The objective of this observational study was to evaluate the association of estrous expression within 40 days in milk (DIM) using a neck-mounted automated activity monitor (Heatime Pro; SCR Engineers Ltd.) with reproductive performance in lactating Holstein cows. A total of 2,077 cows (614 primiparous cows and 1,463 multiparous cows) from 5 commercial dairy farms were included in the statistical analyses. Activity data from the first 7 d after calving were excluded. An estrus event was defined as an activity change index ≥35 for more than 2 h. Cows were classified according to the number of estrus events from d 7 until d 40 postpartum into 3 categories: (1) no estrus event (Estrus0); (2) one estrus event (Estrus1), and (3) 2 or more estrus events (Estrus2). Generalized linear mixed models were used to analyze continuous and categorical data. Shared frailty models were used for time to event data. Overall, 52.7% of cows had no estrus event detected by an automated activity monitor system from d 7 until d 40 postpartum. Herd level prevalence of Estrus0 ranged from 37.5 to 58.4%. Estrous expression from d 7 until d 40 postpartum affected estrous duration and estrous intensity at first artificial insemination (AI). Cows in Estrus0 had the shortest duration (13.2 ± 0.33 h) compared with cows in Estrus1 (13.8 ± 0.36 h) and Estrus2 (14.8 ± 0.41 h). Cows in Estrus2 had a longer estrous duration at first postpartum AI compared with cows in Estrus1. Among Estrus0 cows, 46.2% had an estrus event with high intensity at first postpartum AI. Among cows in Estrus1 and Estrus2, 50.8 and 53.8% had an estrus event with high intensity at first postpartum AI, respectively. There was a significant difference between Estrus2 and Estrus0 and a tendency between Estrus0 and Estrus1. There was no difference between Estrus1 and Estrus2. For Estrus0, Estrus1, and Estrus2 cows, pregnancy per AI was 29.4, 30.9, and 37.8%, respectively. There was a significant difference between Estrus0 and Estrus2 and Estrus0 and Estrus1. Estrous expression from d 7 until d 40 postpartum affected time to first AI and time to pregnancy. Compared with Estrus0 cows, cows in Estrus1 [hazard risk (HR) = 1.74] and Estrus2 (HR = 1.77) had an increased hazard of being inseminated within 100 DIM. There was no difference between Estrus1 and Estrus2. Median DIM to first AI were 70, 59, and 58 for cows in Estrus0, Estrus1, and Estrus2, respectively. Comparing with Estrus0 cows, cows in Estrus1 (HR = 1.28) and Estrus2 (HR = 1.33) had an increased hazard of becoming pregnant within 200 DIM. There was no difference between Estrus1 and Estrus2. Median DIM to pregnancy were 127, 112, and 103 for Estrus0 cows, Estrus1 and Estrus2, respectively. In conclusion, cows with no estrous expression from 7 to 40 DIM had reduced estrous expression at first AI and inferior reproductive performance compared with cows that displayed estrous activity.

**Key words:** estrous expression, automated activity monitor, reproductive performance, dairy cow

**INTRODUCTION**

Reproductive performance has a major effect on profitability of dairy farms (Overton and Cabrera, 2017). Resumption of ovarian cyclicity within the voluntary waiting period (VWP) is associated with improved reproductive performance (Santos et al., 2009; Dubuc et al., 2012). A delayed resumption of cyclicity has been reported to reduce reproductive efficiency in both synchronized and unsynchronized cows (Gümen et al., 2003; Walsh et al., 2007) and was associated with an increased risk for pregnancy loss (Gümen et al., 2003; Sterry et al., 2006). Overall, the prevalence of anovula-
tion was 23.3% (ranging from 7.3 to 41.7%) within 8 US herds including 5,818 cows (Bamber et al., 2009). In a Canadian survey including 1,341 cows from 18 herds, the overall prevalence of anovulation was 19.5%, ranging from 5 to 45% within herds (Walsh et al., 2007). Risk factors for anovulation included parity (i.e., higher risk in primiparous cows), calving problems (e.g., dystocia, stillbirth, twins), excessive BW loss, negative energy balance (i.e., high nonesterified fatty acids, BHB, or both), uterine inflammation and extended dry period length (Walsh et al., 2007; Dubuc et al., 2012; Vercouteren et al., 2015).

Anovular cows are not diagnosed on a routine basis, although it has been shown that an anovulatory condition until the end of the VWP has a strong negative effect on reproductive performance and consequently on the overall farm profitability (Walsh et al., 2007; Galvão et al., 2010; Dubuc et al., 2012). Assessment of anovulation before the end of the VWP is labor intensive, requiring multiple examinations either by analyzing circulating progesterone (P4) concentrations or by visualization of a corpus luteum (CL) using transrectal ultrasound. In-line milk P4 analysis or automated activity monitoring (AAM) systems have the potential to identify cows with poor reproductive performance without additional labor. In-line milk P4 analysis has been used to demonstrate that cows with early postpartum luteal activity have improved reproductive performance (Bruinjé et al., 2017). Using a pedometer system, it has been shown that cows with more than 3 estrus events within 50 d in milk were more likely to become pregnant within 90 DIM (Yániz et al., 2006). The latter study, however, lacks external validity as only a single commercial dairy herd is used. Automated activity monitoring systems based on 3-dimensional accelerometers have become popular in recent years for estrous detection (Fricke et al., 2014). Up to now, these systems have been rarely used to assess the effect of estrous expression in early lactation on reproductive performance (Chebel and Veronese, 2020).

Therefore, the objective of this study was to evaluate the association between estrous expression detected by an AAM within 40 DIM and subsequent reproductive performance of lactating Holstein cows. Specifically, we examined associations between detected estrus events within 40 DIM and subsequent reproduction outcomes after the VWP: (1) estrous expression at first AI; (2) odds of conceiving at first AI; (3) hazard of receiving first postpartum AI by 100 DIM; and (4) hazard of getting pregnant by 200 DIM.

**MATERIALS AND METHODS**

**Animals, Housing, and Nutrition**

This study was an observational cohort study including 2,077 lactating Holstein cows (614 primiparous cows and 1,463 multiparous cows) from 5 commercial dairy farms in northeast Germany calving from May 2018 until September 2019. Inclusion criteria for farms included a herd size of >400 cows and the use of a neck-mounted AAM (Heatime Pro, SCR Engineers Ltd.). Herd size ranged from approximately 558 to 1,224 cows per farm (Table 1). All cows were housed in freestall barns and milked twice or thrice daily. Milk yield ranged from 9,105 to 10,520 kg per 305 d (Table 1). Exclusion criteria for cows were “do-not-breed” status within the VWP, culling before first postpartum AI or before pregnancy diagnosis, and <95% usable AAM data from 7 to 40 DIM.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

**Automated Activity Monitor**

All cows were fitted with a neck-mounted AAM 14 d before their first calving (farm 3), or on the day of their first calving (farms 1, 2, 4, and 5). We evaluated activity data for each cow from calving until d 40

| Table 1. Descriptive information of enrolled commercial dairy farms in northeast Germany |
|-----------------------------------------------|------------------|------------------|------------------|------------------|------------------|
| Variable                                      | Farm             |
| Average herd size                             | 1                | 2                | 3                | 4                | 5                |
| Average 305-d milk yield (kg)                  | 9,339            | 9,677            | 9,105            | 9,938            | 10,520           |
| Milking frequency per day                      | 2                | 2                | 2                | 3                | 3                |
| Voluntary waiting period (DIM)                 | 42               | 40               | 40               | 42               | 40               |
| Heat detection rate (%)                        | 41               | 40               | 49               | 57               | 48               |
| Conception rate (%)                           | 28               | 28               | 36               | 28               | 35               |
| 21 d pregnancy rate (%)                       | 12               | 11               | 18               | 16               | 17               |
| Day of pregnancy diagnosis after AI           | 38               | 28               | 32               | 32               | 35               |
| Method of pregnancy diagnosis                 | Transrectal palpation | Transrectal ultrasound | Transrectal ultrasound | Transrectal ultrasound | Transrectal palpation |

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postpartum and activity data at the first postpartum AI. Individual activity data of each cow were recorded in real time for 2-h periods by a wireless receiver box and transmitted to the on-farm computer, where the accelerometer software (DataFlow II, SCR Engineers Ltd.) was installed. The raw activity data from each cow was converted into an activity change index using a proprietary algorithm. This algorithm calculates the difference of today’s last 2 h of raw activity from the mean of last week’s activity in the same period of the day weighted by the standard deviation of this specific cow. Index values for activity change ranged from 0 to 100 (0 = lowest, 100 = highest). An estrus event within 40 DIM was defined as activity change index ≥35 for >2 h. Activity change index ≥35 was the default setting of the SCR system installed on all participating farms. For each estrus event, peak activity (PA) and duration (DU) were determined. The intensity of an estrus event was represented by the maximum peak value of the activity change index during an estrus event. Onset of estrus was defined as the time a cow reached an activity change index of ≥35. End of estrus was defined by the first instance at which the activity change index fell below 35 during the event. Estrous duration was defined as the interval from onset to end of an estrus event.

Two files were generated for each cow. One included activity data from calving until d 40 postpartum, and the other included activity data from 7 d prior the first AI. Files from the SCR system were exported using DataFlow II. Each file contained the following information: cow ID, lactation number, last breeding date, AI number, DIM, and last calving date. In addition, raw activity data, daily activity, and activity change were recorded for each cow every 2 h. These files were generated in the XLSX format (Office 2013, Microsoft Deutschland Ltd.) using DataFlow II. A software tool (https://doi.org/10.5281/zenodo.3890126), written in the open-source Python programming language (Python Software Foundation), was used to process all XLSX files and generated a single result report XLSX file. The report, formatted in the wide (i.e., one line for each cow) and long format (i.e., one line for each estrus event), included the following information for each cow: cow ID, lactation number, calving data, percentage of usable activity data from d 7 until d 40 postpartum, number of estrus events within 40 DIM, activity change (index value and hour for activity change start, peak, and end), and DU of estrus for each estrus episode. Activity data from the first 7 d after calving were excluded because the system needs time to generate baseline activity data after calving. A large proportion of cows (38.2%) had <95% of usable activity data. These cows were removed from the study. The reason for missing activity data remains speculative, but it may have been due to malfunction of the sensor or data transmission.

Cows were classified according to the number of estrus events from d 7 until d 40 postpartum in 3 categories: (1) no estrus event (Estrus0); (2) one estrus event (Estrus1); (3) two or more estrus events (Estrus2). Estrous intensity was categorized into weak (0–89 activity change index) and strong (90–100 activity change index) PA based on a previous study (Tippenhauer et al., 2021).

Reproductive Management

Lactating Holstein cows were inseminated based on the alert of the AAM, after visual estrous detection or receiving AI after hormonal intervention. A list of cows eligible for breeding with an activity alert was generated on a daily basis by the AAM on each farm. Based on these lists, cows were inseminated predominantly based on an AAM alert. Cows were inseminated once (farms 1, 5) or twice daily (farms 2–4) following the am-pm rule with each cow receiving a single AI based on the AAM alert. Cows that were not bred until a farm specific threshold were either examined by the local veterinarian (farm 2: 80 DIM; farm 3: 70 DIM; farm 5: 100 DIM) and treated accordingly (i.e., cows with a corpus luteum received prostaglandin) or they received a timed AI (TAI) using a simple Ovsynch protocol (GnRH–7 d–PGF–56 h–GnRH–16 h–AI; DIM at enrollment farm 1: 80 DIM; farm 4: 70 DIM).

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 (farm 1) and 35 ± 3 d after AI (farm 5), by transrectal ultrasound beginning at 28 d after AI (farm 2) or at 32 d after AI (farms 3 and 4). 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. Nonpregnancy was based on absence of pregnancy at the day of examination or a rebreeding to an estrus before pregnancy diagnosis. 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 following a nonpregnancy diagnosis and a hormonal intervention. Open cows with a CL received prostaglandin and were bred upon estrous detection. Open cows without a CL received TAI using a simple Ovsynch protocol as described above.
Data Collection and Statistical Analyses

Cow ID, parity, calving date, and breeding information [i.e., DIM, breeding code (estrus vs. hormonal intervention), outcome] were obtained through the on-farm computer software (herdeW and herdeplus, respectively; dsp-Agrosoft GmbH; https://www dsp-agrosoft.de/).

All statistical analyses were performed using SPSS for Windows (version 22.0; IBM; https://www.ibm.com/analytics) or R (version 4.0.2; https://www.r-project.org/).

To evaluate the association between estrous expression detected by an AAM system within 40 DIM and reproductive performance of lactating Holstein cows, a linear regression model (DU at first AI) and 2 logistic regression models [pregnancy per AI (P/AI) at first AI and probability of high intensity estrus event at first AI] were built using the GENLINMIXED procedure of SPSS. Herd was considered a random effect. Cow was nested within farm. Parity was considered as a repeated measure because some cows had more than one calving within the observation period. Model building was conducted as recommended by Dohoo et al. (2009), where each parameter was first analyzed separately in an univariable model using the GENLINMIXED procedures as described above. Only parameters resulting in univariable models with \( P \leq 0.10 \) were included in the final mixed models. Selection of the model that best fit the data was performed using a backward stepwise elimination procedure by removing all variables with \( P > 0.10 \) from the model. The initial models included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), year of AI, season of AI (winter from December 1 to February 28, spring from March 1 to May 31, summer from June 1 to August 31, and autumn from September 1 to November 30), DIM at first AI, estrous activity within 40 DIM (Estrus0 vs. Estrus1 vs. Estrus2), breeding code at first AI (estrus vs. hormonal intervention).

Cox proportional hazards were used to model the time to event outcomes (i.e., time to first AI, time to pregnancy) while accounting for herd as a random effect (shared frailty term; cows within farm) and a random effect for cow for repeated observations of the same cow in different lactations. Cows were censored if they were culled before first insemination or pregnancy diagnosis or at the end of the observation period. The variables parity, year of calving, season of calving, and estrous activity within 40 DIM were tested as risk factors. The proportional hazard assumption was checked using Schoenfeld residuals. Frailty models were fitted in R version 4.0.2 (https://www.r-project.org/) using the R package coxme (version 2.2–16). Survival curves were plotted using the package survminer (version 0.4.8).

The frequency distribution of estrus events from d 7 until d 40 postpartum was tested among parities and among farms using a \( \chi^2 \) test. The same procedure was used for the proportion of cows receiving AI upon heat detection at first AI among different categories of estrus events from d 7 until d 40 postpartum.

Variables were declared to be significant when \( P \leq 0.05 \). A statistical tendency was declared when \( P \) was between 0.05 and \( P \leq 0.10 \).

RESULTS

Descriptive Statistics

Overall, 52.7% (1,095/2,077) of cows had no estrus event detected by the AAM system from d 7 until d 40 postpartum (Table 2). Parity had no effect (\( P = 0.495 \)) on the frequency of estrus events from d 7 until d 40 postpartum. Farm (\( P = 0.001 \)) affected the frequency of estrus events from d 7 until d 40 postpartum. Herd-level prevalence of Estrus0 ranged from 37.5 to 58.4%.

For cows having at least 1 estrus event from d 7 until d 40 postpartum, median DIM for the first estrus event was 23 (Figure 1). There was no difference between primiparous (23 DIM) and multiparous cows (23 DIM). For cows having at least 2 estrus events from d 7 until d 40 postpartum, the interval between the first 2 events is depicted in Figure 2. Of these cows, 24% (73/306) had an interestrus interval between 18 to 24 d.

### Table 2. Number of estrus events\(^1\) detected by an automated activity monitoring system\(^2\) from d 7 until d 40 postpartum for 2,077 cows from 5 farms

<table>
<thead>
<tr>
<th>No. of estrus events</th>
<th>All cows</th>
<th>Primiparous cows</th>
<th>Multiparous cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>0</td>
<td>1,095</td>
<td>52.7</td>
<td>327</td>
</tr>
<tr>
<td>1</td>
<td>676</td>
<td>32.5</td>
<td>190</td>
</tr>
<tr>
<td>≥2</td>
<td>306</td>
<td>14.8</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>2,077</td>
<td>100</td>
<td>614</td>
</tr>
</tbody>
</table>

\(^1\)An estrus event was defined as activity change index value ≥35 for more than 2 h.  
\(^2\)Heatime Pro; SCR Engineers Ltd.
Estrous Expression at First Postpartum AI

Overall, 78.2% (1,625/2,077) of cows received first postpartum AI upon heat detection. Among cows without an estrus event from d 7 until d 40 postpartum, 71.6% (784/1,095) received first postpartum AI upon heat detection. Among cows in Estrus1 and Estrus2, 85.9% (581/676) and 85.0% (260/306) received first postpartum AI upon heat detection, respectively. More cows in Estrus1 and Estrus2 received first postpartum AI upon heat detection ($P = 0.001$).

Activity data on DU at first postpartum AI were available from 1,743 cows. Activity data on DU at first postpartum AI was missing for 334 cows as they did not achieve an activity change index of greater than 35 at first AI; all of these cows received TAI. The mean ($\pm$ standard error of the mean) duration was 13.9 $\pm$ 0.11 h at first AI. Duration of estrous activity at first AI was affected by season of AI ($P = 0.001$) and estrous expression from d 7 until d 40 postpartum ($P = 0.001$). There tended to be an effect of parity on the likelihood of high PA (primiparous cows 52.5% vs. multiparous cows 48.0%; $P = 0.089$). Among Estrus0 cows, 46.2% had high PA at first postpartum AI. Among cows in Estrus1 and Estrus2, 50.8% and 53.8% had high PA at first postpartum AI, respectively. There was a significant difference between Estrus2 and Estrus0 ($P = 0.032$) and a tendency between Estrus0 and Estrus1 ($P = 0.082$). There was no difference between Estrus1 and Estrus2 ($P = 0.427$). Probability for high PA was reduced in summer (35.8%; $P = 0.001$), autumn (55.2%; $P = 0.001$), and winter (55.6%; $P = 0.001$), respectively. Cows with spontaneous estrus (14.2 $\pm$ 0.29 h) at first AI tended ($P = 0.097$) to have longer DU compared with cows after hormonal intervention (13.7 $\pm$ 0.39 h).

A total of 55.6% (1,154/2,077) had high PA at the first postpartum AI. Estrous intensity at first postpartum AI was affected by season of AI ($P = 0.001$), AI code ($P = 0.001$), and estrous expression from d 7 until d 40 postpartum ($P = 0.050$). There tended to be an effect of parity on the likelihood of high PA (primiparous cows 52.5% vs. multiparous cows 48.0%; $P = 0.089$). Among Estrus0 cows, 46.2% had high PA at first postpartum AI. Among cows in Estrus1 and Estrus2, 50.8% and 53.8% had high PA at first postpartum AI, respectively. There was a significant difference between Estrus2 and Estrus0 ($P = 0.032$) and a tendency between Estrus0 and Estrus1 ($P = 0.082$). There was no difference between Estrus1 and Estrus2 ($P = 0.427$). Probability for high PA was reduced in summer (35.8%; $P = 0.001$), autumn (55.2%; $P = 0.001$), and winter (55.6%; $P = 0.001$).
winter (54.9%; \( P = 0.001 \)). There was no difference (\( P > 0.1 \)) in the probability of an estrus event with strong intensity among cows inseminated in spring, autumn, and winter, respectively. Cows with spontaneous estrus (66.8%) at first AI had a greater probability for high PA compared with cows after hormonal intervention (33.7%; \( P = 0.001 \)).

**Pregnancy per AI at First Postpartum AI**

At d 38 after AI, overall P/AI was 30.7% (637/2,077). Pregnancy per AI at first postpartum AI was affected by parity (\( P = 0.001 \)), season of AI (\( P = 0.001 \)), and estrous expression from d 7 until d 40 postpartum (\( P = 0.024 \)). Primiparous cows (36.4%) had greater P/AI compared with multiparous cows (29.1%; \( P = 0.001 \)). Pregnancy per AI was reduced in the summer (25.1%) compared with spring (35.2%; \( P = 0.002 \)), autumn (31.5%; \( P = 0.027 \)), and winter (39.7%; \( P = 0.017 \)). For Estrus0, Estrus1, and Estrus2 cows, pregnancy per AI was 29.4, 30.9, and 37.8%, respectively (Figure 3). There was a significant difference in P/AI at first AI between Estrus0 and Estrus2 (\( P = 0.008 \)) and between Estrus1 and Estrus2 (\( P = 0.039 \)). There was no difference between Estrus0 and Estrus1 (\( P = 0.517 \)).

**Time to First AI**

Estrous expression from d 7 until d 40 postpartum affected time to first AI (\( P = 0.001 \); Figure 4). Compared with Estrus0 cows, cows in Estrus1 [hazard ratio (HR) = 1.74; \( P = 0.001 \)] and Estrus2 (HR = 1.77; \( P = 0.001 \))
had an increased hazard of being inseminated within 100 DIM. There was no difference between Estrus1 and Estrus2 (\(P > 0.1\)). Median DIM to first AI were 70, 59, and 58 for cows in Estrus0, Estrus1, and Estrus2, respectively. Parity (\(P = 0.054\)) tended to affect time to first AI. Primiparous had an increased hazard (HR = 1.07) of being inseminated within 100 DIM. Season of calving (\(P = 0.001\)) affected time to first AI.

**Time to Pregnancy**

Estrous expression from d 7 until d 40 postpartum affected time to pregnancy (\(P = 0.001\); Figure 5). Compared with Estrus0 cows, cows in Estrus1 (HR = 1.28; \(P = 0.001\)) and Estrus2 (HR = 1.33; \(P = 0.001\)) had an increased hazard of becoming pregnant within 200 DIM. There was no difference between Estrus1 and Estrus2 (\(P > 0.1\)). Median DIM to pregnancy were 127, 112, and 103 for Estrus0, Estrus1, and Estrus2 cows, respectively. Parity (\(P = 0.001\)), year of calving (\(P = 0.001\)), and season of calving (\(P = 0.001\)) affected time to pregnancy.

**DISCUSSION**

The objective of this observational study was to evaluate the association between estrous expression detected by an AAM from d 7 until d 40 postpartum and reproductive performance of lactating Holstein cows. Our results suggest that cows with no estrus event detected by an AAM from d 7 until d 40 postpartum had reduced DU at first postpartum AI and a greater chance to receive a synchronized AI. Moreover, cows displaying at least one estrus event from d 7 until d 40 postpartum had improved reproductive performance as shown by an increased hazard of being inseminated within 100 DIM and becoming pregnant within 200 DIM.

The overall herd level prevalence of Estrus0 was 52.7% ranging from 37.5 to 58.4% based on estrous detection using a commercially available AAM system (Heatime Pro; SCR Engineers Ltd.). In a recent study conducted with 467 cows, at least one estrus event was recorded (1 estrus event = 271; 2 estrus events = 168; 3 estrus events = 27; 4 estrus events = 1) using activity data from a neck-mounted activity collar (Heat Ruminination Long Distance, SCR Inc.) from calving until 62 DIM (Chebel and Veronese, 2020). The prevalence of anestrus was similar (42.1%) even though the observation period was longer (62 DIM) and only primiparous cows were enrolled.

The prevalence of anestrus in our study and the study from Chebel and Veronese (2020) seems high compared with a US survey including 8 herds (mean...
23.3%; ranging from 7.3 to 41.7%; Bamber et al., 2009) and a Canadian survey including 17 herds (mean 19.5%; ranging from 5.0 to 45.0%; Walsh et al., 2007) where anovulatory status was determined using serial blood P4 measurements. Although parity had no effect on the frequency of estrous expression from d 7 until d 40 postpartum in our study, it has been shown that primiparous cows have a greater risk for being anovular at the end of the VWP (Bamber et al., 2009). The discrepancy might be due to the definition of anovular cows using serial measurements of circulating blood P4 concentrations in the US and Canadian studies compared with an anovulatory condition identified by behavioral changes using a commercially available AAM system in our study. Measuring P4 in milk or blood is considered the gold standard for defining patterns of estrus cyclicity (Lucy, 2019). A previous study showed only moderate agreement between the timing of the first estrus episode using either serial measurements of milk P4 concentrations or increased activity (Lovendahl and Chagunda, 2010). Although measurement of milk or blood P4 concentrations seems more accurate for phenotyping cyclicity patterns compared with AAM technology, there are a greater number of AAM systems in place on farm compared with commercially available systems that measure in-line milk P4. Using neck collars measuring activity patterns, the genetic correlation (0.96) between calving to first insemination and calving to first high activity was high (Ismael et al., 2015). The estimated heritability of calving to first high activity was 0.16 in that particular study, indicating its potential use for selection of fertility traits based on AAM data. Therefore, calving to first high activity might be a more robust estimate in herds using TAI protocols compared with the calving to first insemination trait (Lucy, 2019).

Median time to the first estrus event was 23 d. This is in agreement with previous studies using either a neck-mounted activity monitor (33.1 d: Lovendahl and Chagunda, 2010) or milk P4 profiles (27.9 d: Nyman et al., 2014). As illustrated in Figure 1, there was a tremendous variation in the occurrence of the first estrus event using an AAM in our study.

It has been shown that the first postpartum estrus cycle may be shorter, which is considered normal (Lucy, 2019). The following cycle length was observed to be 21 d with some variation (Crowe, 2008; Remnant et al., 2015). Using milk P4 profiles, one-quarter of postpartum estrus cycles were found to be irregular (Petersson et al., 2006; Nyman et al., 2014) because of (1) delayed cyclicity, (2) a prolonged luteal phase, or (3) cessation of cyclicity. Based on the interval between the first 2 estrus cycles from d 7 until d 40 postpartum in our study, only 24% had an interval between 18 to 24 d. The majority of cows (43.6%; 340/780) had a short interestrus interval (i.e., <18 d). A small proportion of cows had 3 or more estrus events. Surprisingly, a large number of these cows also had short interestrus intervals. The reason for this remains speculative. However, we also observed short interestrus intervals (i.e., <7 d) in a large data set (5,933 estrus events from 8 commercial dairy farms) in 5% of cows that were past the VWP (Tippenhauer et al., 2021). These cows had reduced fertility and need to be examined more thoroughly in future studies to elucidate the physiological mechanisms (e.g., ovulation failure, delayed ovulation, and hormonal profiles around estrus). The first ovulation is often not accompanied with estrus behavior and followed by a short estrus interval (Crowe et al., 2014). The underlying physiological mechanisms have not yet been fully resolved. It is assumed, however, that high levels of estradiol during late gestation and parturition induce a refractory state to the estrogens present at the first postpartum ovulation. However, P4 from the corpus luteum secreted after the silent ovulation seems to favor estrous expression during the next ovulatory cycle (Allrich, 1994). Also priming of the hypothalamus with P4 by an increased number of estrus cycles before the first insemination might be associated with a better responsiveness of estradiol receptors leading to improved estrus behavior (Thatcher and Wilcox, 1973). In our study we observed that Estrus0 cows displayed shorter DU and were less likely to have high PA, supporting these earlier findings. To the best of our knowledge, this is the first study showing an association between early resumption of estrus cyclicity and estrous expression patterns at the first postpartum AI.

Cows with ≥2 estrus events from d 7 until 40 postpartum had a greater likelihood of conceiving at the first postpartum AI compared with cows in Estrus0 (+8.4%; P = 0.008) and Estrus1 (+6.9%; P = 0.039). This is in agreement with other studies using either milk (Walsh et al., 2007) or blood P4 concentration (Galvão et al., 2010) to determine estrus cyclicity in the early postpartum period, during which anovular cows had reduced odds of conceiving at the first postpartum AI. Another study, however, did not observe any difference in first service conception risk for cows that were cyclic by 21 or 63 d compared with anovular cows (Dubuc et al., 2012).

Postpartum estrous activity influenced time to first AI in our study. Cows with no estrus event from d 7 until d 40 postpartum had reduced hazard of being inseminated until 100 DIM. Median times to first insemination were 70, 59, and 58 d for cows in Estrus0, Estrus1, and Estrus2, respectively. This corresponds with 2 studies in which anovular cows had a reduced likelihood of being inseminated early (Walsh et al., 2007).
2007; Galvão et al., 2010). In the first study (Walsh et al., 2007), median days to first insemination were 72 and 80 for ovular and anovular cows, respectively, using milk P4 profiles in herds with minimum hormonal interventions. In the study from Galvão et al. (2010), a Presynch-Ovsynch protocol with insemination after estrous detection was used to facilitate first postpartum TAI. Median days to first insemination were 71, 76, and 96 for cows cycling at 21 d, 49 d, and anovular cows, respectively, using blood P4 profiles. When comparing different studies regarding the effect of anovulation on insemination risk, one has to be careful as interventions with TAI protocols might confound the effects.

Using time to pregnancy as the ultimate measure of reproductive performance (Lean et al., 2016), cows displaying estrous activity from d 7 until d 40 postpartum had increased hazard of conceiving until 200 DIM compared with Estrus0 cows. Median time to pregnancy was 127, 112, and 103 d for cows in Estrus0, Estrus1, and Estrus2, respectively. This is in agreement with 3 other studies using either milk (Walsh et al., 2007) or blood P4 profiles to identify anovular cows (Galvão et al., 2010; Dubuc et al., 2012). Median time to pregnancy was 126 d and 156 d for cyclic and anovular cows, respectively, in herds with minimum hormonal interventions (Walsh et al., 2007). Median time to pregnancy was 103, 147, and 173 d for cows cycling at 21 d, 49 d, and anovular cows, respectively (Galvão et al., 2010). Interestingly, Dubuc et al. (2012) only observed a detrimental effect of anovulation in third parity or greater cows (cyclic by 21 d: 129 d; cyclic by 63 d: 151 d; anovular by 63 d: 180 d). Overall, this observational study provides evidence that displaying estrous activity in the early postpartum period is beneficial for subsequent reproductive performance. Therefore, activity data in early lactation might be useful in 2 different ways in herd management. They can be used (1) as a descriptive measure (i.e., how many cows are anestrous? Is there a change in the proportion of anestrous cows?), and (2) in a prescriptive way, such that there are different reproductive management strategies for anestrous cows (e.g., enrollment in a TAI protocol such as a Double-Ovsynch protocol) and cows that showed estrous expression in early lactation (i.e., rely on estrous detection after VWP).

**Study Limitations**

One clear drawback of this study is the lack of measurements of milk or blood P4 concentrations in the early postpartum period as they are considered the gold standard to characterize estrus cyclicity patterns (Lucy, 2019). In addition, we did not confirm whether cows identified by the AAM system were truly in estrus before the end of VWP. Although it has been shown that the AAM system used in this study has a high sensitivity and specificity (i.e., ≥90%) to detect ovulation compared with ultrasonography (Valenza et al., 2012; Dolecheck et al., 2015) for cows in mid lactation, there is no study available for cows in early lactation. This should be addressed in future studies. Irrespective of P4 measurements, however, we were able to show that Estrus0 cows have inferior reproductive performance compared with cows with estrus events from d 7 until d 40 postpartum. Therefore, using a distinct, biological outcome such as P/AI at first AI or time to pregnancy might be more useful to evaluate the effect of estrus patterns regardless of the method used compared with using milk or blood P4 profiles.

Furthermore, we had to exclude 38.2% of eligible cows (i.e., enrollment after calving and receiving first AI) due to missing or incomplete activity data. The percentage of cows that had to be removed ranged from 30.4 to 61.7% among the 5 farms. We observed this issue previously in a similar data set using commercial dairy farms (J. L. Plenio, A. Bartel, Freie Universitaet Berlin, Berlin, Germany; A. M. L. Madureira, R. L. A. Cerri, University of British Columbia, Vancouver, BC, Canada; W. Heuwieser, S. Borchardt, Freie Universitaet Berlin, Berlin, Germany; unpublished data). This clearly limits the practical use of this system for early identification of cows with poor predicted reproductive performance and to aid the decision-making process related to reproductive management.

**CONCLUSIONS**

Results from the present study provide strong evidence that early postpartum estrous expression influences fertility in dairy cattle. Cows with no estrous expression from d 7 until d 40 postpartum detected by a commercially available AAM system had inferior reproductive performance compared with cows that displayed estrous activity. Missing or incomplete activity data clearly limits the practical use of this system. Future studies should address risk factors for Estrus0 and evaluate intervention strategies in these cows to improve their reproductive performance.

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