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Timing of artificial insemination using fresh or frozen semen after automated activity monitoring of estrus in lactating dairy cows

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ABSTRACT

The objective of this observational experiment was to determine the association between the time of artificial insemination (AI) and pregnancy per AI (P/AI) in lactating Holstein cows inseminated with either fresh or frozen semen considering different characteristics of an estrus event (i.e., onset, peak, and end) using an automated activity monitoring system. A total of 3,607 AI services based on the alert of an automated activity monitoring system (Heatime; SCR Engineers Ltd., Netanya, Israel) were evaluated from 4 commercial dairy farms in Germany. Pregnancy diagnosis was performed by transrectal palpation 38 ± 3 d after AI or by transrectal ultrasonography 30 ± 3 d after AI. Estrus intensity was categorized based on peak activity of estrus (PAE) into low (35–89 index value) and high (90–100 index value) intensity. The mean (± standard deviation) duration of an estrus event was 14.3 ± 4.6 h. The mean (± standard deviation) interval from onset of estrus (OE; moment where index value was ≥35) to AI was 16.8 ± 8.0 h, from PAE to AI was 11.9 ± 8.1 h, and from end of estrus (EE; moment where index value returned to <35) to AI was 2.5 ± 8.7 h. Primiparous cows had greater P/AI than multiparous cows, whereas first AI postpartum yielded greater P/AI compared with subsequent AI services. Type of semen was not associated with P/AI. Cows with heat stress 1 wk before AI had decreased P/AI. Cows with low estrus intensity (26.0%) were less fertile compared with cows showing high estrus intensity (32.8%). Cows with intermediate 100-d milk yield had decreased P/AI compared with cows with either low or high 100-d milk yield. There was a quadratic effect of the interval from OE to AI on P/AI. At 38 d after AI, P/AI was greatest for cows inseminated from 7 to 24 h after OE, within 18 h after PAE, or from 5 h before EE to 12 h after EE. There was no interaction between the interval from OE to AI and type of semen. There tended to be an interaction between the intervals from PAE to AI and type of semen and from EE to AI and type of semen. Cows inseminated with fresh semen within 5 h before EE had greater P/AI compared with frozen semen, whereas cows inseminated with frozen semen from 13 to 18 h after EE had greater P/AI compared with fresh semen. In conclusion, inseminating cows from 7 to 24 h after OE or 1 to 18 h after PAE yielded greatest P/AI irrespective of type of semen. In addition, high estrus intensity was positively associated with P/AI.
Key words: insemination time, activity monitor, pregnancy, cow

INTRODUCTION

Estrus detection in confined dairy cows is a great challenge as milk production is increased (López-Gatius et al., 2005; Rivera et al., 2010) and cows are less likely to express estrous behavior on concrete surfaces (Britt et al., 1986). Estrus detection rate in a recent survey of Canadian herds was below 50% (Denis-Robichaud et al., 2016). The failure to submit cows for AI not only has a major effect on reproductive performance but also indicates unique potential to improve profitability (Overton and Cabrera, 2017). To improve efficiency and accuracy of estrus detection, automated activity monitoring systems (**AAM**) have been implemented on many farms. A new generation of AAM has been developed using accelerometers capable of detecting motion in all 3 spatial planes in real time. These systems continuously monitor physical activity to predict adequate timing of AI (Fricke et al., 2014). The time of ovulation relative to signs of estrus was similar among studies using AAM (Valenza et al., 2012: 28.7 ± 8.1 h; Stevenson et al., 2014: 25.7 ± 0.4 h; Burnett et al., 2018: 25.8 ± 10.2 h) but highly variable among cows,

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Table 1. Descriptive information of the reproductive management from the 4 participating herds

Item	Herd A	Herd B	Herd C	Herd D
Average herd size (no. of cows)	1,224	716	427	558
Herd average 305-d milk yield (kg)	9,939	9,677	9,461	9,938
Voluntary waiting period (DIM)	42	40	45	42
Heat detection rate (%)	41	40	36	57
Conception rate (%)	28	28	38	28
21-d pregnancy rate (%)	12	11	14	16
Day of pregnancy diagnosis after AI	38 ± 3	28 ± 3	38 ± 3	32 ± 3
Method of pregnancy diagnosis	Transrectal palpation	Transrectal ultrasound	Transrectal palpation	Transrectal ultrasound

leading to potential errors in the timing of AI in relation to ovulation (Roelofs et al., 2006; Valenza et al., 2012).

Precise identification of the optimum timing of AI relative to activity data from AAM, therefore, is challenging, and few studies with this objective are available. In a large US field study, Stevenson et al. (2014) observed that the optimum interval from onset of estrus (**OE**) to AI differed between primiparous cows (13–16 h) and multiparous cows (0–12 h). In a field study from Canada (LeRoy et al., 2018), there was no difference in pregnancy per AI (**P/AI**) when multiparous cows were inseminated 0 to 24 h after OE. For primiparous cows, the odds of pregnancy were greatest if AI occurred 0 to 8 h after the activity alert.

All of these studies used frozen semen for AI; a similar study evaluating optimum timing for AI with fresh semen after spontaneous estrus is missing. Results from a recent study (Borchardt et al., 2018) indicated that the optimum timing of AI might be different for fresh semen compared with frozen semen using 2 different timed AI (**TAI**) protocols. The authors speculated that viability of sperm in the female reproductive tract might be extended using fresh semen in a TAI protocol with a suboptimum timing of AI relative to ovulation (Cosynch-56). In fact, P/AI was greater for cows inseminated with fresh semen compared with frozen semen in a Cosynch protocol (27.5% vs. 20.0%, respectively; $P = 0.03$). Using an Ovsynch protocol with a more consistent timing of AI relative to ovulation, P/AI did not differ between fresh (32.2%) and frozen (28.6%) semen ($P = 0.33$).

Therefore, the objective of this study was to determine the association between the time of AI on P/AI in lactating Holstein cows inseminated with either fresh or frozen semen considering different characteristics of an estrus event (i.e., onset, peak, and end) using an AAM. We tested whether cows inseminated closer to the onset of an estrus event would achieve greater P/AI with fresh semen compared with frozen semen. Furthermore, we tested whether P/AI would differ between fresh and frozen semen for cows inseminated closer to the end of an estrus event.

MATERIALS AND METHODS

Study Design

This study was an observational cohort study including 6,603 AI services from 2,575 lactating Holstein cows (1,049 primiparous and 1,526 multiparous cows) from 4 commercial dairy farms in northeast Germany from July 2018 until May 2019. Inclusion criteria for herds were a herd size above 400 cows and the use of a neck-mounted AAM (Heatime; SCR Engineers Ltd., Netanya, Israel). Herd size ranged from approximately 430 to 1,200 cows per farm (Table 1). All cows were housed in freestall barns and milked twice daily. Milk yield ranged from 9,461 to 9,939 kg/305 d. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

AAM

All cows were fitted with a neck-mounted AAM on the day of their first calving (herds A, B, D) or at approximately 40 DIM (herd C). The AAM was attached to the cows continuously (herds A, B, D) or was removed at 65 d after AI if a cow was diagnosed pregnant (herd C). Individual activity of each cow was recorded every 2 h by a wireless receiver box and transmitted to the accelerometer software (DataFlow II, SCR Engineers Ltd.) installed on the farm computer. The activity raw data from each cow were converted into an activity change index value using a proprietary algorithm (Figure 1). Index values for activity change ranged from 0 to 100 [0 = lowest (baseline), 100 = highest]. Onset of estrus was defined as a cow exceeding an activity change index value of 35 (Madureira et al., 2015). End of estrus (**EE**) was defined by the first instance at which the index value fell below 35 again. The intensity of an estrus event (peak activity of

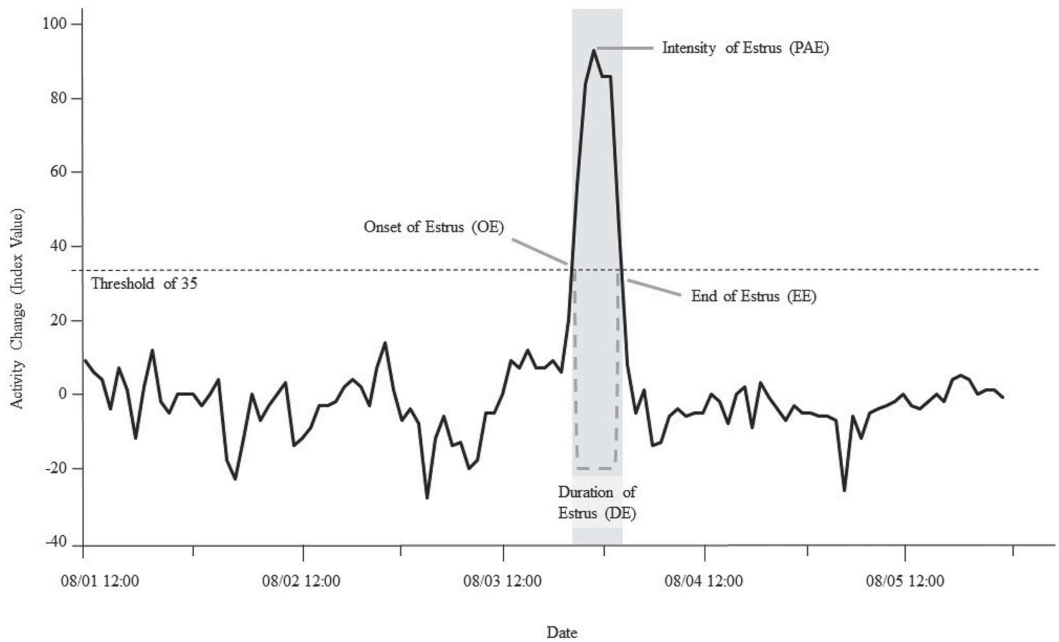

Figure 1. Schematic representation of a cow's activity change at an alert and estrus event characteristics used in the study generated by the DataFlow II software of Heatime (SCR Engineers Ltd., Netanya, Israel). Raw activity of each cow was continuously recorded in 2-h periods (dates are recorded as month/day) and converted into an activity change index value using a proprietary algorithm. Onset of estrus (OE) was defined as a cow exceeding an activity change index value of 35. End of estrus (EE) was defined by the first instance at which the index value fell below 35 again. Intensity of an estrus event (peak activity of estrus; PAE) was represented by the peak of the activity change index value during an estrus event. Duration of an estrus event (DE) was defined as the interval from onset to end of an estrus event.

estrus; **PAE**) was represented by the peak of the activity change index value during an estrus event. Estrus intensity was classified into 2 categories, low (35–89 index value) and high (90–100 index value). Duration of an estrus event (**DE**) was defined as the interval from onset to end of an estrus event. All characteristics were determined for each estrus event, and plausible intervals from OE to AI (0 to 48 h), PAE to AI (−6 to 48 h), and EE to AI (−12 to 48 h) were calculated for each cow.

Files from the SCR system were exported using DataFlow II on a weekly basis for all cows that were inseminated within the last 7 d. These files were generated in the XLSX format (Microsoft Office 2013, Microsoft Corp., Redmond, WA). Each file contained the following information: cow ID, lactation number, last breeding date, AI number, DIM, days from last breeding, and last calving date. In addition, raw activity data, daily activity, and activity change were recorded every 2 h for each cow within the past 7 d. A software tool written in the open-source Python programming language (Python Software Foundation, Wilmington, DE) was used to process the AAM data from all XLSX files and generated a single result report XSLX file (J. L. Plenio, A. Bartel, Institute for Veterinary Epidemiology and Biostatistics, Freie Universitaet Berlin, Berlin, Germany; A. M. L. Madureira, R. L. A. Cerri, Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada; W. Heuwieser, S. Borchardt, Clinic for Animal Reproduction, Freie Universitaet Berlin, Berlin, Germany; unpublished data). The report formatted in the wide (i.e., 1 line for each cow) and long (i.e., 1 line for each estrus event) format included the following information for each cow: cow ID, lactation number, last breeding date, AI number, DIM at AI, raw activity data (peak value and hour of raw activity peak), activity change (index value and hour for activity change start, peak, and end), and DE for each estrus event.

Reproductive Management

Lactating Holstein cows were inseminated based on the alert of the AAM, after visual estrus detection, or as part of a TAI program. Inclusion criteria for cows were as follows: past the voluntary waiting period (Table 1) and eligible for breeding. Exclusion criteria after enrollment in the study were as follows: hormonal treatments before AI (n = 1,900) such as PGF (n = 268), GnRH (n = 978), or an Ovsynch protocol (n = 654); the use of sex-sorted semen (n = 9); and culling before a pregnancy diagnosis (n = 165). A list of cows eligible for breeding based on an activity alert was generated by the AAM. All farms set their threshold for an activity alert at an index of 35. Based on these lists, estrus was verified by the AI technician via transrectal palpation of a highly contractile uterus, or visualization of clear, stringy vaginal discharge, or both. Eligible cows were inseminated at different intervals related to the AAM alert. In herds A and C, lists of cows eligible for breeding were printed at 0700 h, and cows were inseminated once daily (herd A at ~1400 h; herd C at ~0800 h). In herds B and D, lists of cows eligible for breeding were printed twice daily (0300 and 1300 h). Cows in these herds were inseminated twice daily (in the early morning or in the afternoon). Cows were inseminated with either fresh or frozen semen based on individual farm strategies. Cows were not randomly allocated to receive either fresh or frozen semen. Fresh semen was predominantly allocated to cows receiving TAI, and therefore only a limited number of straws were delivered to farms once per week. Therefore, the use of fresh semen for cows inseminated based of the AAM alert in the study was dependent on the number of straws left over from TAI on that specific day. Cows remained in the study until a confirmed pregnancy diagnosis (Table 1), which was performed on a weekly basis by transrectal palpation 38 ± 3 d after AI (herds A and C) or by transrectal ultrasound beginning at 28 ± 3 d after AI (herd B) or at 32 ± 3 d after AI (herd D). For simplicity, the time at which pregnancy diagnosis was conducted is referred to as 38 d after AI throughout this paper. In case of transrectal palpation, pregnancy was based on a verified pregnancy diagnosis defined by the presence of uterine fluid, asymmetry, and a positive fetal membrane slip. Non-pregnancy was based on absence of pregnancy at the day of examination or a rebreeding to an estrus before pregnancy diagnosis (Wiltbank et al., 2015). Positive pregnancy diagnosis performed by ultrasound was based on visualization of an embryo with a heartbeat. Cows diagnosed not pregnant were reassigned to breeding after spontaneous estrus or TAI.

Semen for this study was predominantly produced by genomically selected Holstein-Friesian bulls from 2 local AI centers (RinderAllianz GmbH, Woldegk, Germany, and Rinderproduktion Berlin-Brandenburg GmbH, Groß Kreutz, Germany). Sires were not randomly allocated to the farms due to farm-specific breeding objectives. Ejaculates for producing fresh and frozen semen were collected using an artificial vagina per standard operating procedure twice a week (Monday and Wednesday) between 0600 and 0800 h. Ejaculates meeting the required quality standards (i.e., >70% progressive motility; >1.5 mL volume; $>500 \times 10^6$ spermatozoa) were used for semen production. All ejaculates failing to meet these criteria were excluded from the study. The amount of fresh semen produced from each ejaculate was based on the requested number of straws for that day. The remaining semen was used for the production of frozen semen. Fresh semen was diluted using Caprogen liquid semen extender (Vishwanath and Shannon, 2000) to 10×10^6 spermatozoa/straw. After sealing, the 0.25-mL straws of fresh semen were dispatched to the farm technicians in the evening of the day of collection. Fresh semen was transported and stored in thermal insulated canisters with a temperature of 10 to 15°C until insemination, which happened within 3 d after delivery. Frozen semen was diluted using OptiXcel liquid semen extender (IMV Technologies, Normandy, France) to 20×10^6 spermatozoa/straw. Frozen semen was stored in 0.25-mL straws within a canister filled with liquid nitrogen at −196°C and allocated to the farms as required. For thawing, straws were placed in a water bath at 37°C for 15 s.

Data Collection and Statistical Analyses

Cow ID, parity, DIM, breeding date and time, number of AI, milk yield for the first 100 DIM, AI code (insemination according to AAM alert or hormone treatment), and type of semen used for AI were obtained through the on-farm computer software (herdeW and herdeplus, respectively; dsp-Agrosoft GmbH, Ketzin/Havel, Germany). If information about breeding time was not captured in the herd management software, it was documented on a list that was obtained on a monthly basis. All data were transferred to Microsoft Excel (Microsoft Corp.). The ambient temperature and relative humidity within each pen where eligible cows were housed were recorded using a Tinytag Plus II logger (Gemini Loggers Ltd., Chichester, UK). The logger was secured at a beam 3 m from the ground and every hour measured ambient temperature from −25°C to 85°C with an accuracy of ± 0.3°C and a resolution of 0.01°C. Relative humidity was measured every hour from 0 to 100% with an accuracy of ±3.0% and a resolution of 0.3%. The temperature-humidity index (**THI**) was calculated following the equation reported by NRC (1971):

$$\mathrm{THI} = (1.8 \times \mathrm{T} + 32) - [(0.55 - 0.0055 \times \mathrm{RH}) \times (1.8 \times \mathrm{T} - 26)],$$

where T = dry bulb temperature and RH = relative humidity. Data from the logger were transferred to Mi-

Table 2. Quartiles (Q1–Q4) of cumulative milk yield (kg) within the first 100 DIM from enrolled herds stratified by herd and parity

Quartile	Herd A	Herd B	Herd C	Herd D
Primiparous cows				
Q1	≤3,152	≤3,013	≤2,446	≤3,073
Q2	3,153–3,410	3,014–3,324	2,447–2,946	3,074–3,381
Q3	3,411–3,729	3,325–3,676	2,947–3,172	3,382–3,703
Q4	>3,729	>3,676	>3,172	>3,703
Multiparous cows				
Q1	≤4,050	≤4,018	≤3,438	≤3,416
Q2	4,051–4,483	4,019–4,472	3,439–3,698	3,417–3,822
Q3	4,484–4,899	4,473–4,881	3,699–4,261	3,823–4,322
Q4	>4,899	>4,881	>4,261	>4,322

crosoft Excel. The mean THI measured 1 wk before AI was used and classified into the categories low (<60), medium (60–68), and high (>68). For each of the 4 farms, cumulative milk yield within the first 100 DIM was classified into quartiles stratified by parity (Table 2).

All statistical analyses were performed using SPSS for Windows (version 22.0; SPSS Inc., Chicago, IL). To determine optimum timing of AI considering 3 intervals (from OE to AI, from PAE to AI, and from EE to AI), 3 different logistic regression models using the GENLINMIXED procedure of SPSS were built. Herd was considered a random effect. The unit of interest was the insemination, and cow (nested within farm) was included as a random effect because some cows contributed more than 1 AI. A chi-squared test was used to evaluate whether there was circadian dynamic of OE and whether parity was associated with the frequency of high estrus intensity. Model building was conducted as recommended by Dohoo et al. (2009), where each parameter was first analyzed separately in a univariable model. Only parameters resulting in univariable models with $P \leq 0.10$ were included in the final mixed model. Because there was a strong correlation (r = 0.66; $P < 0.01$) between estrus intensity and estrus duration, we only considered estrus intensity in the statistical model. Selection of the model that best fit the data was performed by using a backward stepwise elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), type of semen (fresh vs. frozen semen), interval to AI (OE to AI vs. PAE to AI vs. EE to AI), estrus intensity (low vs. high), AI number (first AI vs. subsequent AI), THI (<60 vs. 60–68 vs. >68), and cumulative milk yield within 100 DIM [quartile (**Q**) 1 to Q4].

We tested all biologically plausible 2-way interactions. Because there were no interactions between type of semen and parity ($P = 0.42$), type of semen and THI ($P = 0.39$), type of semen and estrus intensity ($P = 0.35$), type of semen and 100-d milk yield ($P = 0.45$), parity and the interval from OE to AI ($P = 0.47$), parity and the interval from PAE to AI ($P = 0.77$), parity and the interval from EE to AI ($P = 0.30$), or parity and estrus intensity ($P = 0.40$) on P/AI, these interactions were not included in the final statistical model. Regardless of the significance level, type of semen, interval (OE, PAE, or EE) to AI, and the interaction between interval (OE, PAE, or EE) to AI and type of semen were forced to remain in the final model. The final model therefore contained the following fixed effects: parity, interval (OE, PAE, or EE) to AI, estrus intensity, AI number, THI, cumulative 100-d milk yield, type of semen, and the interaction between the type of semen and the interval (OE, PAE, or EE) to AI. A Bonferroni adjustment was used to account for multiple comparisons.

Variables were declared to be significant when $P < 0.05$. A statistical tendency was declared when $P \geq 0.05$ and $P \leq 0.10$.

RESULTS

Overall, 2,575 cows were enrolled in this experiment, including activity information from 6,603 AI services. After exclusion of 2,996 AI services (45.4%) due to hormonal treatments before AI (n = 1,900), AI before an activity alert or services with incomplete activity data (n = 922), use of sex-sorted semen (n = 9), and culling before a pregnancy diagnosis (n = 165), a total of 3,607 AI services representing 1,979 cows (805 primiparous and 1,174 multiparous cows) were included in the final statistical analyses.

The mean (±SD) DE was 14.3 ± 4.6 h. The circadian distribution of cows reaching OE was similar during the day ($P = 0.13$). The mean interval from OE to AI was 16.8 ± 8.0 h, from PAE to AI was 11.9 ± 8.1 h, and from EE to AI was 2.5 ± 8.7 h. The mean index value of PAE was 90.7 ± 14.6; 31.3% of cows had low estrus intensity and 68.7% had high estrus intensity. There was no association ($P = 0.88$) between parity and the frequency of high estrus intensity.

Overall P/AI was 29.3%. Primiparous cows (31.4%) had greater P/AI compared with multiparous cows (27.3%; $P = 0.01$). Number of AI was associated with P/AI ($P = 0.01$), as cows receiving first AI (31.5%) had greater P/AI compared with cows receiving second or greater AI (27.2%). Pregnancy per AI did not differ ($P = 0.66$) between P/AI for cows inseminated with frozen semen (28.8%) or cows inseminated with fresh semen (29.8%). Intensity of estrus was associated with P/AI such that cows with low estrus intensity (26.0%) had decreased P/AI ($P < 0.01$) compared with cows with high estrus intensity (32.8%). Temperature-humidity index measured 1 wk before AI had an effect on P/AI ($P < 0.01$). A low THI of <60 (36.1%) was associated with increased P/AI compared with a medium THI of 60 to 68 (28.6%; $P < 0.01$) and a high THI of >68 (23.9%; $P < 0.01$), whereas P/AI did not differ ($P = 0.14$) between medium and high THI. There was an association between cumulative milk yield within 100 DIM and P/AI ($P = 0.03$), as cows within a Q2 100-d milk yield had decreased P/AI (25.7%) compared with cows within a Q1 (29.8%; $P = 0.04$), Q3 (30.3%; $P = 0.03$), or Q4 (31.6%; $P = 0.01$) 100-d milk yield.

Interval from OE to AI

There was an association between the interval from OE to AI and P/AI ($P < 0.01$; Figure 2A), as cows inseminated from 7 to 24 h after OE had greatest P/AI. Cows inseminated within 6 h after OE and 25 h or later after OE showed suboptimum P/AI (0–6 h: 20.8%; 7–12 h: 33.1%; 13–18 h: 34.9%; 19–24 h: 32.9%; 25–48 h: 26.2%). There was no interaction between the interval from OE to AI and type of semen ($P = 0.56$).

Interval from PAE to AI

There was an association between the interval from PAE to AI and P/AI ($P < 0.01$), as cows inseminated within 18 h after PAE had greatest P/AI. Cows inseminated before PAE and 19 h or later after PAE showed suboptimum P/AI (−6 to 0 h: 17.8%; 1–6 h: 33.0%; 7–12 h: 32.1%; 13–18 h: 36.4%; 19–24 h: 27.5%; 25–48 h: 21.2%). Overall, there tended to be an interaction between the interval from PAE to AI and type of semen ($P = 0.09$; Figure 2B). Cows inseminated within 6 h before PAE with frozen semen had greater P/AI compared with those inseminated with fresh semen ($P = 0.01$).

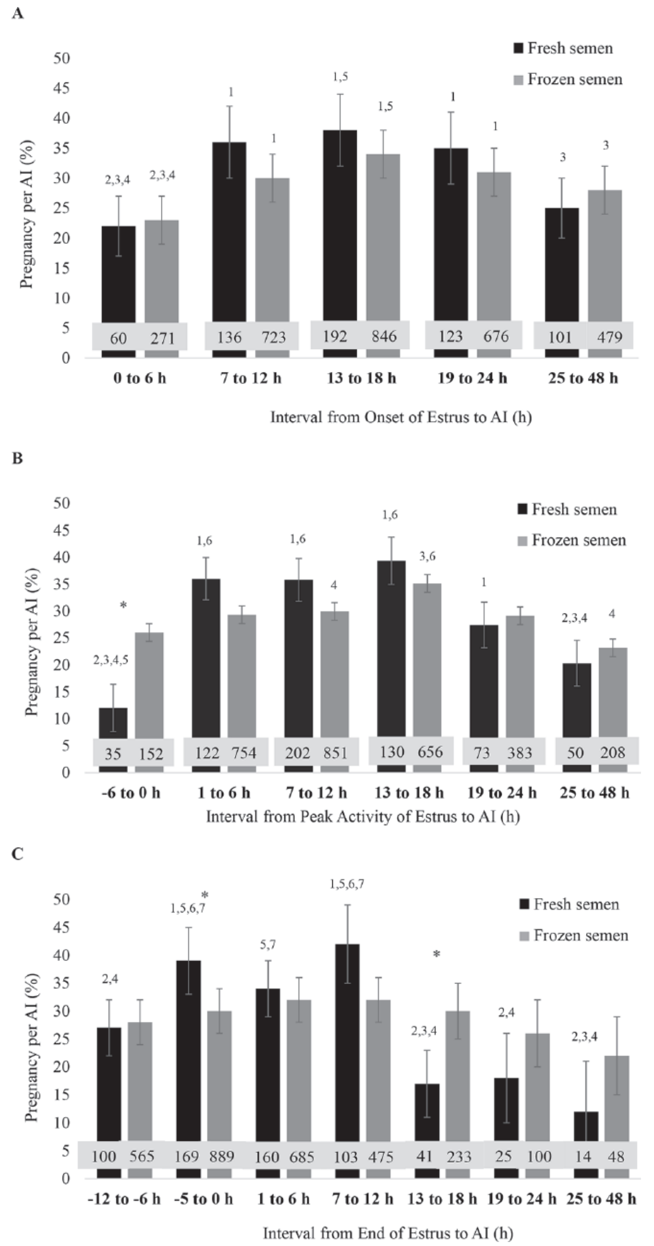

Figure 2. Pregnancy per artificial insemination (± SEM) for cows (n = 1,979) inseminated with either fresh semen (black bars) or frozen semen (gray bars) considering onset of estrus to AI (A), peak activity of estrus to AI (B), and end of estrus to AI (C) using a neck-mounted accelerometer system for estrus detection (Heatime; SCR Engineers Ltd., Netanya, Israel). Onset of estrus was defined as a cow exceeding an activity change index value of 35. Intensity of an estrus event was represented by the peak of the activity change index value during an estrus event. End of estrus was defined by the first instance at which the index value fell below 35 again. A Bonferroni adjustment was used to account for multiple comparisons. An asterisk indicates a significant difference ($P < 0.05$) between type of semen (i.e., fresh vs. frozen semen) within a time interval. Bars with different numbers indicate a significant difference ($P < 0.05$) among time intervals for either fresh or frozen semen. Therefore, time intervals were classified into numbers (1 to 5 for panel A, 1 to 6 for panel B, 1 to 7 for panel C), with number 1 beginning at the first time interval in ascending order. The number within each bar is the number of cows inseminated with this type of semen within that specific time interval.

Interval from EE to AI

There was an association between the interval from EE to AI and P/AI ($P < 0.01$). Cows inseminated from 5 h before EE to 12 h after EE had greatest P/AI. Cows inseminated more than 5 h before EE and more than 13 h after EE showed suboptimum P/AI (−12 to −6 h: 27.2%; −5 to 0 h: 34.4%; 1–6 h: 32.5%; 7–12 h: 35.8%; 13–18 h: 21.6%; 19–24 h: 21.4%; 25–48 h: 15.8%). There tended ($P = 0.07$) to be an interaction between the interval from EE to AI and type of semen (Figure 2C). Cows inseminated with fresh semen within 5 h before EE had greater P/AI ($P = 0.03$) compared with cows inseminated with frozen semen. In addition, cows inseminated with fresh semen from 7 to 12 h after EE tended ($P = 0.09$) to have greater P/AI compared with cows inseminated with frozen semen. Cows inseminated from 13 to 18 h after EE showed greater P/AI ($P = 0.03$) when inseminated with frozen semen compared with fresh semen.

DISCUSSION

The objective of this study was to determine the association between the time of AI and P/AI in lactating Holstein cows inseminated with either fresh or frozen semen considering different characteristics of an estrus event (i.e., onset, peak, and end) using an AAM. We tested (1) whether cows inseminated close to the beginning of an estrus event would achieve greater P/AI with fresh semen compared with frozen semen and (2) whether P/AI would differ between fresh and frozen semen for cows inseminated closer to the end of an estrus event.

Overall, P/AI did not differ for cows inseminated with fresh or frozen semen. This is consistent with 2 previous studies that also compared pregnancy outcomes for AI with fresh or frozen semen (Bucher et al., 2009; Murphy et al., 2017). Similar P/AI for both types of semen was observed in beef cows allocated to TAI in a progesterone (**P4**)-supplemented 7-d Cosynch protocol (Bucher et al., 2009) and in grazing dairy cows in Ireland (Murphy et al., 2017). In both studies they used a reduced number of sperm per straw for fresh semen compared with frozen semen. For fresh semen, the authors therefore assumed an extended viability in the female reproductive tract and a higher sperm quality compared with frozen semen. A case report (Crespilho et al., 2012), however, showed increased P/AI for cooled semen (5°C) compared with frozen semen after TAI (61.5% vs. 45.7%, respectively). Collectively, studies evaluating reproductive performance of fresh and frozen semen are difficult to compare due to differences in sperm number per straw, semen diluents, storage time, temperature of semen, and type of AI (i.e., spontaneous estrus vs. TAI; Bucher et al., 2009; Crespilho et al., 2009; Murphy et al., 2017). Compromised sperm function after cryopreservation and its consequences for fertility have been widely studied (Watson, 1995; Gravance et al., 1998; Curry, 2000). Nevertheless, poor fertility resulting from compromised sperm viability in the female reproductive tract can be overcome by precise timing of insemination relative to OE and ovulation (Bombardelli et al., 2016; Borchardt et al., 2018). In a study by Borchardt et al. (2018), the optimum timing of AI was influenced by type of semen, as cows enrolled in a TAI protocol with a long interval from AI to ovulation (~24 h; Cosynch-56) achieved greater P/AI when inseminated with fresh compared with frozen semen (27.5% vs. 20.0%, respectively; $P = 0.03$). However, when cows were enrolled in a TAI protocol with a shorter interval from AI to expected ovulation (12–16 h; Ovsynch-56) no effect of type of semen ($P = 0.33$) was observed. These results further support the idea that viability of sperm in the female reproductive tract is extended using fresh semen compared with frozen semen. In our study, there was no interaction between the type of semen and the interval from OE to AI, but there was a tendency for an interaction between the type of semen and the intervals from PAE and EE to AI. Discrepancies from the latter study might be due to the wide variation of ovulation timing among cows inseminated based on estrous behavior compared with cows receiving TAI (Roelofs et al., 2006; Valenza et al., 2012).

Similar intervals from onset of increased activity to ovulation were determined in 3 studies using neck-mounted AAM (Heatime), ranging from 25.7 ± 1.2 h to 28.7 ± 8.1 h (Valenza et al., 2012: 28.7 ± 8.1 h; Aungier et al., 2015: 27.0 ± 1.0 h; Bombardelli et al., 2016: 25.7 ± 1.2 h). These results are also consistent with the mean interval of 27.6 h from onset of standing estrus to ovulation determined with an electronic pressure-sensing system (Walker et al., 1996). All of these studies, however, reported a wide range of variation among cows observed for the interval from onset of activity to ovulation. In addition, one study reported 1.6 to 3.7 h longer intervals from OE, PAE, and EE to ovulation for multiparous cows compared with primiparous cows (Stevenson et al., 2014). Furthermore, variability in the DE and timing of AI relative to ovulation could contribute to poor fertility. In our experiment, the observed DE agreed with that in other studies (Dalton et al., 2001; Yoshioka et al., 2010; Burnett et al., 2018) and the circadian distribution of cows reaching OE was equal throughout the day. Whereas some studies support the latter finding (Dransfield et al., 1998; Xu et al., 1998; Bombardelli et al., 2016), others found estrous behavior to be more likely during the evening and nighttime (Hurnik et al., 1975; Esslemont and Bryant, 1976) or early morning and daylight hours (Van Vliet and van Eerdenburg, 1996).

The results from the current study underline the importance of careful timing of AI relative to specific characteristics of an estrus event identified by an AAM as the intervals from OE to AI, PAE to AI, and EE to AI were associated with pregnancy outcomes. The data suggest that adequate P/AI could be achieved when AI is performed only once daily, as cows showed greatest P/AI when inseminated from 7 to 24 h after OE. This corroborates older findings from Foote (1978) and Nebel et al. (1994) that demonstrated similar P/AI for once-daily versus twice-daily AI. Based on mounting behavior, however, the greatest pregnancy outcomes resulted from AI between 4 and 12 h after the first standing event measured by an electronic pressure-sensing system (Dransfield et al., 1998). In other studies, the greatest P/AI was found when AI was performed between 9 and 15 h after OE (Aungier et al., 2015) or after PAE (Bar, 2010), which agrees with our findings that showed increased P/AI for cows inseminated within 18 h after PAE.

Although we did not observe an interaction between parity and the intervals from OE, PAE, or EE to AI, 2 studies described differences in the optimum timing of AI for primiparous and multiparous cows using AAM (Stevenson et al., 2014; LeRoy et al., 2018). One study found greatest P/AI for primiparous cows inseminated from 13 to 16 h and for multiparous cows inseminated within 12 h after OE (Stevenson et al., 2014). Another study observed greatest P/AI for primiparous cows inseminated within 8 h after OE, whereas for multiparous cows P/AI did not differ when inseminated up to 24 h (LeRoy et al., 2018). The relative discrepancy in the results of studies is likely caused by differences in experimental design, types of AAM, and different classification intervals (our experiment: mainly 5-h intervals; Stevenson et al., 2014: 3-h intervals; LeRoy et al., 2018: 8-h intervals), as the frequency of data transfer between AAM and on-farm computer differed.

Approximately two-thirds of the cows (68.7%) expressed high estrus intensity, whereas one-third (31.3%) showed low estrus intensity. This is noteworthy because estrus intensity had a marked effect on P/AI. The positive association between high estrous expression and P/AI has been described earlier for lactating dairy cows and beef cows (Madureira et al., 2015; Davoodi et al., 2016; Polsky et al., 2017). Cows with a greater PAE had an approximately 12 percentage units greater P/AI compared with those with lower PAE (Madureira et al., 2015). Estrous expression was reported to be influenced by various factors, such as milk yield, negative energy balance, stage of lactation, parity, and THI at time of estrus (Aungier et al., 2012; Madureira et al., 2015; Polsky et al., 2017). Furthermore, higher P4 concentrations on d 10 after AI were found in cows displaying high estrus intensity at AI (Madureira et al., 2019). Cows with low estrus intensity, on the other hand, had a higher risk of ovulation failure, abnormal timing of ovulation, and pregnancy losses (Pereira et al., 2016; Burnett et al., 2018; Madureira et al., 2019). Only few studies exist, however, that found no association between estrus intensity and pregnancy outcomes (Aungier et al., 2012; Bombardelli et al., 2016). Further research is warranted to better use data from AAM to support on-farm decision making (e.g., by using PAE as a characteristic to select cows with superior estrus intensity to optimize pregnancy outcomes).

In the present study, P/AI decreased by 12.2 percentage units for cows exposed to a THI >68 1 wk before AI compared with cows exposed to a THI <60 1 wk before AI. Negative effects of heat stress include hormonal imbalances (Wilson et al., 1998), lower quality of the follicle and oocyte, and negative effects on embryo development and survival (Wolfenson et al., 1995; De Rensis and Scaramuzzi, 2003). In moderate climates, heat stress decreased P/AI at a THI threshold of 73 (Schüller et al., 2014), with the greatest negative effect on P/AI observed 21 to 1 d before AI. Exposure to a mean THI of 73 or more during this period decreased P/AI by 19 percentage units, which is similar to our results. In contrast to other studies that found a reduced likelihood of pregnancy during periods of heat stress depending on the type of semen used (Schüller et al., 2016), we did not observe an interaction between type of semen and THI.

Interestingly, cows with intermediate (Q2) cumulative milk yield within 100 DIM had decreased P/AI compared with the other quartiles. The reason for this observation remains speculative. Previous research on the effect of milk yield on P/AI has been controversial, as some studies found no effect on P/AI (Dransfield et al., 1998; López-Gatius et al., 2005; Madureira et al., 2015), whereas others found a negative effect of milk yield on P/AI (Valenza et al., 2012; Bombardelli et al., 2016; Burnett et al., 2017). When evaluating an effect of milk yield on reproductive performance, other variables closely associated with the probability of pregnancy, such as feeding management, housing, or skilled labor, are often ignored (LeBlanc, 2010). In addition, low-producing cows might be sick from diseases that also affect the reproductive tract, whereas high-producing cows are more likely to be healthy and inseminated and less likely to be culled (Gröhn and Rajala-Schultz, 2000; Santos et al., 2009).

One limitation of our study was that because we conducted this experiment as an observational cohort study, there was no option of randomizing type of semen and timing of AI. Yet our findings broaden the understanding about AAM-based estrus characteristics and subsequent timing of AI. External validity was improved by enrolling cows from 4 commercial farms in contrast to a more controlled study design on a single farm.

Another limitation of our study was that cows were not systematically confirmed as being in estrus after the alert of the AAM and thus before AI by means of P4 concentration or transrectal ultrasonography of the ovaries. Studies measuring P4 concentrations of cows detected in estrus by an AAM, however, found only few cows to be false positive (Holman et al., 2011; LeRoy et al., 2018). In the study from Holman et al. (2011), prevalence of cows that were flagged by a neck-mounted AAM and inseminated having low serum P4 ranged between 86.3% and 97.6%. As our study was a field trial with 6,603 AI services, measurement of P4 concentrations was not feasible.

CONCLUSIONS

Using an AAM, inseminating cows from 7 to 24 h after OE or 1 to 18 h after PAE yielded greatest P/AI irrespective of the type of semen (fresh vs. frozen). Cows inseminated within 6 h before PAE with frozen semen had greater P/AI compared with those inseminated with fresh semen. Inseminating cows from 5 h before to 12 h after EE yielded the greatest P/AI. Cows inseminated with fresh semen within 5 h before EE had greater P/AI compared with those inseminated with frozen semen, whereas cows inseminated with frozen semen from 13 to 18 h after EE had greater P/AI compared with those inseminated with fresh semen. Our results confirm the well-known practice of once-daily AI. About two-thirds of cows expressed high estrus intensity, which yielded the greatest P/AI. Further research is warranted to determine factors that affect estrus intensity.

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