increase, decreased behavioral estrus and incidence of ovulation with no behavioral estrus increases (Fricke et al., 2014). The

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economic impact of poor visual detection of estrus and infertility continues to be an economic burden of \$360 per missed estrus (Lucy, 2001; De Vries, 2006). A potential solution to improve estrous detection rates is use of precision dairy monitoring technologies (PDMT).

Precision dairy monitoring technologies measure physiological, behavioral, or production variables of individual animals to improve management strategies and overall efficiency (Bewley, 2010). Precision dairy monitoring technologies are commonly used for estrous detection (Nebel et al., 2000) because of their ability to monitor and measure behavioral and physiological changes that typically occur during estrus 24 h per day, year round. Novel research beginning with Kiddy (1977), Esslemont et al. (1980), and Liu and Spahr (1993) used pedometers located on the rear legs to monitor activity at estrus and found an increase in activity. Dohi et al. (1993) found that pressure sensors located on the rump could be used for continuous monitoring of behavioral estrus. Trimberger's (1948) view for the need for continuous recordings of standing estrus is still used in research today. Quantifying behavioral and physiological variables with automated estrous detection improves estrus detection rates (Rorie et al., 2002; Michaelis et al., 2014; Stevenson et al., 2014) compared with visual observation. Completed research on commercial PDMT used for detecting estrus, however, is limited to one or a few systems in the same group of cows (reference was standing estrus) or varying sample sizes (Ginther et al., 2013; Rutten et al., 2013; Rorie et al., 2002). The reference (gold) standard used to determine efficacy of estrous alerts is often standing estrus, which only occurs in 30 to 80% of lactating dairy cows without timed AI (Fricke et al., 2014). Silent ovulating, cows that are in estrus but do not express behavioral estrus, can be detected in estrus. Sauls et al. (2017) synchronized cows to ovulate with only 62 to 77% detected in estrus by neck collar or automated rump pressure devices. Thus, using temporal progesterone patterns as a reference standard to confirm ovulation is necessary to accurately assess the efficacy of PDMT systems. Thus, the objectives of this study were to (1) determine the relationship of physiological and behavioral changes peri-estrus with generation of alerts for estrus by commercial PDMT and (2) evaluate the efficacy of estrous alerts generated by commercially available PDMT systems on the same cows. Our hypotheses were (1) the PDMT used would correctly identify more cows in estrus and not in estrus than visual observation and (2) variables measured by PDMT other than activity would undergo marked changes on the day of estrus.

MATERIALS AND METHODS

This study was performed with approval of the University of Kentucky Institutional Animal Care and Use Committee (Institutional Animal Care and Use Committee experiment number: 2013–1199).

Animals, Feeding, and Housing

Lactating Holstein cows ($n = 109$) at the University of Kentucky Coldstream Dairy (Lexington) were enrolled in the study between January 2014 and May 2015. Cows were enrolled in the experiment in groups of 6 to 10 cows between 45 to 85 DIM. Lactating cows were housed in 2 freestall pens and beds were covered with sawdust. The freestall pens had grooved concrete alleys with full roofs over the beds and partial roofs over the feedbunks. Cows had ad libitum access to water in each pen and shared a feedbunk between pens. Lactating cows were fed the same ration at 0600 and 1330 h daily. The lactating cow ration was balanced for level of milk production and cow size. Cows were milked 2× at 0430 and 1530 h.

Before and during the study, cows were balanced between pens by DIM and parity. Calving dates, breeding dates, and DIM were obtained from PCDART management software (Dairy Records Management Systems, Raleigh, NC). Parity ranged from 1 through 7. Mean cow parity was 1.99 ± 1.30 . The average milk yield of enrolled cows during the experiment was 37.7 ± 9.8 kg/d. Mean DIM at initiation of the synchronization protocol was 66.5 ± 11.4 . Mean DIM at estrus was 85.5 ± 11.4 .

A weather station (HOBO U23 Pro v2 External Temperature/Relative Humidity Data Logger U23–002, Onset, Bourne, MA) was located inside each freestall pen that measured relative humidity and temperature every 15 min. Temperature humidity index was computed using the following formula (NOAA, 1976): $THI = \text{temperature } (^{\circ}\text{F}) - [0.55 - (0.55 \times \text{relative humidity}/100)] \times [\text{temperature } (^{\circ}\text{F}) - 58.8]$.

Synchronization of Estrus

A modified G7G-Ovsynch (Figure 1; PGF_{2α}; 25 mg, Lutalyse, Zoetis US, New York, NY; GnRH: 100 µg, Cystorelin, Merial Limited, Duluth, GA) was used to synchronize cows into sexually active groups, making it easier to visually observe estrus in groups of 6 to 10 cows at a time. Fricke et al. (2014) reported a lack of declines in pregnancies per AI in cows that were

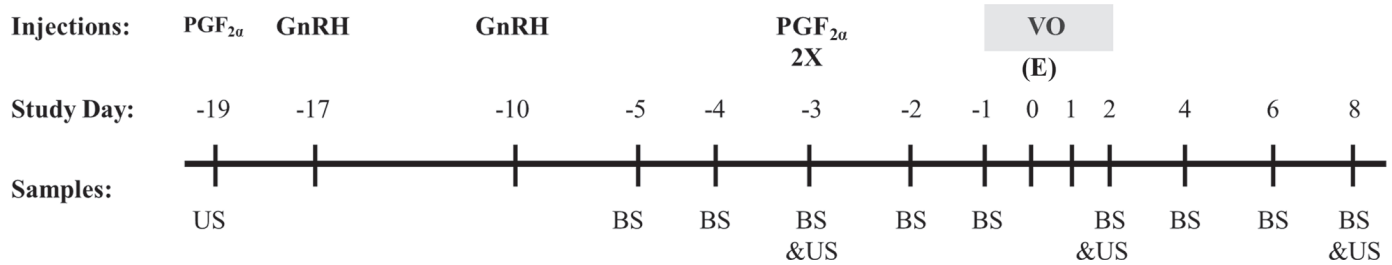


Figure 1. Experimental design employed (28 d) to assess the efficacy of 8 commercial precision dairy monitoring technologies (PDMT) during synchronized estrus in cows ($n = 109$) for visual observation of estrus and verification of ovulation and estrus with temporal patterns in plasma progesterone ($n = 109$). AfiAct Pedometer Plus (AfiMilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, the Netherlands); Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg by GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems LLC, Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton (Embedded Data Systems, Lawrenceburg, KY); and Track a Cow (ENG Systems Innovative Dairy Solutions, Rosh Pina, Israel). The last GnRH injection of a traditional Ovsynch experiment was withheld to permit expression of estrus. Visual observation (VO) for estrous behaviors occurred during a 4-d period (d -1 to 2; d 0 = estrus after the final PGF_{2α}) 4 times a day (0330, 1000, 1430, and 2200 h) for 30 min during each observation period. Mode day of estrus (E) based on reference (gold) standard of either temporal progesterone or standing estrus; estrus = d 0. Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/mL on d -5, -4, and -3, then <1.0 ng/mL on d -1, and >1.0 ng/mL on d 6 and 8). The PGF_{2α} was administered twice on d -3, 6 h apart at 0800 and 1400 h. Transrectal ultrasonography (US) was performed at 0800 h to verify resumption of ovarian cyclicity at enrollment (d -19), presence of a corpus luteum (CL) on the day of the final injection (designated experimental d -3), regression of the CL by d 2, and presence of a new CL on d 8. Blood samples (BS) were collected at 0800 h to obtain plasma for progesterone RIA.

synchronized for estrus compared with those without synchronization.

Ultrasound and Sampling

Transrectal ultrasonography was performed on d -19, -3, 2, and 8 (d 0 = day of estrus) in the experiment using an Ibex Pro Portable Ultrasound (E.I. Medical Imaging, Loveland, CO). Resumption of estrous cycles at enrollment was verified by the presence of a corpus luteum (CL) on experiment d -19. On the day of PGF_{2α} injection (designated experiment -3), presence of a CL and a preovulatory follicle verified cyclicity and response to synchronization. Regression of the CL and ovulation of the preovulatory follicle were recorded on d 5. Presence of a new CL on d 11 verified ovulation and served as the reference standard for ovulation in comparison with estrous detection.

Blood samples were collected by coccygeal venipuncture from the median caudal vein or artery into a Monoject tube containing 100 μ L of a 15% solution of EDTA (K3; Covidien, Minneapolis, MN) on d -5, -4, -3, -2, -1, 0, 2, 4, 6, and 8 (d 0 = day of estrus) to quantify plasma progesterone at the University of Kentucky for verification of luteal regression and ovulation (Figure 1). Potential periods of estrus before ovulation were defined by the temporal progesterone pattern (>1.0 ng/mL on d -5, -4, and -3, and then <1.0 ng/mL on d -1 and >1.0 ng/mL on d 6 and 8). Temporal progesterone patterns were the primary standard used to compare the efficacy of automated

estrous detection systems and variables measured on the day of estrus. Cows that met the requirements for progesterone concentrations on the designated experiment day were classified as positive for having ovulated. Cows that failed to ovulate according to progesterone concentrations, less than 1.0 ng/mL, on d 2, 6, or 8 were classified as negative for ovulation. Cows with progesterone concentrations less than 1.0 ng/mL on d -5 or -4 and progesterone concentrations greater than 1.0 ng/mL on d 6 or 8 were confirmed positive for ovulation. Transrectal ultrasonography results were only used in the final analyses for final verification of ovulation on d 6 or 8 when cows were expected to have a new CL after ovulation.

Automated Estrus-Detection Systems

Each cow was equipped with AfiAct Pedometer Plus (AfiMilk, Kibbutz Afikim, Israel), CowScout S Leg [Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg by GEA Farm Technologies GmbH, Bönen, Germany)], DVM Bolus (DVM Systems LLC, Greeley, CO), HR Tag (SCR Engineers Ltd., Netanya, Israel), CowManager SensOor (Agis Automatisering, Harmelen, the Netherlands), IceQube (IceRobotics Ltd., Edinburgh, Scotland), and Track a Cow (ENG Systems Innovative Dairy Solutions, Rosh Pina, Israel) devices (Table 1) before study enrollment to allow for a 2-wk adjustment period. First-calf heifers were equipped with all devices at least 10 to 14 d before their predicted calving date. Devices were

placed and attached according to recommendations of each company (Table 1). Leg devices were placed on the same leg for each technology for every cow. The DVM boluses were inserted into the reticulorumen orally, using a bolus gun. Ear tags were positioned using an ear tagger provided by each technology company to fit the respective device. Thermochron iButtons (Embedded Data Systems, Lawrenceburg, KY) were placed in an intravaginal device (CIDR, Zoetis US, New York, NY) lacking progesterone supplement, inserted into cows 7 d before the final injection of PGF_{2α} to measure vaginal temperature at estrus. It is important to note that all devices used in this study have since had updated versions come out on the market.

The AfiMilk Milking Point Controller (AfiMilk) was used to collect individual milk yield and milking time for each milking. Body weights were recorded by AfiWeigh (AfiMilk) placed in a common exit alley. Cows were sorted into their respective groups using AfiSort (AfiMilk) after each milking.

All computer clocks were set to synchronize with NIST Internet Time Service (National Institute of Standards and Technology, Gaithersburg, MD) automatically, and time was checked on all computers manually on a weekly basis. Raw data, including measurements and recordings of behavioral and physiological variables, and alerts for estrus generated by each PDMT software program were downloaded daily. Company default settings for report and alert generation for each system were used during the study. Proprietary algorithms and individual animal thresholds for each system were used to generate estrous alerts. All systems were used with the default settings recommended by each manufacturer.

Visual Observation for Estrus

Cows were observed for estrus during 30 min 4 times daily (0330, 1000, 1430, and 2200 h) or until all cows stood to be mounted on d −1 through 2). Breeding

Table 1. Commercial precision dairy monitoring technologies (PDMT) evaluated in addition to visual detection (4× daily) for estrous detection^{1,2} efficacy and consistent variable measurements in lactating Holstein cows (n = 109)

PDMT	Location on animal	Variables measured	Year purchased	Measurement frequency	Data reporting frequency
AfiAct Pedometer Plus, (AfiMilk, Kibbutz Afikim, Israel)	Right rear leg	Activity (steps) Lying time (min) Lying bouts	2012	Continuously	End of milking
CowManager SensoOr, (Agis Automatisering, Harmelen, the Netherlands)	Left ear	Rumination time (min) Eating time (min) Time not active (min) Time active (min) Time high active (min)	2013	Every minute	Every hour
Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg, GEA Farm Technologies GmbH, Bönen, Germany)	Left front leg	Activity (steps)	2013	Continuously	15-min intervals
HR Tag, (SCR Engineers Ltd., Netanya, Israel)	Neck	Neck activity Rumination time (min)	2011	Continuously	Every 2 h
IceQube (IceRobotics Ltd., Edinburgh, Scotland)	Left rear leg	Lying time (min) Steps Motion index Lying bouts Bout duration (min)	2013	Continuously	15-min intervals
Track a Cow (ENG Systems Innovative Dairy Solutions, Rosh Pina, Israel)	Right front leg	Activity unit Lying time (min) Lying bouts Bout duration (min) Time spent at feed bunk	2013	Continuously	Every 5 min
AfiMilk MPC Analyzer (AfiMilk, Kibbutz Afikim, Israel)	Not applicable	Milk yield (kg) Milk flow Milk conductivity	2012	Each milking	End of each milking
DVM bolus (DVM Systems LLC, Greeley, CO)	Reticulorumen	Reticulorumen temperature (°C)	2013	Every 5 min	Hourly
Thermochron iButton (Embedded Data Systems, Lawrenceburg, KY)	Vagina	Temperature ³	2013	Every 5 min	Every 5 min

¹PDMT with estrous alerts: AfiAct Pedometer Plus, CowManager SensoOr, CowScout S Leg, HR Tag, IceQube, and Track a Cow were used in assessing efficacy of systems for automated estrous detection. The PDMT devices are randomly assigned numbers for result reporting.

²Estrus was verified by the temporal patterns of plasma progesterone indicating ovulation and estrus (reference standard).

³Thermochron iButtons were attached to an intravaginal device to continuously collect vaginal temperature a week before and a week after estrus in cows.

decisions were left up to the herd manager, but most frequently insemination events were based on standing estrus. Pregnancies per AI were not considered in this study because of insufficient power for reproductive management studies. In replicate 12, inclement weather prevented observers from watching cows for estrus during 3 periods. Because all cows displayed other behaviors related to estrus, including sniffing and chin resting, during the 1000 h period the day before the snow storm, these cows were included in the final analyses.

Pen lights were turned on for the 0330 and 2200 h observation periods and turned off at the end of each observation period. Cows were adjusted to this routine before the study started to avoid differences in routine behavior. Cows were released to a dirt exercise lot for each pen separately for 1 h each day during the 1000 h observation period.

Cows were identified with neck strap digits and numbers spray-painted on each side of the body. The Van Eerdenburg et al. (1996) scoring scale for visual observations of estrus, including modifications used by Roelofs et al. (2005), was used to quantify intensity of estrus. Behaviors of estrus were assigned the following points: 100 for standing to be mounted, 45 for front mounting, 35 for attempting to mount other cows, 15 for chin resting, 10 for mounted but not standing, 10 for vulva sniffing, 5 for restlessness, and 3 for flehmen response. When a cow reached a score of 100 points, the animal was considered in estrus. Once a cow received greater than or equal to 100 points, instead of 2 consecutive visual detection periods required, the cow was defined as being in estrus. One observer per pen watched for behaviors during each observation period. Each observer (trained before first observation session) recorded behaviors by hand and recorded all standing heat times using a satellite powered watch (WV58A-1AV Atomic Digital Watch, Casio, Shibuya, Tokyo, Japan) synchronized with the PDMT system computers.

Data Handling of PDMT System Alerts

Each PDMT system software, except Thermochron iButtons and DVM boluses, generated alerts for both (1) cows that should be inseminated and (2) cows that were suspected of being in estrus. Cows identified for insemination were those that met the numerical threshold of a specific variable or variables as specified in the PDMT system software. Thresholds or alert requirements are typically regarded as confidential and proprietary by the PDMT system manufacturers. If alerts were classified other than those mentioned, the company clarified which reports identified cows in es-

trus ready for insemination. Manufacturers of PDMT systems specified which report and alerts to use before final analysis.

Potential estrous periods (reference standard) were defined using the temporal pattern of progesterone (>1.0 ng/mL on d -5, -4, and -3, then <1.0 ng/mL on d -1 and >1.0 ng/mL on d 6 and 8). Cows that ovulated according to the temporal pattern of progesterone were considered to have been in estrus regardless of visual observation. Estrous alerts were categorized by the comparison of the alert provided by each PDMT system to the reference standard. Temporal progesterone patterns indicative of ovulation verified estrus. The performance of PDMT were assessed with reference standards (1) temporal progesterone patterns to confirm ovulation, and (2) standing estrus independently to confirm detection of estrus. True positives (TP) were estrous alerts generated for cows that were confirmed in estrus. False positives (FP) were estrous alerts generated for cows confirmed not in estrus. True negatives (TN) were estrous alerts not generated for cows confirmed not in estrus. False negatives (FN) were estrous alerts not generated for cows that were confirmed in estrus.

Statistical Analyses

Working devices were defined as those devices having 80% (19 of 24 h) of raw data for the day before estrus and day of estrus for the variables identified for each device by the respective company as necessary for estrous alerts. Data from broken devices and devices those with less than 80% of raw data for the days before and after day of estrus were removed before final analysis of estrous alerts. Cows missing data 7 d before and after the day of estrus were removed for the final analysis of relative change in the variables of interest.

Automated Estrus-Detection System Alerts. The FREQUENCY procedure of SAS 9.3 (SAS Institute Inc., Cary, NC) was used to determine the frequency of TP, FP, TN, and FN for each PDMT system alerts. Sensitivity, the proportion of cows that ovulated or were in estrus that correctly gave a PDMT system alert for estrus, was calculated by $TP/(TP + FN) \times 100$. Specificity, the proportion of cows that did not ovulate and correctly did not give a PDMT system alert for estrus, was calculated by $TN/(TN + FP) \times 100$. Accuracy, the proportion of cows that were correctly identified in estrus or not in estrus, was calculated by $(TP + TN)/(TP + TN + FP + FN) \times 100$. The positive predicted value, the proportion of cows with an alert and that were in estrus or ovulated, was calculated by $TP/(TP + FP) \times 100$. The negative predictive value,

the proportion of cows that correctly did not give a PDMT system alert for estrus, was calculated by $TN / (TN + FN) \times 100$.

Variable Changes for Estrus vs. Non-Estrus.

The MIXED procedure of SAS 9.3 (SAS Institute Inc.) was used to analyze the repeated measure cow, independent effect of ($\alpha = 0.05$) estrus state with 26 variables recorded by 8 PDMT devices. Independent variables were removed by manual backward stepwise elimination if their estimate was considered statistically nonsignificant ($P > 0.05$). Parity, daily or peri-estrus milk yield, season, and BCS were found to be nonsignificant so they were not used in the final model. Cow was a repeated measure nested within estrus-status. The final models were

$$Y_{ijk} = \mu + P_4 \text{ estrus-state}_i + \text{Cow}_{j(i)} + \varepsilon_{ij(k)},$$

$$Y_{ijk} = \mu + \text{St estrus-state}_i + \text{Cow}_{j(i)} + \varepsilon_{ij(k)},$$

where Y_{ijk} is the k th observation from the j th cow nested within the i th estrus-state based on temporal progesterone (P_4) or standing estrus (St), μ is the intercept, and $\varepsilon_{ij(k)}$ is the residual error.

Variable Percentage Changes at Estrus. Based on temporal progesterone patterns and ultrasound exams, cows that were not in estrus and did not ovulate were removed from the final analysis. The final analysis removed cows with less than 80% (19 h of 24 h) of raw data for the day before estrus and day of estrus for the variables recorded by each device. The EXPAND procedure of SAS 9.3 was used to create a baseline using the backward moving average of the 7 d before the day of estrus for 26 variables measured by all PDMT devices. The percentage change in each variable on the day of estrus and each experimental day compared with the 7-d baseline was calculated as follows:

$$\begin{aligned} & \frac{(\text{experimental day measurement} \\ & - \text{baseline measurement}) / \text{baseline measurement} \times 100, \\ & \frac{(\text{estrus day measurement} - \text{baseline measurement}) / \\ & \text{baseline measurement} \times 100. \end{aligned}$$

The UNIVARIATE procedure of SAS 9.3 was used to eliminate the 1st and 99th percentile outliers of percentage change for each PDMT variable. Fifty cows had data for the 7-d baseline and day of estrus for all PDMT; cows without missing data were used to evaluate changes in variables from PDMT.

RESULTS AND DISCUSSION

All cows were included in the analysis classified by progesterone pattern and standing events. Ninety-four (86.2%) of the 109 cows had a temporal progesterone pattern indicative of ovulation during the presumptive period of estrus. The remaining 15 cows did not follow the same pattern and were classified as negatives. Only 51 of the 109 cows (47%) stood to be mounted during visual observation. The mean number of steps for the duration of the experiment was $3,827 \pm 2,902$ (AfiAct Pedometer Plus; mean \pm SD), $4,410 \pm 1,815$ (Nedap Realtime Leg), $2,270 \pm 991$ (Track a Cow), and $1,138 \pm 613$ (IceQube). The mean measure of neck movements was 415 ± 137 (HR Tag), time of head movement was 52 ± 40 min/d (CowManager SensOor), and motion index was 51 ± 241 (IceQube). Mean lying time was 9.1 ± 2.8 h/d (IceQube), 9.4 ± 3.0 h/d (Track a Cow), and 8.9 ± 2.9 h/d (AfiAct Pedometer Plus). Mean lying bouts per day were 16.2 ± 7.5 (IceQube) and 10.7 ± 5.6 per d (Track a Cow). Mean rumination time (h/d) was 7.8 ± 1.4 (HR Tag; neck based) and 9.2 ± 1.9 (CowManager SensOor; ear based). The mean hours per day eating time was 3.5 ± 1.6 (CowManager SensOor). Mean vaginal, ear skin, and reticulorumen temperature were $39.0 \pm 0.47^\circ\text{C}$, $22.2 \pm 6.6^\circ\text{C}$, and $39.02 \pm 0.4^\circ\text{C}$, respectively.

Precision Dairy Monitoring Technology Alerts for Estrus

The variables measured by PDMT devices quantified activity, rumination, feeding, and lying behaviors and temperature. The results from this study provide confirmation that 4 of the 6 commercial estrus-detection devices had greater sensitivity (correctly detecting animals in estrus) than visual observations (Table 2). When temporal progesterone patterns only were used as the reference standard in comparison with standing estrus as the reference standard, lesser FP increased the specificity, but greater FN decreased the sensitivity. If only standing estrus events were used as a reference standard, the number of FP increased (Table 3). The difference in sensitivity and specificity between reference standards is most likely due to not all animals in estrus displaying standing estrus.

The goal of continuous monitoring with automated systems is to detect animals in estrus to predict ovulation time. Predictors of ovulation time should have high sensitivity (89%) for detecting estrual behaviors by 18 h before ovulation (Trimberger, 1948). Intervals between estrous detection, insemination, and ovula-

Table 2. Commercial precision dairy monitoring technologies¹ (PDMT) evaluated for estrous alert and visual observation² efficacy³ with a reference standard of temporal plasma progesterone patterns⁴ for ovulation in partially synchronized⁵ Holstein cows (n = 109)

Detection method	TP	FP	TN	FN	Total no. of cows ⁶	Sn (%)	Sp (%)	Acc (%)	PPV (%)	NPV (%)
AfiAct Pedometer	76	2	13	18	109	80.9	86.7	81.7	97.4	41.9
CowScout S Leg	72	0	14	21	107	77.4	100.0	80.4	100.0	40.0
IceQube	45	2	10	34	91	57.0	83.3	60.4	95.7	22.7
HR Tag	33	1	11	46	91	41.8	91.7	48.4	97.1	19.3
CowManager	51	0	8	6	65	89.5	100.0	90.8	100.0	57.1
Track a Cow	35	1	10	15	61	70.0	90.9	73.8	97.2	40.0
Standing	51	0	15	43	109	54.3	100.0	60.6	100.0	25.9
Behavioral score ⁷	62	0	15	32	109	66.0	100.0	70.6	100.0	31.9

¹AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, the Netherlands); Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg by GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube (IceRobotics Ltd., Edinburgh, Scotland); Track a Cow (ENGs Systems Innovative Dairy Solutions, Rosh Pina, Israel).

²Visual observation for estrous behaviors occurred during a 4-d period (d -1 to 2; d 0 = estrus after the final PGF_{2α}) for 4 times a day (0330, 1000, 1430, and 2200 h) for 30 min during each observation period.

³Sensitivity (Sn) = TP/(TP + FN) × 100, specificity (Sp) = TN/(TN + FP) × 100, accuracy (Acc) = (TP + TN)/(TP + TN + FP + FN) × 100, positive predictive value (PPV) = TP/(TP + FP) × 100, and negative predictive value (NPV) = TN/(TN + FN) × 100; where TP = true positive, TN = true negative, FP = false positive, and FN = false negative.

⁴Periods of estrus were defined by the temporal pattern of progesterone (reference standard; >1.0 ng/mL on d -5, -4, and -3 then <1.0 ng/mL on d -1 and >1.0 ng/mL on d 6 and 8). Blood samples were taken at 0800 h for measurement of progesterone in blood plasma by radioimmunoassay.

⁵The last GnRH injection of a traditional Ovsynch experiment (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the experiment.

⁶Lactating Holstein cows (n = 109) 45 to 85 DIM were enrolled in the study. However, only cows who had functioning devices are reflected for each device. Devices were considered broken for cows with less than 80% (19/24 h) of raw data for the day before estrus and day of estrus for the variables identified by each company as necessary for estrus alerts.

⁷Scoring system as defined by van Eerdenburg et al. (1996) and modified to detect a cow in estrus once total points for one observation period ≥ 100 points.

tion are often longer when using visual observation for estrous detection. The first observed standing heat is often noted as the onset of estrus. Standing estrus is not expressed by all animals and thus is not the best standard for estrous detection (Roelofs et al., 2004). Synchronization programs combined with automated

Table 3. Commercial precision dairy monitoring technologies¹ (PDMT) evaluated for estrous alert efficacy² with a reference standard of standing estrus³ partially synchronized⁴ Holstein cows (n = 109)

Detection method	TP	FP	TN	FN	Total no. of cows ⁵	Sn (%)	Sp (%)	Acc (%)	PPV (%)	NPV (%)
AfiAct Pedometer	47	31	27	4	109	92.2	46.6	67.9	60.3	87.1
CowScout S Leg	47	25	31	4	107	92.2	55.4	72.9	65.3	88.6
IceQube	29	18	32	12	91	70.7	64.0	67.0	61.7	72.7
HR Tag	24	10	33	24	91	50.0	76.7	62.6	70.6	57.9
CowManager	30	21	14	0	65	100.0	40.0	67.7	58.8	100.0
Track a Cow	22	14	18	7	61	75.9	56.3	65.6	61.1	72.0

¹AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensoOr (Agis Automatisering, Harmelen, the Netherlands); Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg by GEA Farm Technologies GmbH, Bönen, Germany); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube (IceRobotics Ltd., Edinburgh, Scotland); Track a Cow (ENGs Systems Innovative Dairy Solutions, Rosh Pina, Israel).

²Sensitivity (Sn) = TP/(TP + FN) × 100, specificity (Sp) = TN/(TN + FP) × 100, accuracy (Acc) = (TP + TN)/(TP + TN + FP + FN) × 100, positive predictive value (PPV) = TP/(TP + FP) × 100, and negative predictive value (NPV) = TN/(TN + FN) × 100, where TP = true positive, TN = true negative, FP = false positive, and FN = false negative.

³Visual observation for estrous behaviors occurred during a 4-d period (d -1 to 2; d 0 = estrus after the final PGF_{2α}) for 4 times a day (0330, 1000, 1430, and 2200 h) for 30 min during each observation period.

⁴The last GnRH injection of a traditional Ovsynch experiment (Pursley et al., 1995) was withheld to permit expression of estrus. A presynchronization of G7G (Bello et al., 2006) was initiated when cows were enrolled into the experiment.

⁵Lactating Holstein cows (n = 109) 45 to 85 DIM were enrolled in the study. However, only cows who had functioning devices are reflected for each device. Devices were considered broken for cows with less than 80% (19/24 h) of raw data for the day before estrus and day of estrus for the variables identified by each company as necessary for estrus alerts.

estrous detection have also been explored (Neves et al., 2012; Stevenson et al., 2014; Dolecheck et al., 2016). Fricke et al. (2014) reported using hormones for initial grouping and then using automated estrous detection with a collar-based system or using automated estrous detection then hormonal intervention for problem cows yielded similar days open and conception rates. Using a stochastic dynamic simulation model, automated estrous detection was generally profitable (\$3,509 for a herd of 130 animals), but it was assumed that an increase in sensitivity of estrous detection was achieved (Rutten et al., 2013). To increase the sensitivity of estrous alerts, baseline behavior should be measured at least a week before accurate comparisons of estrous alerts. Duration of behavioral estrus is often associated with duration of increased number of steps or activity compared with the baseline number of steps or activity in dairy cows (Silper et al., 2015).

Variable Percent Changes at Estrus

Activity. Activity monitoring is the most commonly tested automated estrous detection system used commercially (Firk et al., 2002; Van Eerdenburg, 2008; Stevenson et al., 2014). Alerts based on activity measurements have been shown to improve reproductive performance, even in detecting up to 54% of estrous events without standing mounts (Galon, 2010). Previous research reported a significant ($P < 0.05$) increase in walking activity on the day of estrus compared with a baseline with pedometers or accelerometers (Liu and Spahr, 1993; Roelofs et al., 2005; Michaelis et al., 2014).

The percentage change in steps (Table 4) on the day of estrus was greater ($P < 0.01$) in estrual cows compared with non-estrual cows recorded by AfiAct Pedometer Plus ($73.2 \pm 7.9\%$), CowScout S Leg ($13.4 \pm 1.4\%$), and Track a Cow ($80.1 \pm 10.2\%$). The percentage change in proprietary measures of activity on the day of estrus was also greater ($P < 0.05$) in estrual cows compared with non-estrual cows: motion index ($76.3 \pm 11.9\%$), high activity ($230 \pm 25.1\%$), and neck activity ($50.0 \pm 5.9\%$) recorded by IceQube, SensOor, and HR Tag, respectively. Devices in this study measuring activity in steps, neck movement, high activity of head movement, or a proprietary motion index increased on the day of estrus 69 to 170% from the baseline 7 d before estrus. Activity outside of the 1 to 2 d peri-estrus was consistent indicating that a reliable baseline was established for devices before estrus. The current study shows similar increases in activity for all PDMT devices. López-Gatius et al. (2005) reported an increase of 75 to 500% in activity on the day of estrus in 5,883 services on 2 commercial dairy farms. López-Gatius et

al. (2005) calculated the increase using day of estrus number of steps divided by the threshold determined by the AfiAct system, which differs from percentage change calculated in the current study. However, regardless of the PDMT used, our study reported large increases in activity.

Rumination and Eating Time. Percentage change in rumination time on the day of estrus was lesser ($P < 0.01$) in estrual cows compared with non-estrual cows recorded by SensOor (ear based, $-20.2 \pm 2.3\%$), but not by the HR Tag (Table 4). Percentage change in eating time on the day of estrus was greater in estrual cows ($P < 0.001$) compared with non-estrual cows measured by SensOor ($62.6 \pm 7.8\%$). We speculate that the SensOor device may not be doing a good job distinguishing between eating behavior and head activity during estrus. The number of visits to the feedbunk did not differ between estrual and non-estrual cows (Table 4). We speculate that there was an increase in feedbunk visits due to high activity (e.g., pacing and mounting 4.6 m from the feedbunk rather than actual eating at the feedbunk). Both devices also recorded an average 22% increase in rumination the day after estrus compared with the day of estrus ($P < 0.05$). Rumination time measured by the SCR HR Tag is commonly used in research for disease (Soriani et al., 2012; Stangaferro et al., 2016), DMI (Clément et al., 2014), and recently estrus (Kamphuis et al., 2012; Reith and Hoy, 2012; Elischer et al., 2013). Reith and Hoy (2012) reported 5.92 h spent ruminating, with an average 17% decrease on the day of estrus. The current study reports only a 4.22% decrease in daily rumination time. This may be due to substantial cow to cow variation previously reported by Reith and Hoy (2012), with the change in rumination time -71 to $+16\%$. Rumination time decreased in 94% of 265 estrus events and decreased as much as 247 min per d on the day of estrus (Reith and Hoy, 2012). Published studies for CowManager SensOor estrous detection do not yet exist. However, recent validations (Borchers et al., 2016) show moderate to strong correlations ($r = 0.69$ to 0.93 ; CowManager SensOor and SmartBow, respectively) between rumination time with visual observation of rumination time ($r = 0.93$; Bikker et al., 2014).

Temperature. The average vaginal temperature percentage change of estrual cows on the day of estrus was greater compared with non-estrual cows ($P < 0.01$) recorded by iButtons ($0.3 \pm 0.1\%$) embedded in blank CIDR. The maximum vaginal temperature percentage change of estrual cows on the day of estrus had a tendency to be greater ($P = 0.09$) compared with non-estrual cows recorded by iButtons ($0.6 \pm 0.1\%$). The ear skin temperature and reticulorumen temperature

percentage changes were not different between estrual and non-estrual cows on the day of estrus (Table 4). An unexplained decrease in ear skin temperature 4 d before estrus may be due to placement of the CowManager SensOor or removal of the tag due to animals rubbing the tag against wood or metal. Cows often lost these ear tags due to the plastic material easily breaking on metal bars and cow brushes, and thus were replaced within the week before observation of estrus. The difference in mean temperatures recorded by CowManager SensOor and the other devices recorded may be because of ambient temperature or inconsistent placement of the ear tag.

Dairy cow core body temperature is often in agreement with other temperatures including the reticulorumen (Bewley et al., 2008; Rose-Dye et al., 2011), ear skin (Redden et al., 1993), and vagina (Redden et al.,

1993). Suthar et al. (2013) found a strong relationship ($r = 0.92$, $P < 0.001$; 2013) with vaginal temperature and estrus using progesterone concentration as a standard. Detecting estrus and predicting ovulation with estrual rises in temperature is not a novel concept (Wrenn et al., 1958). Wrenn et al. (1958) and Redden et al. (1993) reported a 1.0 to 1.6°C decrease in vaginal temperatures the day before estrus and a similar increase the day of estrus and the day after ovulation.

McArthur et al. (1992) found that milk temperature increased $0.4 \pm 0.1^\circ\text{C}$ at estrus. Temperatures were highly variable, however, and temperature increases lasted for short periods with a mean increased time of 9 h. Gil et al. (1997) reported a strong correlation between increased rectal temperature and increased milk temperature ($r = 0.90$) in 78.9% of 38 silent ovulations based on visual observation of standing to be mounted.

Table 4. Differences in commercial precision dairy monitoring technology¹ (PDMT) variables, activity, and lying behavior, between Holstein cows ($n = 109$) in estrus² and not in estrus² on the predicted day of synchronized estrus

Variable	PDMT ¹	Total no. of cows ³	Mean % change \pm SE ⁴		P-value
			Estrus	Nonestrus	
No. of steps	AfiAct Pedometer Plus	109	73.2 \pm 7.9	1.0 \pm 18.6	<0.001
No. of steps	CowScout S Leg	87	13.4 \pm 1.4	-0.6 \pm 3.4	<0.001
No. of steps	Track a Cow	75	80.1 \pm 10.2	2.5 \pm 24.0	<0.01
Motion index	IceQube	86	76.3 \pm 11.9	0.5 \pm 23.3	<0.01
Active time	SensoOr	50	25.6 \pm 6.0	-10.4 \pm 13.3	0.02
High activity	SensoOr	50	230.0 \pm 25.1	-1.1 \pm 55.6	<0.001
Neck activity	HR Tag	55	50.0 \pm 5.9	11.4 \pm 14.6	0.02
Lying time	AfiAct Pedometer Plus	109	-18.0 \pm 4.7	2.7 \pm 11.1	0.09
Lying bouts	AfiAct Pedometer Plus	109	-12.3 \pm 7.7	-1.2 \pm 18.3	0.58
Lying time	IceQube	86	-12.0 \pm 3.6	5.9 \pm 7.0	0.03
Lying bouts	IceQube	86	-9.1 \pm 3.7	-0.6 \pm 7.3	0.30
Bout duration	IceQube	86	-11.0 \pm 3.7	6.9 \pm 7.2	0.03
Time not active	SensoOr	50	-29.6 \pm 4.2	-6.8 \pm 9.2	0.03
Lying time	Track a Cow	70	-18.8 \pm 3.7	1.7 \pm 8.4	0.03
Lying bouts	Track a Cow	69	19.4 \pm 5.3	-0.9 \pm 12.0	0.13
Rumination time	SensoOr	50	-20.2 \pm 2.3	-3.6 \pm 5.1	<0.01
Rumination time	HR Tag	55	-2.5 \pm 3.8	10.5 \pm 9.3	0.20
Eating time	SensoOr	50	62.6 \pm 7.8	-13.7 \pm 17.2	<0.001
Feedbunk visits	Track a Cow	50	56.4 \pm 17.3	3.7 \pm 35.9	0.19
Mean vaginal temperature	Thermochron iButton	76	0.3 \pm 0.1	-0.1 \pm 0.1	<0.01
Maximum vaginal temperature	Thermochron iButton	76	0.6 \pm 0.1	0.1 \pm 0.3	0.09
Ear skin temperature	SensoOr	45	10.3 \pm 3.0	18.2 \pm 6.1	0.25
Reticulorumen temperature	DVM bolus	47	0.4 \pm 0.1	0.5 \pm 0.2	0.46
Milk yield	Afimilk MPC Analyzer	109	-3.8 \pm 1.2	1.0 \pm 2.8	0.12

¹AfiAct Pedometer Plus (Afimilk, Kibbutz Afikim, Israel); CowManager SensOor (Agis Automatisering, Harmelen, the Netherlands); Nedap Realtime Leg (Nedap Livestock Management, the Netherlands, marketed as CowScout S Leg by GEA Farm Technologies GmbH, Bönen, Germany); DVM bolus (DVM Systems LLC, Greeley, CO); HR Tag (SCR Engineers Ltd., Netanya, Israel); IceQube (IceRobotics Ltd., Edinburgh, Scotland); Thermochron iButton (Embedded Data Systems, Lawrenceburg, KY); Track a Cow (ENG Systems Innovative Dairy Solutions, Rosh Pina, Israel).

²Potential periods of estrus (reference standard) were defined by the temporal pattern of progesterone (>1.0 ng/mL on d -5, -4, and -3, then <1.0 ng/mL on d -1 and >1.0 ng/mL on d 6 and 8). Progesterone was measured in blood plasma by RIA.

³Lactating Holstein cows ($n = 109$) 45 to 85 DIM were enrolled in the study. However, only cows with functional devices $>80\%$ (19 of 24 h) of the time 1 d before the day of suspected estrus were included.

⁴Percentage change was determined for each day of the experiment using a backward 7-d average not including the experiment day then calculated by [(experiment day measurement - baseline measurement)/baseline measurement] \times 100 for the day of observed standing mount for cows in estrus. The second day of visual observation served as the day of estrus for calculation purposes for cows that did not display estrus..

Vaginal, ear skin (Redden et al., 1993), and tympanic (Scott et al., 1983) temperatures increased at estrus. The effectiveness, determined by comparisons to visual observation, of these variables to generate an alert for estrus were like visual observation, but all PDMT had 1 to 2 FP when temporal progesterone was used as the reference standard.

Lying Time and Bouts. The percentage change in lying time (Table 4) on the day of estrus was less ($P < 0.05$) in estrual cows compared with non-estrual cows that stayed the same in lying time recorded by AfiAct Pedometer Plus ($-18 \pm 4.7\%$; $P = 0.09$), IceQube ($-12.0\% \pm 3.6\%$), and Track a Cow ($-18.8\% \pm 3.7\%$). The percentage change in lying bouts was not different between the estrual and non-estrual cows on the day of estrus (Table 4). The percentage change of the IceQube bout duration was lesser ($P < 0.05$) in estrual cows compared with non-estrual cows. The percentage change in time not active recorded by Track a Cow was lesser in estrual cows ($-29.6\% \pm 4.2\%$; $P < 0.05$). These differences may be due to different definitions of a day. The AfiAct Pedometer Plus only reported data twice daily when the cows entered the parlor for milking, giving a sum of approximately 12 h between parlor visits. The remaining devices reported data hourly. A day was defined as the time periods of 1200 to 2400 h instead of 0500 to 1700 h for the AfiAct Pedometer Plus. Further research is necessary for changes in all variables by hour surrounding observed estrus. Lying time alone in algorithms for estrous detection resulted in 50% sensitivity (McGowan et al., 2007). In contrast, when combined with number of steps, sensitivity was 88.9% with 20 cows (Silper et al., 2017). Time not active includes time standing still or null head movement. Time not active recorded by CowManager SensOor and IceQube decreased on the day of estrus (Table 4). Lying bouts decreased 9 and 11% on the day of estrus measured by IceQube and AfiAct, respectively (Table 4) and lying bout duration was 11% shorter measured by IceQube. Lying bouts increased 19% on the day of estrus, however, measured by Track a Cow. We do not know why an increase occurred on the day of estrus for this PDMT. An increase in lying bouts may be explained by proprietary algorithms to determine what behavior pattern counts as a lying bout. Overall, lying behaviors may be helpful as historic information for future alert generation indicating estrus because lying behavior decreases the day after estrus (Rorie et al., 2002). Secondary behaviors, including feeding behaviors and lying time, change on the day of estrus and day after estrus. Lying time typically decreased by about 10% on the day of estrus and increased by 20% the day following estrus in previous studies (McGowan et

al., 2007). Even though lying time, lying bouts, rumination, eating time, feedbunk visits, and temperature results in the current study were not strong variables as independent predictors of estrus, other studies have shown success generating new algorithms using these PDMT sources of data. Silper et al. (2015) reported a strong relationship between day of estrus activity and standing or lying time on d 2 or 7 before estrus ($r = 0.52$ and 0.66 , respectively; $P < 0.001$).

CONCLUSIONS

Four of the 6 PDMT that reported estrous alerts correctly detected 15 to 35% more cows than visual observation. Another important take-home message is that the reference standard of temporal progesterone patterns is better than using standing estrus because a large proportion of cows ovulated without standing estrus. Silent ovulation is still a challenge for automated estrous detection systems that rely on behavior changes. Sensitivity previously reported for automated estrous detection systems may be greater because standing estrus was used as the reference standard, and hence verification of ovulation may be a more objective reference standard for future studies. Most measures of activity increased on the day of estrus in agreement with previous literature. Independently, rumination and eating time are predictors of estrus in dairy cows. Temperature tended to change on the day of estrus but requires constant monitoring and real time reporting to be effective. Others have shown the addition of lying time and lying bouts to predict day of estrus as beneficial, but we did not observe this. Larger animal sample sizes are required to develop new algorithms with variables other than activity such as lying time, lying bouts, feeding time, and rumination.

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