Estrous characteristics and reproductive outcomes of Holstein heifers treated with 2 prostaglandin formulations and detected in estrus by an automated estrous detection or mounting device

Anderson Veronese,¹ Odinei Marques,¹ Rafael Moreira,¹ Anna L. Belli,¹ Todd R. Bilby,² and Ricardo C. Chebel^{1,3*}

¹Department of Large Animal Clinical Sciences, University of Florida, Gainesville 32610

²Merck Animal Health, Madison, NJ 07940

³Department of Animal Sciences, University of Florida, Gainesville 32610

ABSTRACT

Dinoprost tromethamine (DIN), a molecule similar to endogenous PGF_{2α}, has a half-life of approximately 9 min. Cloprostenol sodium (CLO), a synthetic analog of PGF_{2α}, has a half-life of approximately 3 h. We hypothesized that treatment of Holstein heifers with CLO would improve estrous detection rate, estrous characteristics, service rate, and overall reproductive performance compared with DIN. Currently in the United States, heifers are largely inseminated based on signs of estrus, which is detected visually or with the aid of mounting detection devices (MD). Automated estrous detection devices (AED) are becoming more accessible to producers, but it is not clear whether they present advantages in the reproductive management of heifers. Therefore, we hypothesized that the use of an AED would improve service and pregnancy rates compared with detection of estrus with the aid of a MD. Holstein heifers (n = 1,019) were enrolled in the experiment at 10 to 11 mo of age, when they were fitted with a Heatime HR LD System (SCR Ltd., Netanya, Israel). At 12 mo of age, we paired heifers according to estrous cycle phase and randomly assigned them to treatments in a 2 × 2 design: PGF_{2α} formulation (CLO vs. DIN) and estrous detection treatment (AED vs. MD). Heifers in the AED treatment were detected in estrus only by the Heatime HR LD System, whereas heifers in the MD treatment were detected in estrus only by the Kamar Heatmount Detector (Kamar Products Inc., Zionsville, IN). Treatments with the same PGF_{2α} formulations were repeated 14 d after the first treatment if heifers had not been detected in estrus. A sub-group of heifers had blood sampled on the day of PGF_{2α} treatment and within 24 h of onset of estrus to determine progester-

one and estradiol concentrations. Treatment with CLO reduced the progesterone concentration within 24 h of onset of estrus compared with DIN (0.04 ± 0.01 vs. 0.11 ± 0.01 ng/mL). Among heifers in mid diestrus on the day of PGF_{2α} treatment, CLO reduced the interval to estrus compared with DIN (72.0 ± 2.2 vs. 82.4 ± 2.4 h). Prostaglandin F_{2α} formulation and estrous detection treatment did not affect pregnancy to the first service. The interval between the first and second services tended to be reduced for the AED treatment compared with the MD treatment (24.4 ± 0.5 vs. 25.7 ± 0.6 d). Prostaglandin F_{2α} formulation and estrous detection treatment did not affect the hazard of pregnancy. Although CLO treatment may shorten the interval to estrus in heifers at mid diestrus compared with DIN, PGF_{2α} formulation did not affect reproductive performance. In the current experiment, no advantages in reproductive performance were observed when estrous detection was based on an AED compared with a MD. **Key words:** prostaglandin F_{2α}, automated estrous detection, estrous behavior, reproductive performance

INTRODUCTION

Proper reproductive management of dairy heifers is critical to ensure that they calve at the proper age and weight (Stevenson et al., 2008), which have been associated with first lactation performance (Heinrichs and Heinrichs, 2011). Prostaglandin F_{2α} treatments 14 d apart may be used to increase the service rate and improve the overall reproductive performance of dairy heifers (Stevenson et al., 2000; Lopes et al., 2013). Dinoprost tromethamine (DIN) is a molecule similar to endogenous PGF_{2α} and has a half-life (T_{1/2}) of approximately 9 min (Shrestha et al., 2012). Cloprostenol sodium (CLO) is a synthetic analog of PGF_{2α} and has a T_{1/2} of approximately 3 h (Reeves, 1978). Despite differences in T_{1/2} between CLO and DIN, results published to date comparing the effects of CLO and

Received November 7, 2018.

Accepted February 11, 2019.

*Corresponding author: rcchebel@ufl.edu

DIN treatments on luteolysis and hazard of estrus and pregnancy of lactating dairy cows are not consistent.

Martins et al. (2011a,b) and Pursley et al. (2012) observed that treatment of lactating cows with CLO resulted in a faster decrease in progesterone concentration, greater estrous detection rate among primiparous cows, greater likelihood of pregnancy among cows inseminated 3 and 4 d after PGF_{2α} treatment, and greater overall pregnancy rate compared with treatment with DIN. Conversely, Stevenson and Phatak (2010) observed a decrease in luteolysis among lactating cows treated with CLO compared with cows treated with DIN and no differences between the 2 treatments regarding fertility outcomes. The experiments comparing CLO and DIN cited herein used lactating dairy cows and some compared the effects of CLO and DIN within an ovulation synchronization protocol (Stevenson and Phatak, 2010; Martins et al., 2011a,b). The use of heifers to determine the differences in hazard of estrus and estrous characteristics in response to CLO and DIN would eliminate the possible confounding effects of differences in milk yield, feed intake, and steroidal hormone concentrations (Sangsritavong et al., 2002; Wiltbank et al., 2006).

Reproductive management of dairy heifers that does not employ ovulation synchronization protocols and fixed time AI is highly dependent on efficient and accurate estrous detection (Lopes et al., 2013) that lead to synchronous service and ovulation (Fricke et al., 2014a,b; Stevenson et al., 2014). Automated estrous detection devices (AED) that detect estrus indirectly according to changes in walking, activity, and rumination patterns are becoming more accessible to and adopted by dairy farmers (Denis-Robichaud et al., 2016). Such devices determine the approximate time of onset of estrus and the ideal window of time to inseminate cattle after the onset of estrus, eliminating human subjectivity (Fricke et al., 2014a). The benefits of using AED to manage the reproduction of lactating dairy cows compared with visual detection of estrus or ovulation synchronization protocols are highly dependent on the current reproductive performance of the herd, the cost of the system, and the longevity of the system (Fricke et al., 2014a; Rutten et al., 2014; Stevenson et al., 2014; Giordano et al., 2015; Dolecheck et al., 2016). The advantages of using AED to detect estrus in dairy heifers compared with the use of more traditional mounting detection devices (MD), however, have not been evaluated.

In the current experiment, we tested the hypotheses that (1) dairy heifers treated with CLO would have increased estrous detection rate and more intense signs of estrus compared with heifers treated with DIN, and (2) that heifers managed with the aid of an AED would

have greater hazard of first and second services and greater hazard of pregnancy than heifers managed with the aid of a MD. Therefore, the objectives of the current experiment were to determine the effects of different PGF_{2α} formulations on hazard of estrus, characteristics of estrus, and hazard of pregnancy. Additionally, the current experiment aimed at determining whether the use of an AED for the reproductive management of dairy heifers would improve hazard of service and pregnancy compared with the use of a MD.

MATERIALS AND METHODS

We conducted this experiment from March to December 2016 in a commercial dairy herd with approximately 4,200 replacement heifers, located in north central Florida. All procedures involving animals were approved by the animal care and use committee of the University of Florida (protocol #201609559).

Animals, Housing, and Management

One thousand nineteen heifers were enrolled in the study between 10 and 11 mo of age. Starting at 12 mo of age, farm personnel weighed heifers weekly using an electronic scale. Heifers ≥ 340 kg of BW were moved to a breeding pen (d 0). Heifers were housed in pasture, with natural shade and no artificial cooling. The breeding pens had self-locking head stanchions on the feeding area. Heifers were fed twice daily (0700 and 1630) a TMR formulated to meet or exceed the nutritional requirements of Holsteins heifers weighing 340 kg of live BW and gaining 800 to 1,000 g of live BW per day (NRC, 2001). We calculated the percentage of days with average temperature humidity index ≥ 72 (PctTHI72) and cumulative precipitation (CPrec) from d 0 to 30 and from d 30 to 60 using data from the Gainesville airport (<https://www.wunderground.com/weather/us/fl/gainesville>), located approximately 40 miles (64.4 km) east of the dairy.

Automated Estrous Monitoring Device and Estrous Characteristics

Heifers were fitted with an AED (Heatime HR LD System, SCR Inc., Netanya, Israel) on the left cranial area of the neck at enrollment. The AED records rumination and activity for every 2-h period. Estrus is determined according to changes in activity and rumination within a 2-h period compared with the average activity and rumination of the same 2-h period in the previous 5 and 7 d, respectively (DataFlow2 software, SCR Inc., Netanya, Israel). Daily at 0600 and 1530, study personnel evaluated the activity and rumination

patterns of heifers determined to be in estrus by the DataFlow2 software. Heifers with heat index <50 (0 = minimum, 100 = maximum), duration of estrus <6 h, and no change in rumination time on the day of a pen movement were determined to have changes in activity pattern due to pen movement and not due to estrus. When heifers were in estrus (heat index ≥ 70), study personnel recorded rumination nadir (maximum difference in rumination time within a 2-h period during the estrus compared with the average rumination on the same period in the previous 7 d), activity peak (0 = no change in activity, 100 = maximum change in activity), and heat index generated by the DataFlow2 software. Heat index is calculated based on an internal algorithm of the DataFlow2 software that takes into consideration the magnitudes of changes in activity and rumination and the interval from a previously recorded estrus. Study personnel evaluated the activity graph of each heifer individually and determined the time of onset (2-h period when the activity threshold was surpassed), peak (2-h period when the activity change was maximum), and end (2-h period when the activity change fell below the activity threshold) of estrus. The intervals from onset of estrus to activity peak and from onset of estrus to the end of estrus (duration) were calculated. The activity threshold did not change throughout the experiment.

Spontaneous estruses occurring before heifers were treated with $\text{PGF}_{2\alpha}$ were recorded to determine the phase of the estrous cycle when heifers were treated with $\text{PGF}_{2\alpha}$. The AED was removed from heifers 28 d after service, when heifers were re-inseminated, or when heifers were not detected in estrus within 28 d after the start of the reproductive program. Heifers not detected in estrus within 28 d after the start of the reproductive program were submitted to a fixed-time AI protocol and were not included in the analysis of pregnancy outcomes ($n = 11$).

Treatments and Reproductive Management

Once weekly, heifers eligible to start the reproductive program (≥ 12 mo of age and ≥ 340 kg of BW) were classified according to the interval from a previously recorded estrus by the AED as being in metestrus (d 0 to 3), early diestrus (d 4 to 6), mid diestrus (d 7 to 12), late diestrus (d 13 to 17), proestrus (d 18 to 23), and unknown (d ≥ 24 or no estrus recorded). Heifers were then paired according to estrous cycle phase (**EstCycPh**) and were randomly allocated to treatments in a 2×2 design ($\text{PGF}_{2\alpha}$ formulation and estrous detection treatment) according to a previously prepared randomized spreadsheet created in Excel software (2010, Microsoft

Corporation, Redmond, WA). Heifers assigned to the CLO treatment received 500 μg of cloprostenol sodium (Estrumate, Merck Animal Health, Madison, NJ), and heifers assigned to the DIN treatment received 25 mg of dinoprost tromethamine (Lutalyse, Zoetis, Parsippany, NJ). Heifers in metestrus on the day of treatment assignment were treated with $\text{PGF}_{2\alpha}$ 96 h later, when they were in early diestrus. Heifers that were not detected in estrus within 14 d of the first $\text{PGF}_{2\alpha}$ treatment were treated for a second time with the original $\text{PGF}_{2\alpha}$ formulation. Heifers assigned to the MD treatment were fitted with a Kamar Heatmount Detector (Kamar Products Inc., Zionsville, IN), whereas heifers assigned to the AED treatment were not fitted with a Kamar Heatmount Detector.

Heifers were selected to be artificially inseminated or to receive an embryo according to the genetic selection program of the dairy, namely genomic merit for milk yield, productive life, and net merit. Heifers detected in estrus were artificially inseminated on the same morning or received an embryo 6 to 9 d after estrous detection when confirmed to have a corpus luteum by the herd veterinarian. Following the first service, heifers were monitored daily according to the estrous detection treatment and those detected in estrus were re-serviced.

Blood Sampling and Progesterone and Estradiol Assays

Blood samples were collected from a sub-group of heifers on the day of $\text{PGF}_{2\alpha}$ treatment (CLO = 83, DIN = 31) and within 24 h after the onset of estrus (CLO = 78, DIN = 29). The disparity in the number of heifers that had blood sampled according to $\text{PGF}_{2\alpha}$ formulation was because the primary objective of the sampling strategy was to assess differences in progesterone and estradiol concentrations according to genomic estimated breeding values for fertility traits, which is reported elsewhere (Veronese et al., 2019). Therefore, we needed a large number of heifers treated with one of the $\text{PGF}_{2\alpha}$ formulations (CLO), and to compare the effects of $\text{PGF}_{2\alpha}$ formulations on progesterone concentration, we sampled a smaller sub-group of heifers treated with DIN. Heifers used for blood sampling were selected within the same week of enrollment to ensure that management and environmental conditions did not confound the findings.

Blood was sampled by puncture of the coccygeal vein or artery into evacuated tubes containing K2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Immediately upon collection, tubes were placed in ice and kept refrigerated until transported to the laboratory within 2 to 3 h for processing. Blood tubes were

centrifuged at $1,500 \times g$ for 15 min at 4°C and aliquots of plasma were frozen at -80°C until assayed. Progesterone concentrations in plasma were determined by radioimmunoassay using a commercial kit (Coat-a-Count, MP Biomedical LLC, Solon OH). Plasma harvested from cows on d 4 (~1 ng/mL) and 10 (~4 ng/mL) of the estrous cycle were incorporated into each assay and used to calculate the coefficients of variation. Intra- and interassay coefficients of variation were 5.8 and 10.5%, respectively. Heifers with progesterone concentration ≥ 1.0 ng/mL on the day of PGF_{2α} treatment that displayed estrus within 96 h after treatment were considered to have had complete luteolysis. Serum concentration of estradiol-17β was quantified by RIA (Jinks et al., 2013) and the intraassay coefficient of variation was 2.73%.

Pregnancy Diagnoses and Reproductive Data

All heifers were examined for pregnancy by palpation per rectum of uterine contents at 38 ± 3 d after the detected estrus that resulted in AI or ET. Pregnant heifers were re-examined by palpation per rectum of the uterine contents at 73 ± 3 d of gestation. Pregnancy per service was calculated by dividing the number of heifers pregnant 38 ± 3 and 73 ± 3 d after service by the number of heifers serviced. Pregnancy loss was calculated by dividing the number of heifers pregnant 73 ± 3 d after service by the number of heifers pregnant 38 ± 3 d after service. Data regarding sire of insemination, sire and dam of embryo transfer, service technician, information regarding embryos (type: multiple ovulation embryo transferred fresh or frozen/thawed, in vitro-produced embryo transferred fresh or frozen/thawed; grade: 1 = excellent/good, 4 = fair; stage: 4 = morula, 8 = hatched blastocyst), interval from estrus to embryo transfer, and reproductive outcomes were collected from the dairy management software (PCDART, Dairy Records Management System, Chapel Hill, NC).

Statistical Analysis

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Continuous outcomes were analyzed by ANOVA using the MIXED procedure. Data were evaluated for normality and homogeneity of residuals after fitting the model. Data violating the assumptions of normality were transformed. Rumination nadir values were multiplied by -1 and transformed to the natural log before analysis. Thus, positive rumination nadir values were excluded (CLO = 4, DIN = 12). Outlier detection was performed, and rumination nadir transformed values < 2 (CLO = 1, DIN = 2) were con-

sidered outliers and removed from the analysis. Interval from onset of estrus to peak of activity was transformed to the square root. Rumination nadir and interval from onset of estrus to peak of activity data presented are back-transformed values.

Binary outcomes were analyzed by logistic regression using the GLIMMIX procedure. Heifers were classified as having activity peak ≥ 80 and heat index ≥ 80 because the data were skewed to the right and because these thresholds were associated with pregnancy outcomes. Hazard of estrus, first service, second service, and pregnancy were analyzed by the Cox proportional hazard ratio using the PHREG procedure of SAS. When PGF_{2α} formulation or estrous detection treatment affected hazard of an outcome, their effect on the interval to the outcome was analyzed by the Wilcoxon test of equality using the LIFETEST procedure.

The models to evaluate progesterone concentration on the day of PGF_{2α} treatment and estradiol concentration within 24 h of onset of estrus included PGF_{2α} formulation, EstCycPh on the day of PGF_{2α} treatment, the interaction between PGF_{2α} formulation and EstCycPh on the day of PGF_{2α} treatment, genomic merit for daughter pregnancy rate (DPR) and heifer conception rate (HCR), and PctTHI72 and CPrec from d 0 to 30. Additionally, the interval from onset of estrus to collection of blood sample was added to the model to determine the effect of PGF_{2α} formulation on estradiol concentration. Progesterone concentration within 24 h of onset of estrus was analyzed by Kruskal-Wallis test using the NPARIWAY procedure.

The models to evaluate the effects of PGF_{2α} formulation on characteristics of estrus recorded by the AED and hazard of estrus included PGF_{2α} formulation, EstCycPh on the day of PGF_{2α} treatment, the interaction between PGF_{2α} formulation and EstCycPh on the day of PGF_{2α} treatment, DPR, HCR, and PctTHI72 and CPrec from d 0 to 30. The effect of PGF_{2α} formulation on the hazard of estrus was analyzed from the moment of the PGF_{2α} treatment that resulted in estrus. Heifers in proestrus on the day of PGF_{2α} treatment ($n = 142$) and heifers detected in estrus < 12 h ($n = 9$) and > 168 h ($n = 114$) after PGF_{2α} treatment were excluded from these analyses because we assumed that they displayed estrus not in response to the PGF_{2α} treatment.

Hazard of first service (from the moment of the first PGF_{2α} treatment) and pregnancy outcomes to first service were evaluated with models that included PGF_{2α} formulation, estrous detection treatment, EstCycPh on the day of PGF_{2α} treatment, DPR, HCR, and PctTHI72 and CPrec from d 0 to 30. The following interactions were included in the model: PGF_{2α} formulation $\times$ estrous detection treatment, PGF_{2α} formulation $\times$

EstCycPh on the day of PGF_{2α} treatment, and estrous detection treatment × EstCycPh on the day of PGF_{2α} treatment. The models to evaluate pregnancy outcomes included service technician, sire (random effect), and for heifers receiving embryo transfer, the type, grade, and stage of the embryo and the interval from estrus to embryo transfer.

The models to determine the effects of estrous detection treatment on the hazard of second service and pregnancy outcomes to second service included estrous detection treatment, DPR, HCR, and PctTHI72 and CPrec from d 30 to 60. The models to evaluate pregnancy outcomes to second service also included service technician, sire (random effect), and for heifers receiving embryo transfer, the type, grade, and stage of the embryo and the interval from estrus to embryo transfer.

The effects of PGF_{2α} formulation and estrous detection treatment on hazard of pregnancy were evaluated with a model that included PGF_{2α} formulation, estrous detection treatment, EstCycPh on the day of PGF_{2α} treatment, breeding strategy (100% AI, 100% embryo transfer, mixed), DPR, HCR, and PctTHI72 and CPrec from d 0 to 30. The following interactions were included in the model: PGF_{2α} formulation × estrous detection treatment, PGF_{2α} formulation × EstCycPh on the day of PGF_{2α} treatment, estrous detection treatment × EstCycPh on the day of PGF_{2α} treatment, PGF_{2α} formulation × breeding strategy, and estrous detection treatment × breeding strategy.

In all models we eliminated (backward stepwise) variables with $P > 0.10$ until only variables with $P \leq 0.10$ remained. To control for family-wise error rate due to multiple-comparison, the Tukey-Kramer method was applied and statistical significance was considered at adjusted $P \leq 0.05$ and a tendency was considered when $0.05 < \text{adjusted } P \leq 0.10$. Data are reported as least squares means ± standard error of the mean, unless otherwise stated.

RESULTS

No differences were observed among treatments regarding DPR (1.66 ± 0.04 ; $P = 0.23$) and HCR (1.34 ± 0.04 ; $P = 0.30$). Heifers in the CLO treatment (376.8 ± 0.3 d) had a tendency ($P = 0.07$) to be younger at enrollment than heifers in the DIN treatment (377.5 ± 0.3 d), but we did not ($P = 0.20$) observe differences in age at enrollment between heifers assigned to the MD and AED treatments. Heifers assigned to the CLO treatment were ($P < 0.01$) heavier at enrollment than heifers assigned to the DIN treatment (388.0 ± 1.2 vs. 383.1 ± 1.3 kg), but BW at enrollment was not ($P = 0.11$) different between heifers assigned to the MD and AED treatments.

Effects of PGF_{2α} Formulation on Progesterone and Estradiol Concentrations and Expression and Characteristics of Estrus

Estrous cycle day ($P = 0.82$) and progesterone concentration ($P = 0.27$) on the day of PGF_{2α} treatment were not different between CLO and DIN treatments (Table 1). Progesterone concentration on the day of PGF_{2α} treatment was ($P \leq 0.01$) lower for heifers treated at early diestrus (1.23 ± 0.65 ng/mL) and proestrus (2.52 ± 0.63 ng/mL) compared with heifers treated at mid diestrus (5.05 ± 0.50 ng/mL) and late diestrus (5.43 ± 0.58 ng/mL). Treatment with CLO resulted in lower ($P < 0.01$) progesterone concentrations within 24 h of onset of estrus than treatment with DIN, but PGF_{2α} formulation did not ($P = 0.23$) affect the likelihood of luteolysis (Table 1). An association ($P < 0.01$) was observed between EstCycPh on the day of PGF_{2α} treatment and progesterone concentration within 24 h of treatment (early diestrus = 0.20 ± 0.01 , mid diestrus = 0.03 ± 0.01 , late diestrus = 0.03 ± 0.01 , proestrus = 0.06 ± 0.01 , unknown = 0.06 ± 0.01 ng/mL). Furthermore, EstCycPh on the day of PGF_{2α} treatment was ($P < 0.01$) associated with the likelihood of luteolysis (early diestrus = 50%, mid diestrus = 87.9%, late diestrus = 100%, proestrus = 100%, and unknown = 100%). Prostaglandin F_{2α} formulation did not ($P = 0.49$) affect estradiol concentration within 24 h of onset of estrus (Table 1). Estrous cycle phase at PGF_{2α} treatment was not ($P = 0.90$) associated with estradiol concentration within 24 h of onset of estrus.

The interaction between PGF_{2α} formulation and EstCycPh affected ($P < 0.01$) the hazard of estrus. Among heifers in mid diestrus, CLO resulted in shorter ($P < 0.01$) interval from the PGF_{2α} treatment to estrus compared with DIN (Figure 1). Prostaglandin F_{2α} formulation did not ($P \geq 0.26$) affect the interval from the PGF_{2α} treatment to estrus among heifers in early diestrus, late diestrus, proestrus, and unknown estrous cycle phase. Similarly, the likelihood of heifers displaying estrus within 7 d of PGF_{2α} treatment was ($P = 0.01$) affected by the interaction between PGF_{2α} formulation and EstCycPh (Figure 2). Among heifers in mid diestrus on the day of PGF_{2α} treatment, CLO resulted in greater ($P < 0.01$) likelihood of estrus than DIN. Prostaglandin F_{2α} formulation did not ($P \geq 0.99$) affect the likelihood of estrus among heifers in early diestrus, late diestrus, and unknown EstCycPh.

Prostaglandin F_{2α} formulation did not affect the duration of estrus ($P = 0.99$), the interval from the onset of estrus to activity peak ($P = 0.63$), the rumination nadir ($P = 0.25$), and the likelihood of heifers with activity peak ≥ 80 ($P = 0.41$) and heat index ≥ 80 ($P = 0.50$; Table 1). Heifers treated with PGF_{2α} during early

Figure 1. Effect of PGF_{2α} formulation on the interval from the first PGF_{2α} treatment to onset of estrus among heifers in mid diestrus at the PGF_{2α} treatment (n = 332). CLO: heifers treated with 500 µg of cloprostenol sodium (Estrumate, Merck Animal Health, Madison, NJ); DIN: heifers treated with 25 mg of dinoprost tromethamine (Lutalyse, Zoetis, Parsippany, NJ). Effect of PGF_{2α} formulation ($P < 0.01$). Mean ($\pm$ SEM) and median, respectively, interval from the first PGF_{2α} treatment to onset of estrus: CLO = 72.0 ± 2.2 and 46 h, DIN = 82.4 ± 2.4 and 61.5 h. Heifers censored at CLO = 18.4%, DIN = 25.9%.

diestrus had ($P \leq 0.01$) the shortest estrus, whereas heifers treated with PGF_{2α} during mid diestrus had ($P = 0.02$) shorter estrus than those treated during late diestrus (Table 2). Similarly, heifers treated with PGF_{2α} during early diestrus had ($P < 0.01$) the shortest interval from the onset of estrus to activity peak and heifers treated with PGF_{2α} during mid diestrus had ($P < 0.01$) shorter interval from the onset of estrus to activity peak than those treated during late diestrus

Figure 2. Effects of PGF_{2α} formulation and estrous cycle phase on the likelihood of estrus within 7 d after PGF_{2α} treatment. Excluding heifers treated with PGF_{2α} during proestrus and heifers detected in estrus < 12 h after PGF_{2α} treatment. CLO: heifers treated with 500 µg of cloprostenol sodium (Estrumate, Merck Animal Health, Madison, NJ); DIN: heifers treated with 25 mg of dinoprost tromethamine (Lutalyse, Zoetis, Parsippany, NJ). Estrous cycle phase (estrus = d 0): early diestrus = d 4 to 6 (ED; n = 145), mid diestrus = d 7 to 12 (MD; n = 331), late diestrus = d 13 to 17 (LD; n = 213), and unknown = d ≥ 24 or no estrus recorded (Unk; n = 105). Effect of PGF_{2α} formulation ($P = 1.00$), estrous cycle phase ($P < 0.01$), and the interaction between PGF_{2α} formulation and estrous cycle phase ($P = 0.01$). * $P < 0.01$. Error bars represent the SEM.

(Table 2). Rumination nadir on the day of estrus was ($P < 0.01$) highest for heifers treated with PGF_{2α} during early diestrus, whereas a tendency ($P = 0.09$) was observed for heifers treated with PGF_{2α} during mid diestrus to have higher rumination nadir than those treated during late diestrus (Table 2). Heifers treated

Table 1. Effects of prostaglandin formulation¹ on mean ($\pm$ SEM) hormonal concentrations and estrous characteristics of Holstein heifers

Variable	CLO	DIN	P-value
Day of the estrous cycle on the day of the first PGF _{2α} treatment	11.2 $\pm$ 0.3	11.3 $\pm$ 0.3	0.82
Progesterone on the day of the first PGF _{2α} treatment, ² ng/mL	5.13 $\pm$ 0.33	5.81 $\pm$ 0.51	0.27
Progesterone within 24 h of onset of estrus, ² ng/mL	0.04 $\pm$ 0.01	0.11 $\pm$ 0.01	< 0.01
Likelihood of complete luteolysis, ³ %	90.6 $\pm$ 3.6	81.5 $\pm$ 7.5	0.23
Estradiol within 24 h of onset of estrus, ² pg/mL	4.26 $\pm$ 0.28	4.64 $\pm$ 0.46	0.49
Heifers detected in estrus within 7 d of the first PGF _{2α} treatment, ⁴ %	99.7 $\pm$ 4.5	99.7 $\pm$ 4.8	1.00
Duration of estrus, ⁵ h	16.22 $\pm$ 0.38	16.21 $\pm$ 0.37	0.99
Interval from onset of estrus to activity peak, ⁵ h	6.90 $\pm$ 0.37	6.77 $\pm$ 0.36	0.63
Rumination nadir at estrus, ⁵ min	-31.94 $\pm$ 0.71	-30.98 $\pm$ 0.69	0.25
Heifers with activity peak ≥ 80 at estrus, ⁵ %	71.4 $\pm$ 3.6	74.2 $\pm$ 3.3	0.41
Heifers with heat index ≥ 80 at estrus, ⁵ %	86.6 $\pm$ 2.3	84.1 $\pm$ 2.9	0.50

¹CLO (n = 517): heifers treated with 500 µg of cloprostenol sodium (Estrumate, Merck Animal Health, Madison, NJ); DIN (n = 502): heifers treated with 25 mg of dinoprost tromethamine (Lutalyse, Zoetis, Parsippany, NJ).

²Sub-sample of heifers sampled on the day of PGF_{2α} treatment (CLO = 81, DIN = 31) and within 24 h of onset of estrus (CLO = 78, DIN = 29).

³Heifers with progesterone concentration ≥ 1.0 ng/mL on the day of PGF_{2α} treatment (CLO = 64, DIN = 27) that displayed estrus within 96 h after treatment were considered to have had complete luteolysis.

⁴Excluding heifers in proestrus on the day of PGF_{2α} treatment and heifers detected in estrus < 12 h (CLO = 387, DIN = 372).

⁵Excluding heifers in proestrus on the day of PGF_{2α} treatment and heifers detected in estrus < 12 and > 168 h after PGF_{2α} treatment (CLO = 343, DIN = 312).

Table 2. Association between estrous cycle phase¹ at PGF_{2α} treatment and estrous characteristics (±SEM) of Holstein heifers²

Variable	Early diestrus	Mid diestrus	Late diestrus	Unknown	P-value
n	89	285	213	68	
Duration of estrus, h	14.7 ± 0.5 ^a	16.7 ± 0.3 ^b	18.0 ± 0.4 ^c	17.8 ± 0.6 ^{bc}	<0.01
Interval from onset of estrus to peak of activity, h	5.7 ± 0.4 ^a	7.2 ± 0.3 ^b	8.8 ± 0.4 ^c	7.6 ± 0.7 ^{bc}	<0.01
Rumination nadir at estrus, min	-26.2 ± 1.1 ^a	-31.5 ± 0.7 ^{b,A}	-34.1 ± 0.9 ^{b,B}	-31.8 ± 1.5 ^b	<0.01
Heifers with activity peak ≥80 at estrus, %	51.9 ± 5.3 ^a	75.9 ± 2.6 ^b	84.1 ± 2.5 ^b	82.2 ± 4.7 ^b	<0.01
Heifers with heat index ≥80 at estrus, %	63.0 ± 5.1 ^a	83.6 ± 2.2 ^b	90.2 ± 2.0 ^b	89.6 ± 3.7 ^b	<0.01

^{a-c}Within an item, values with different superscripts differ ($P \leq 0.05$).

^{A,B}Within an item, values with different superscripts tended to differ ($0.05 < P \leq 0.10$).

¹Estrus = d 0, early diestrus = d 4 to 6, mid diestrus = d 7 to 12, late diestrus = d 13 to 17, proestrus = d 18 to 23, and unknown = d ≥24 or no estrus recorded.

²Excluding heifers in proestrus on the day of PGF_{2α} treatment and heifers detected in estrus <12 and >168 h after PGF_{2α} treatment.

with PGF_{2α} during early diestrus were ($P < 0.01$) least likely to have activity peak ≥80 and heat index ≥80, but we did not ($P \geq 0.12$) observe differences among heifers treated with PGF_{2α} during mid diestrus, late diestrus, and unknown EstCycPh (Table 2).

Effects of PGF_{2α} Formulation and Estrous Detection Treatment on Reproductive Performance

Hazard of first service was not affected by PGF_{2α} formulation ($P = 0.21$), estrous detection treatment ($P = 0.11$), and by the interaction between PGF_{2α} formulation and estrous detection treatment ($P = 0.81$). The mean (±SEM) and median interval from the first PGF_{2α} treatment to first service were 4.8 ± 0.2 and 3.0 d, respectively. Heifers in early diestrus had ($P < 0.01$) and tended ($P = 0.06$) to have reduced hazard of first service than heifers in proestrus and unknown estrous phase, respectively, whereas heifers in mid diestrus and late diestrus had ($P < 0.01$) reduced hazard of first service than heifers in proestrus.

The likelihood of pregnancy following the first AI was not affected by PGF_{2α} formulation ($P \geq 0.12$) and estrous detection treatment ($P \geq 0.65$; Table 3). We did not detect an effect of PGF_{2α} formulation ($P = 0.31$) and estrous detection treatment ($P = 0.54$) on the likelihood of pregnancy loss from 38 ± 3 to 73 ± 3 d after first AI. Heifers treated with PGF_{2α} during early diestrus and mid diestrus were ($P \leq 0.05$) less likely to be pregnant 38 ± 3 and 73 ± 3 d after AI compared with heifers treated with PGF_{2α} during late diestrus and proestrus. Estrous cycle phase on the day of PGF_{2α} treatment was not ($P = 0.96$) associated with pregnancy loss from 38 ± 3 to 73 ± 3 d after first AI (Table 4). Prostaglandin F_{2α} formulation ($P \geq 0.28$) and estrous detection treatment ($P \geq 0.14$) did not affect the likelihood of pregnancy and the likelihood of pregnancy loss following the first embryo transfer (Table 3).

Heifers enrolled in the AED treatment tended ($P = 0.09$) to have greater hazard of second service [adjusted hazard ratio (AHR) = 1.17, 95% CI = 0.98, 1.39]

Table 3. Effects of PGF_{2α} formulation and estrous detection treatment (ED) on first service outcomes (±SEM)

Variable	PGF _{2α} formulation ¹		Estrous detection treatment ²		P-value		
	CLO	DIN	MD	AED	PGF _{2α}	ED	PGF _{2α} × ED
First AI							
n	244	252	236	260			
Pregnant on d 38, %	47.1 ± 3.9	53.5 ± 3.9	50.6 ± 4.0	50.6 ± 3.8	0.22	1.00	0.62
Pregnant on d 73, %	43.2 ± 3.8	51.1 ± 3.9	46.1 ± 4.0	48.4 ± 3.7	0.12	0.65	0.98
Pregnancy loss, %	8.5 ± 2.6	5.2 ± 1.9	7.7 ± 2.5	5.8 ± 2.0	0.31	0.54	0.17
First embryo transfer							
n	270	242	240	272			
Pregnant on d 38, %	26.5 ± 4.3	31.1 ± 4.8	26.7 ± 4.3	31.1 ± 4.8	0.28	0.30	0.24
Pregnant on d 73, %	16.8 ± 3.8	19.5 ± 4.4	15.7 ± 3.7	20.4 ± 4.4	0.40	0.14	0.70
Pregnancy loss, %	24.0 ± 9.2	28.2 ± 10.5	30.0 ± 11.9	22.1 ± 9.5	0.62	0.38	0.34

¹CLO: heifers treated with 500 µg of cloprostenol sodium (Estrumate, Merck Animal Health, Madison, NJ); DIN: heifers treated with 25 mg of dinoprost tromethamine (Lutalyse, Zoetis, Parsippany, NJ).

²MD: heifers fitted with and inseminated based on a mounting detection device (Kamar Heatmount Detector, Kamar Products Inc., Zionsville, IN); AED: heifers not fitted with a mounting detection device and inseminated based on the automated estrous detection device (Heatime HR LD System, SCR Inc., Netanya, Israel).

Table 4. Association between estrous cycle phase¹ at PGF_{2α} treatment and pregnancy outcomes (±SEM) of Holstein heifers after the first AI

Variable	Early diestrus	Mid diestrus	Late diestrus	Proestrus	Unknown	P-value
n	141	164	105	93	55	
Pregnant on d 38, %	36.3 ± 5.5 ^a	44.3 ± 4.1 ^a	62.8 ± 5.1 ^b	62.8 ± 5.2 ^b	49.5 ± 8.2 ^{ab}	<0.01
Pregnant on d 73, %	36.6 ± 5.5 ^a	39.8 ± 4.0 ^a	58.2 ± 5.1 ^b	59.0 ± 5.3 ^b	44.4 ± 8.1 ^{ab}	<0.01
Pregnancy loss, %	0.0 ± 0.0	6.2 ± 4.0	4.3 ± 3.0	4.7 ± 3.1	7.9 ± 6.5	0.96

^{a,b}Within an item, values with different superscripts differ ($P \leq 0.05$).
¹Estrus = d 0, early diestrus = d 4 to 6, mid diestrus = d 7 to 12, late diestrus = d 13 to 17, proestrus = d 18 to 23, and unknown = d ≥ 24 or no estrus recorded.

than heifers enrolled in the MD treatment (referent). Heifers enrolled in the AED treatment tended ($P = 0.06$) to have reduced interval between the first and second services than heifers enrolled in the MD treatment (Figure 3). Estrous detection treatment did not affect the likelihood of pregnancy ($P = 0.26$) and the likelihood of pregnancy loss ($P = 0.74$) after the second AI (Table 5). Similarly, estrous detection treatment did not affect the likelihood of pregnancy ($P \geq 0.45$) and the likelihood of pregnancy loss ($P = 0.28$) after the second embryo transfer (Table 5).

Hazard of pregnancy after the first PGF_{2α} treatment was not affected by PGF_{2α} formulation ($P = 0.51$), estrous detection treatment ($P = 0.14$), and the interaction between PGF_{2α} formulation and estrous detection treatment ($P = 0.65$). Estrous cycle phase at the first PGF_{2α} treatment was ($P = 0.05$) associated with the hazard of pregnancy. Heifers in early diestrus (AHR =

0.74, 95% CI = 0.59, 0.93) and mid diestrus (AHR = 0.79, 95% CI = 0.64, 0.97) had ($P \leq 0.02$) reduced hazard of pregnancy compared with heifers in late diestrus (referent). No differences were noted ($P > 0.10$) among heifers in proestrus (AHR = 0.94, 95% CI = 0.72, 1.22), late diestrus, and unknown EstCycPh (AHR = 0.75, 95% CI = 0.53, 1.06).

DISCUSSION

In the current experiment, the interaction between PGF_{2α} formulation and EstCycPh affected the hazard of estrus because, among heifers in mid diestrus, CLO increased the hazard of estrus compared with DIN. Although it is not possible to conclude from the current experiment, we speculate that the longer $T_{1/2}$ of CLO may have improved luteolysis and consequently the hazard of estrus among heifers in mid diestrus. Among heifers in early diestrus and late diestrus, however, PGF_{2α} formulation did not affect the hazard of estrus. During early diestrus the corpus luteum is less sensitive to exogenous PGF_{2α} (Valldcabres-Torres et al., 2012; Ferraz et al., 2016). Although we expected that treatment of heifers in early diestrus with CLO would result in greater hazard of estrus compared with DIN, we conclude that CLO given during early diestrus

Figure 3. Effect of estrous detection treatment on the interval between first and second services (n = 606). MD: heifers fitted with and inseminated based on a mounting detection device (Kamar Heatmount Detector, Kamar Products Inc., Zionsville, IN); AED: heifers not fitted with a mounting detection device and inseminated based on the automated estrous detection device (Heatime HR LD System, SCR Inc., Netanya, Israel). Effect of estrous detection treatment ($P = 0.06$). Mean (±SEM) and median, respectively, interval between first and second service: MD = 25.7 ± 0.6 and 22 d; AED = 24.4 ± 0.5 and 21 d. Heifers censored at MD = 15.6%, AED = 10.0%.

Table 5. Effects of estrous detection treatment¹ on pregnancy after the second service (±SEM)

Variable	MD	AED	P-value
Second AI			
n	131	136	
Pregnant on d 38, %	28.8 ± 4.3	35.8 ± 4.4	0.26
Pregnant on d 73, %	27.5 ± 4.2	34.3 ± 4.4	0.26
Pregnancy loss, %	5.1 ± 3.5	3.7 ± 2.6	0.74
Second embryo transfer			
n	166	173	
Pregnant on d 38, %	29.7 ± 4.9	26.0 ± 4.5	0.45
Pregnant on d 73, %	25.5 ± 4.7	23.3 ± 4.4	0.65
Pregnancy loss, %	9.4 ± 4.0	3.9 ± 2.7	0.28

¹MD: heifers fitted with and inseminated based on a mounting detection device (Kamar Heatmount Detector, Kamar Products Inc., Zionsville, IN); AED: heifers not fitted with a mounting detection device and inseminated based on the automated estrous detection device (Heatime HR LD System, SCR Inc., Netanya, Israel).

was not able to overcome the blunted response of the young corpus luteum to exogenous $\text{PGF}_{2\alpha}$. The lack of differences in hazard of estrus among heifers treated with $\text{PGF}_{2\alpha}$ during late diestrus may be a consequence of the greater variability in the stage of development of ovarian follicles at the time of luteolysis. Sartori et al. (2004) demonstrated that almost half of heifers have estrous cycles with more than 3 follicular waves and that the emergence of the second follicular wave occurs from d 6 to 10 of the estrous cycle. Luteal regression is required for ovarian cyclic activity, but the interval between $\text{PGF}_{2\alpha}$ treatment and estrus is dependent on the rate of decrease of progesterone concentration, the presence, size, and dominance of an ovarian follicle, and the consequent rise in estradiol (Goravanahally et al., 2009).

The lack of differences in estradiol concentration within 24 h of onset of estrus may explain the lack of differences in estrous characteristics of heifers treated with CLO and DIN. Estrous characteristics measured by AED have been associated with physiological signs of estrus such as clear vaginal mucus, uterine tone, visual mounting activity, and standing to be mounted behavior (Silper et al., 2015). Secretion of estradiol from ovarian follicles is critical for the onset of estrus, and estradiol concentration has been positively associated with the duration of estrus and heat index of lactating cows (Jinks et al., 2013; Aungier et al., 2015; Madureira et al., 2015; Reith and Hoy, 2018). Additionally, the lack of difference between CLO and DIN treatments regarding estradiol concentration may explain the lack of differences between the $\text{PGF}_{2\alpha}$ formulations in rumination nadir. Mice treated with estradiol benzoate had reduced feed intake compared with untreated controls (Butera et al., 2010), and estradiol decreases neuropeptide-Y secretion and increases the potency of cholecystokinin satiety systems in mice (Bonavera et al., 1994), which have been associated with DMI in cattle (Abo-Ismael et al., 2013).

The hazard of first service was not affected by $\text{PGF}_{2\alpha}$ formulation and estrous detection treatment. Although CLO-treated heifers were detected in estrus approximately 15.5 h sooner than DIN-treated heifers, heifers were serviced once per day only, which likely limited our ability to detect differences in hazard of service. The challenges to detect estrus are generally associated with housing conditions, labor available for detection of estrus, method of estrous detection, and milk yield. Lopez et al. (2004) estimated that visual detection of estrus every 6 h would result in probability of detecting estrus between 50 and 100% when milk yield was 55 and 25 kg/d, respectively. The estimated probability of detecting estrus when visual estrous detection was performed every 24 h was 15 and 65% for cows produc-

ing 55 and 25 kg/d, respectively (Lopez et al., 2004). Elevated milk yield increases DMI, which has been proposed to increase the clearance of steroidal hormones in the liver (Sangsrivong et al., 2002), reducing the estradiol concentration at estrus in high-producing cows. Britt et al. (1986) demonstrated that duration of estrus and mounting and standing activities were greater in lactating cows housed in dirt compared with concrete. In the current experiment, we compared an AED to a MD in heifers that were housed in pasture, animals and housing conditions that are not negatively associated with estrous expression.

A tendency was observed for the hazard of second service to be greater for heifers enrolled in the AED treatment compared with heifers enrolled in the MD treatment. Helmer and Britt (1985) demonstrated that a greater number of heifers in estrus within a group results in more intense estrual behavior. Therefore, we speculate that the large number of heifers in estrus within a short period of time after the first $\text{PGF}_{2\alpha}$ treatment (96 h) may have facilitated the detection of estrus based on MD by farm personnel, limiting our ability to detect differences, if any, between the AED and MD treatments. When heifers spontaneously returned to estrus after the first service, fewer heifers within a group were in estrus at the same time. Therefore, we speculate that detection of heifers returning to estrus based on a MD by farm personnel was more difficult because of fewer heifers in estrus within a group and lesser intensity of estrus, which may have been overcome by the AED device.

Pregnancy per service were not different between heifers treated with CLO and DIN. The effects of $\text{PGF}_{2\alpha}$ formulation on pregnancy per AI of lactating dairy cows are inconsistent, with some groups reporting greater pregnancy per AI in cows treated with CLO (Martins et al., 2011a; Pursley et al., 2012) and others not detecting differences between CLO- and DIN-treated cows (Stevenson and Phatak, 2010). The most likely mechanism through which $\text{PGF}_{2\alpha}$ formulation would affect pregnancy per AI would be through differences in progesterone concentration at AI (Giordano et al., 2012). In the current experiment, a sub-sample of CLO-treated heifers had reduced progesterone concentration within 24 h of estrus, but this difference was likely not biologically important. We should note that the pregnancy to first AI in the current experiment was considerably lower than the pregnancy to first AI reported by others (Kuhn et al., 2006; Stevenson et al., 2008). Other collaborating herds, located approximately 50 and 100 miles (80.5 and 160.9 km) from the herd in which this experiment was conducted, had average pregnancy to first AI during the same period between 55% (sex-sorted semen) and 60% (conventional semen;

R. C. Chebel, University of Florida, Gainesville, FL, personal communication). In fact, one of the reasons why we selected the collaborating herd for this experiment was the hypothesis that the low pregnancy per AI of heifers was in part due to inaccurate estrous detection based on a MD by farm personnel. The lack of difference between the AED and MD suggests that heifers housed in pasture express sufficiently strong estrus such that both estrous detection systems would result in similar estrous detection accuracy. Ultimately, neither the PGF_{2α} formulation nor the estrous detection treatment affected the hazard of pregnancy of dairy heifers in the current experiment.

Although not the focus of this experiment, significant differences were observed in characteristics of estrus and reproductive parameters according to EstCycPh on the day of PGF_{2α} treatment. Heifers treated with PGF_{2α} at early diestrus and mid diestrus had less intense signs of estrus than those treated during late diestrus. Furthermore, the likelihood of estrus within 168 h of PGF_{2α} treatment and the pregnancy per service were reduced among heifers treated during early diestrus and mid diestrus. This may be explained by the greater progesterone concentration and reduced luteolysis within 24 h of PGF_{2α} treatment among heifers treated during early diestrus and mid diestrus. As discussed previously, the corpus luteum's response to exogenous PGF_{2α} is highly dependent on EstCycPh. Several experiments with lactating dairy cows have demonstrated the negative effect of elevated progesterone concentration at AI on pregnancy outcomes (Wiltbank et al., 2015). The results from the current experiment provide evidence that greater reproductive efficiency and more judicious use of PGF_{2α} may be achieved when the EstCycPh of dairy heifers is known.

CONCLUSIONS

In the current experiment, CLO resulted in interval to first estrus 15.5 h shorter than DIN among heifers treated during mid diestrus. No differences between the 2 PGF_{2α} formulations were observed in interval to first service. Although CLO resulted in reduced progesterone concentration 24 h after treatment compared with DIN, PGF_{2α} formulation did not affect pregnancy outcomes. Thus, we conclude that the selection of PGF_{2α} formulation for heifers may be based on convenience and cost. Although the use of an AED shortened the interval between first and second services, such gains were not sufficient to improve the hazard of pregnancy compared with reproductive management based on a MD. Adoption of AED for reproductive management of heifers may not be advantageous compared with MD

when significant improvements in reproductive parameters are not observed.

ACKNOWLEDGMENTS

The authors thank the owner and staff of North Florida Holsteins (Bell, FL) for allowing the experiment and all farm staff for the help provided. Gratitude is extended to Merck Animal Health (Madison, NJ) for financial support and to SCR Inc. (Netanya, Israel) for partial financial support.

REFERENCES

- Abo-Ismael, M. K., M. J. Kelly, E. J. Squires, K. C. Swanson, S. Bauck, and S. P. Miller. 2013. Identification of single nucleotide polymorphisms in genes involved in digestive and metabolic processes associated with feed efficiency and performance traits in beef cattle. *J. Anim. Sci.* 91:2512–2529. <https://doi.org/10.2527/jas.2012-5756>.
- Aungier, S. P. M., J. F. Roche, P. Duffy, S. Scully, and M. A. Crowe. 2015. The relationship between activity clusters detected by an automatic activity monitor and endocrine changes during the periestrous period in lactating dairy cows. *J. Dairy Sci.* 98:1666–1684. <https://doi.org/10.3168/jds.2013-7405>.
- Bonavera, J. J., M. G. Dube, P. S. Kalra, and S. P. Kalra. 1994. Anorectic effects of estrogen may be mediated by decreased neuropeptide-Y release in the hypothalamic paraventricular nucleus. *Endocrinology* 134:2367–2370.
- Britt, J. H., R. G. Scott, J. D. Armstrong, and M. D. Whitacre. 1986. Determinants of estrous behavior in lactating Holstein cows. *J. Dairy Sci.* 69:2195–2202.
- Butera, P. C., D. M. Wojcik, and S. J. Clough. 2010. Effects of estradiol on food intake and meal patterns for diets that differ in flavor and fat content. *Physiol. Behav.* 99:142–145. <https://doi.org/10.1016/j.physbeh.2009.10.009>.
- Denis-Robichaud, J., R. L. A. Cerri, A. Jones-Bitton, and S. J. LeBlanc. 2016. Survey of reproduction management on Canadian dairy farms. *J. Dairy Sci.* 99:9339–9351. <https://doi.org/10.3168/jds.2016-11445>.
- Dolecheck, K. A., G. Heersche Jr., and J. M. Bewley. 2016. Retention payoff-based cost per day open regression equations: Application in a user-friendly decision support tool for investment analysis of automated estrus detection technologies. *J. Dairy Sci.* 99:10182–10193.
- Ferraz, M. V. C. Jr., A. V. Pires, M. V. Biehl, M. H. Santos, D. M. Polizel, D. D. Nepomuceno, R. Sartori, J. B. Barreto Filho, J. R. S. Gonçalves, and M. L. Day. 2016. Luteolysis in *Bos indicus* cows on Days 5 and 7 of estrous cycle with varying doses of PGF_{2α}. *Theriogenology* 86:1268–1274. <https://doi.org/10.1016/j.theriogenology.2016.04.067>.
- Fricke, P. M., P. D. Carvalho, J. O. Giordano, A. Valenza, G. Lopes Jr., and M. C. Amundson. 2014a. Expression and detection of estrus in dairy cows: The role of new technologies. *Animal* 8:134–143. <https://doi.org/10.1017/S1751731114000299>.
- Fricke, P. M., J. Giordano, A. Valenza, G. Lopes, M. Amundson, and P. Carvalho. 2014b. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity-monitoring system. *J. Dairy Sci.* 97:2771–2781. <https://doi.org/10.3168/jds.2013-7366>.
- Giordano, J. O., M. Stangaferro, R. Wijma, W. Chandler, and R. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. *J. Dairy Sci.* 98:2488–2501. <https://doi.org/10.3168/jds.2014-8961>.

- Giordano, J. O., M. C. Wiltbank, J. N. Guenther, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. *J. Dairy Sci.* 95:639–653. <https://doi.org/10.3168/jds.2011-4418>.
- Goravanahally, M. P., M. Salem, J. Yao, E. K. Inskeep, and J. A. Flores. 2009. Differential gene expression in the bovine corpus luteum during transition from early phase to midphase and its potential role in acquisition of luteolytic sensitivity to prostaglandin F₂ alpha. *Biol. Reprod.* 80:980–988. <https://doi.org/10.1095/biolreprod.108.069518>.
- Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336–341. <https://doi.org/10.3168/jds.2010-3170>.
- Helmer, S. D., and J. H. Britt. 1985. Mounting behavior as affected by stage of estrous cycle in Holstein heifers. *J. Dairy Sci.* 68:1290–1296.
- Jinks, E. M., M. F. Smith, J. A. Atkins, K. G. Pohler, G. A. Perry, M. D. MacNeil, A. J. Roberts, R. C. Waterman, L. J. Alexander, and T. W. Geary. 2013. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. *J. Anim. Sci.* 91:1176–1185. <https://doi.org/10.2527/jas.2012-5611>.
- Kuhn, M. T., J. L. Hutchison, and G. R. Wiggans. 2006. Characterization of Holstein heifer fertility in the United States. *J. Dairy Sci.* 89:4907–4920.
- Lopes, G., Jr., C. R. Johnson, L. G. D. Mendonça, P. R. B. Silva, J. G. N. Moraes, A. Ahmadzadeh, J. C. Dalton, and R. C. Chebel. 2013. Evaluation of reproductive and economic outcomes of dairy heifers inseminated at induced estrus or at fixed time after a 5-day or 7-day progesterone insert-based ovulation synchronization protocol. *J. Dairy Sci.* 96:1612–1622. <https://doi.org/10.3168/jds.2012-5971>.
- Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. *Anim. Reprod. Sci.* 81:209–223.
- Madureira, A. M., B. F. Silper, T. A. Burnett, L. Polsky, L. H. Cruppe, D. M. Veira, J. L. Vasconcelos, and R. L. Cerri. 2015. Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. *J. Dairy Sci.* 98:7003–7014. <https://doi.org/10.3168/jds.2015-9672>.
- Martins, J. P. N., R. K. Policelli, L. M. Neuder, W. Raphael, and J. R. Pursley. 2011a. Effects of cloprostenol sodium at final prostaglandin F_{2α} of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. *J. Dairy Sci.* 94:2815–2824. <https://doi.org/10.3168/jds.2010-3652>.
- Martins, J. P. N., R. K. Policelli, and J. R. Pursley. 2011b. Luteolytic effects of cloprostenol sodium in lactating dairy cows treated with G6G/Ovsynch. *J. Dairy Sci.* 94:2806–2814. <https://doi.org/10.3168/jds.2010-3650>.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Pursley, J. R., J. P. N. Martins, C. Wright, and N. D. Stewart. 2012. Compared to dinoprost tromethamine, cloprostenol sodium increased rates of estrus detection, conception and pregnancy in lactating dairy cows on a large commercial dairy. *Theriogenology* 78:823–829. <https://doi.org/10.1016/j.theriogenology.2012.03.032>.
- Reeves, P. R. 1978. Distribution, elimination, and residue studies in the cow with the synthetic prostaglandin estrumate. *J. Agric. Food Chem.* 26:152–155. <https://doi.org/10.1021/jf60215a001>.
- Reith, S., and S. Hoy. 2018. Review: Behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle. *Animal* 12:398–407. <https://doi.org/10.1017/S1751731117001975>.
- Rutten, C. J., W. Steeneveld, C. Inchaisri, and H. Hogeveen. 2014. An ex ante analysis on the use of activity meters for automated estrus detection: To invest or not to invest? *J. Dairy Sci.* 97:6869–6887.
- Sangsrivong, S., D. K. Combs, R. Sartori, L. E. Armentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *J. Dairy Sci.* 85:2831–2842. [https://doi.org/10.3168/jds.S0022-0302\(02\)74370-1](https://doi.org/10.3168/jds.S0022-0302(02)74370-1).
- Sartori, R., J. M. Haughian, R. D. Shaver, G. J. M. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J. Dairy Sci.* 87:905–920. [https://doi.org/10.3168/jds.S0022-0302\(04\)73235-X](https://doi.org/10.3168/jds.S0022-0302(04)73235-X).
- Shrestha, H. K., M. A. Beg, R. R. Burnette, and O. J. Ginther. 2012. Plasma clearance and half-life of prostaglandin F_{2α}: A comparison between mares and heifers. *Biol. Reprod.* 87:18. <https://doi.org/10.1095/biolreprod.112.100776>.
- Silper, B. F., A. M. L. Madureira, M. Kaur, T. A. Burnett, and R. L. A. Cerri. 2015. Short communication: Comparison of estrus characteristics in Holstein heifers by 2 activity monitoring systems. *J. Dairy Sci.* 98:3158–3165.
- Stevenson, J. S., S. L. Hill, R. L. Nebel, and J. M. DeJarnette. 2014. Ovulation timing and conception risk after automated activity monitoring in lactating dairy cows. *J. Dairy Sci.* 97:4296–4308. <https://doi.org/10.3168/jds.2013-7873>.
- Stevenson, J. L., J. A. Rodrigues, F. A. Braga, S. Bitente, J. C. Dalton, J. E. P. Santos, and R. C. Chebel. 2008. Effect of breeding protocols and reproductive tract score on reproductive performance of dairy heifers and economic outcome of breeding programs. *J. Dairy Sci.* 91:3424–3438. <https://doi.org/10.3168/jds.2007-0804>.
- Stevenson, J. S., and P. Phatak. 2010. Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. *Theriogenology* 73:1127–1138. <https://doi.org/10.1016/j.theriogenology.2010.01.014>.
- Stevenson, J. S., J. F. Smith, and D. E. Hawkins. 2000. Reproductive outcomes for dairy heifers treated with combinations of prostaglandin F_{2α}, norgestomet, and gonadotropin-releasing hormone. *J. Dairy Sci.* 83:2008–2015. [https://doi.org/10.3168/jds.S0022-0302\(00\)75079-X](https://doi.org/10.3168/jds.S0022-0302(00)75079-X).
- Valdecabres-Torres, X., E. García-Roselló, A. García-Muñoz, and J. Cuervo-Arango. 2012. Effects of d-cloprostenol dose and corpus luteum age on ovulation, luteal function, and morphology in nonlactating dairy cows with early corpora lutea. *J. Dairy Sci.* 95:4389–4395. <https://doi.org/10.3168/jds.2011-5284>.
- Veronese, A., O. Marques, F. Penagaricano, R. S. Bisinotto, K. G. Pohler, T. R. Bilby, and R. C. Chebel. 2019. Genomic merit for reproductive traits II: Physiological responses of Holstein heifers. *J. Dairy Sci.* 102:6639–6648. <https://doi.org/10.3168/jds.2018-15245>.
- Wiltbank, M., H. Lopez, R. Sartori, S. Sangsrivong, and A. Gümen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology* 65:17–29. <https://doi.org/10.1016/j.theriogenology.2005.10.003>.
- Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglandin F_{2α} during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *J. Dairy Sci.* 98:8644–8654. <https://doi.org/10.3168/jds.2015-9353>.