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Effect of transition cow health and estrous expression detected by an automated activity monitoring system within 60 days in milk on reproductive performance of lactating Holstein cows

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ABSTRACT

The objective of this observational study was to evaluate the association of transition cow health and estrous expression, detected by an automated activity monitoring system (Smarttag Neck, Nedap Livestock Management), with reproductive performance in lactating Holstein cows. A total of 3,750 lactating Holstein cows (1,563 primiparous cows and 2,187 multiparous cows) from a commercial dairy farm in Slovakia calving from January 2020 until July 2021 were enrolled on an ongoing basis. Activity data were recorded from d 7 until d 60 postpartum. Within this observational period, cows were classified into 3 categories: (1) no estrus event (Estrus0), (2) 1 estrus event (Estrus1), or (3) 2 or more estrus events (Estrus2+). Transition cow health was assessed by farm personnel within the first 30 d in milk (DIM) using standard operating procedures. Generalized linear mixed models were used to analyze continuous and categorical data. Cox proportional hazard models were used for time to event data. The overall prevalence of anestrus was 20.8%. Multiparous cows had a greater risk for anestrus compared with primiparous cows [odds ratio (OR) = 1.4]. Cows with stillbirth (OR = 1.76), retained placenta (OR = 2.19), puerperal metritis (OR = 1.48), or subclinical ketosis (OR = 1.51) had a greater risk for anestrus. In addition, cows calving in summer (OR = 0.82), autumn (OR = 0.38), or winter (OR = 0.56) had a higher incidence of anestrus than cows calving in spring. Estrous expression from d 7 until d 60 postpartum was associated with estrous duration (DU) and estrous intensity at first artificial insemination (AI). Cows in Estrus0 had the shortest DU at first postpartum AI (9.4 ± 0.18 h) compared with cows in Estrus1 (10.5 ± 0.13 h) and Estrus2+ (11.4 ± 0.12 h). Cows in Estrus2+ had a longer DU at first postpartum AI compared with cows in Estrus1. For Estrus0, Estrus1, and Estrus2+ cows, pregnancy per AI at first service was 42.5%, 50.9%, and 55.4%, respectively. Estrous expression from d 7 until d 60 postpartum was associated with time to first AI and time to pregnancy. Compared with Estrus0 cows, Estrus1 [hazard ratio (HR) = 1.43] and Estrus2+ cows (HR = 1.62) had an increased hazard of being inseminated within 100 DIM. Compared with Estrus2+, Estrus1 cows had a reduced hazard of being inseminated within 100 DIM (HR = 0.89). Compared with Estrus0 cows, Estrus1 (HR = 1.24) and Estrus2+ cows (HR = 1.46) had an increased hazard of becoming pregnant within 200 DIM. Median DIM to pregnancy were 121, 96, and 92 for Estrus0, Estrus1, and Estrus2+ cows, respectively. In conclusion, cows with transition cow disorders (i.e., stillbirth, retained placenta, puerperal metritis, or subclinical ketosis) had a greater chance for anestrus compared with healthy cows. Cows in Estrus0 had reduced estrous expression at first AI and inferior reproductive performance compared with cows that displayed estrous activity from d 7 until d 60.

Key words: estrous expression, automated activity monitoring, transition cow health, reproductive performance

INTRODUCTION

An early resumption of ovarian cyclicity within the voluntary waiting period (**VWP**) is a major prerequisite for good reproductive performance (Santos et al., 2009; Dubuc et al., 2012) and therefore has a crucial influence on the economic efficiency of dairy farms (Overton and Cabrera, 2017). Overall, the prevalence of anovulatory cows in North American dairy herds was 23% at approximately 50 DIM (Bamber et al., 2009; Dubuc et al., 2012); within herds, prevalence ranged from 5 to 45% (Walsh et al., 2007; Dubuc et al., 2012). Detection of anovulatory cows within the VWP has not been implemented in commercial dairy herds on a routine basis. Identifying anovulatory cows

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within the VWP requires at least 2 examinations (at least 1 wk apart), either by analyzing circulating progesterone (**P4**) concentrations in blood serum (Santos et al., 2009; Dubuc et al., 2012) or by visualizing a corpus luteum (**CL**) via transrectal ultrasound. In-line milk P4 analysis or estrus detection by an automated activity monitoring (**AAM**) system would represent more practical alternatives. When using in-line milk P4 analysis, it has been shown that cows with early luteal activity have better reproductive performance (Bruinjé et al., 2017). An increasing number of commercial dairy farms, however, are adopting AAM systems as a reliable tool to identify anestrous cows. Using AAM systems, it has been shown that cows with no estrous expression from 7 to 40 DIM have reduced estrous expression at first AI and inferior reproductive performance compared with cows that displayed estrous activity within the VWP (Borchardt et al., 2021).

Several factors have been associated with anovulation and anestrus in the VWP, including parity, digestive disorders, ketosis, displaced abomasum (**DA**), BW loss (>28 kg), negative energy balance, higher blood concentrations of nonesterified fatty acids or haptoglobin (Dubuc et al., 2012), dystocia, and twins (Walsh et al., 2007; Vercouteren et al., 2015; Stevenson et al., 2020). Inflammation of the reproductive tract seems to have a major impact on anovulation within the VWP by causing altered luteal function, reduced estrus detection rates, and delayed resumption of cyclicity (Bruinjé et al., 2021a,b,c). Therefore, metritis (Vercouteren et al., 2015; Stevenson et al., 2020), cytological endometritis (Dubuc et al., 2012), and purulent vaginal discharge (Bruinjé et al., 2021a,c) are important risk factors.

In comparison with a previous study from our group (Borchardt et al., 2021), the major differences are the VWP (40 d vs. 60 d), and therefore, the observational period (7–40 d vs. 7–60 d); the study population (2,077 cows from 5 farms vs. 3,750 cows from 1 farm); and the neck-mounted AAM system (Heatime Pro, SCR Engineers Ltd. vs. Smarttag Neck, Nedap Livestock Management). New to this study is the association of transition cow health with estrous expression in the VWP.

Therefore, the objective of this study was to describe the association of transition cow health and estrous expression within 60 DIM, detected by an AAM system, with the reproductive performance of lactating dairy cows. In addition, we aimed to evaluate risk factors for anestrus within the VWP. Our hypotheses were that cows with transition cow disorders have a greater risk for anestrus within the VWP and, consequently, impaired reproductive performance.

MATERIALS AND METHODS

This study was an observational, retrospective cohort study. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

Animals, Housing, and Management

This experiment included health and AAM data from 3,750 lactating Holstein cows (1,563 primiparous and 2,187 multiparous) from a Slovakian commercial dairy farm housing approximately 2,700 cows. Cows were included if they were freshened between January 2020 and July 2021 and had complete AAM data from calving until first AI. Cows were excluded if they were culled before receiving AI or a pregnancy diagnosis (n = 757), or if they had incomplete activity data (n = 455). Cows were housed in a freestall barn with concrete flooring and had ad libitum access to feed and water. The TMR was formulated to meet or exceed the dietary requirements for dairy cows (NRC, 2001). All cows were fed a TMR twice daily according to their transition status (far-off, close-up, and fresh-cow diets) and feed was pushed up 10 times per day. Prepartum cows were fed a moderate negative DCAD diet.

Cows were milked twice daily, with a 305-d milk yield of 11,500 kg. All cow-related events (e.g., AI, treatments, pregnancy outcome) were entered into herd-management software (DairyComp 305, Valley Agriculture Software) by farm personnel.

Automated Activity Monitoring

Twenty-one days before their first calving, all cows were equipped with a neck-mounted AAM system (Smarttag Neck, Nedap Livestock Management) (Roelofs and Van Erp-Van der Kooij, 2015). This accelerometer recorded activity data in real time and stored it as aggregated average activity blocks of 2 h time periods (12 blocks of 2 h per day) per cow. The relative change in activity was measured by using a z-score transformation (Roelofs et al., 2005) and a proprietary algorithm. The z-score represents the deviation of the current activity from the mean activity within a certain time period (i.e., comparing the number of neck movements within every 2 h time period with the same 2 h time period of the preceding 10 d for each cow). The z-score forms the basis for the intensity of an estrus event, quantified by the x-factor from 0 (lowest index value) to 100 (highest index value). When the x-factor exceeds a threshold for multiple consecutive periods, a cow is considered in estrus, and an AAM alert (i.e., attention) is generated. Values for attention were 0 (no estrus) or 1 (estrus). Onset of estrus was defined as the first instance at which the attention changed from 0 to 1. End of estrus was defined by the first instance at which the attention changed from 1 to 0. In summary, an estrus event was defined as an attention change from 0 to 1 and remained at 1 for more than 2 time periods (>4 h) based on a proprietary algorithm. For each estrus event within the VWP, peak activity (**PA**) and estrous duration (**DU**) were determined. The estrous intensity defined as PA was represented by the maximum value of the x-factor within an estrus event during the observational period (i.e., d 7–60). The peak value was divided into 10 equal categories and pregnancy per AI (**P/AI**) was calculated for each increment. Two increments of P/AI significantly differed from the other categories and thus, PA was divided into the categories of low (x-factor = 0–10), medium (x-factor = 11–20), and high (x-factor = 21–100). Estrous duration was defined as the interval from onset to end of an estrus event.

A file for each cow, including cow ID, raw activity data, the x-factor, and attention every 2 h, was generated from the AAM system on the farm computer. Each file was generated, then exported into XLSX format (Office 2013, Microsoft Deutschland Ltd.). Processing of the activity data was conducted as previously described (Tippenhauer et al., 2023). The processed and cleaned report was formatted in the wide (i.e., one line for each cow) and long format (i.e., one line for each estrus event), and included the following information for each cow: cow ID number, lactation number, last breeding date, last calving date, AI number, DIM at AI, x-factor (index value and date and time of PA), attention (date and time for start and end of an estrus event), and DU of each estrus event.

Cows were classified according to the number of estrus events detected by the AAM system from d 7 until d 60 postpartum into 3 categories: (1) no estrus event (Estrus0), (2) 1 estrus event (Estrus1), or (3) 2 or more estrus events (Estrus2+).

Reproductive Management

Lactating cows were inseminated based on visual estrus detection following an alert of the AAM system or received timed artificial insemination (**TAI**) after hormonal intervention. The VWP was 60 DIM. On a daily basis, the AAM system generated a list of cows eligible for breeding based on the attention value. Estrus was verified by the AI technician via transrectal palpation of a highly contractile uterus, visualization of clear, stringy vaginal discharge, or both. If cows were confirmed in estrus, AI was conducted on the same day. Inseminations were performed either with conventional or sexed semen. The selection of cows receiving sexed semen was based on the pedigree information for the cow and the relative value of the cow, which is a computed item in DairyComp 305. The relative value is a comparison of the cow's 305-d mature-equivalent milk yield with the herd-average 305-d mature-equivalent milk yield. A relative value of 100 signifies a cow with a production level similar to the herd average. Cows showing signs of estrus at any time after an AI (e.g., first AI) received further AI services. Cows that were not bred until 80 ± 3 DIM received TAI using a modified 7 d Ovsynch protocol including a second $PGF_{2\alpha}$ treatment on d 8 (GnRH, 7 d later $PGF_{2\alpha}$, followed 24 h later with $PGF_{2\alpha}$, followed 32 h later with GnRH, and concluded 16 to 18 h later with TAI). Cows not re-inseminated at detected estrus underwent pregnancy diagnosis through transrectal ultrasound (Easi-Scan:GO, IMV Imaging) 39 ± 3 d after AI. All cows received a single GnRH treatment at 32 ± 3 d after AI. Open cows with at least 1 CL ≥ 15 mm received 2 $PGF_{2\alpha}$ treatments 24 h apart followed by a GnRH treatment 32 h after the second $PGF_{2\alpha}$, and TAI 16 to 18 h later. Cows that did not meet the criteria to be included in the CL group received a modified Ovsynch protocol (i.e., GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16–18 h later TAI). A second pregnancy diagnosis was performed 80 d after AI. All pregnancy diagnoses were conducted by trained farm personnel.

Transition Cow Management

Cows and heifers were transferred to a bedded pack barn at 55 and 21 d before expected parturition, respectively. Prepartum cows and heifers were monitored every hour to detect signs of imminent parturition (i.e., restlessness, vaginal discharge with bloody traces, lying lateral with abdominal contractions, a visible or broken amniotic sac, or feet of the emerging calf outside the vulva). Animals were moved into an individual maternity pen (8 × 8 m and bedded with fresh straw) when the amniotic sac was visible or broken outside the vulva, or feet of the emerging calf were detected outside the vulva. If the cow had not delivered the calf within 1 h after that, calving assistance was provided. Parameters recorded for the study were calving ease on a 5-point scale (0 = physiological calving, 1 = calving assisted by 1 person, 2 = calving assisted by 2 people, 3 = calving assisted by 3 people, or 4 = calving assisted by more than 3 people), stillbirth (yes or no), twins (yes or no), and calf sex (female or male). Dystocia was defined as a calving ease of 3 or greater. All multiparous cows received prophylactic calcium supplementation (BOVIKALC, Boehringer Ingelheim) at parturition and 12 to 24 h later.

All infectious and metabolic diseases occurring after calving were recorded until 30 DIM [retained placenta (**RP**), puerperal metritis (**PM**), subclinical ketosis, mastitis, and clinical milk fever] and 60 DIM (clinical mastitis and left DA), and diagnosed based on specific protocols, respectively. Until 10 DIM, every cow enrolled in this study was examined daily by farm personnel following standard operating procedures created by the herd manager. These examinations included monitoring of general appearance, attitude, presence of fetal membranes outside the vulva, vaginal discharge, udder health, lameness, and manure consistency. Rectal temperature was measured using a digital thermometer. Percussion of the left flank was performed by using a stethoscope. Transrectal massage of the uterus was conducted at 3, 6, and 9 DIM to obtain and evaluate uterine discharge. Blood ketone concentration was evaluated at d 5 and 10 using a handheld electronic ketone meter (TaiDoc, Pharmadoc). In addition, daily milk yield was used to identify potentially diseased cows. A definition of each disease of interest was provided to farm personnel. After 11 DIM, cows were screened for health disorders based on visual observation by farm personnel every morning at 0800 h and on deviations in their daily milk production (day-to-day reduction of >4.5 kg; Guterbock, 2004). The affected cows were separated and examined more thoroughly as described previously. Cows were diagnosed with RP when fetal membranes were not expulsed until 24 h after calving (Nordlund and Cook, 2004). Cows with RP were not treated but were monitored for pyrexia for 10 d. Puerperal metritis was defined as an animal having an abnormally enlarged uterus and a fetid, watery, red-brown uterine discharge associated with a sign of systemic illness such as decreased milk yield, dullness, or other signs of toxemia, and a rectal body temperature >39.5°C within 21 d after parturition (Sheldon et al., 2006). Cows diagnosed with PM were treated with ceftiofur hydrochloride (2.2 mg/kg of BW; Excenel RTU, Zoetis) i.m. for 3 to 5 consecutive days. Based on Vasquez et al. (2017), clinical mastitis was defined by visible signs of inflammation in an affected mammary gland, such as redness, swelling, pain, or heat, and an altered milk secretion that may include the appearance of clots, flakes, discoloration, or abnormal consistency. Udder health was assessed by farm personnel twice per day during regular milking hours. Cows were considered to have a left DA when percussion of the left flank resulted in tympanic resonance detected with a stethoscope. The treatment of choice was the "roll and toggle" method (Grymer and Sterner, 1982); in cases of unsuccessful treatment, cows were surgically treated with abomasopexy. Subclinical ketosis was defined as serum BHB concentrations >1.2 mmol/L (McArt et al., 2013). Ketotic cows received 250 mL of propylene glycol orally for 5 d. Clinical hypocalcemia was defined as a multiparous cow showing recumbency and paresis (Nordlund and Cook, 2004). Cows with clinical hypocalcemia were treated i.v. with 500 mL of a 23% calcium borogluconate solution (10.5 g of calcium).

Statistical Analyses

Cow ID, parity, calving date, milk yield for the first 100 DIM, and breeding information [i.e., DIM, number of AI, AI code (estrus vs. hormonal intervention), type of semen, pregnancy outcome] was obtained through the on-farm computer software (DairyComp 305). All data were transferred to Microsoft Excel and merged with the aforementioned activity data report. All statistical analyses were performed using SPSS for Windows (version 28.0, IBM Corp.).

In order to evaluate the association between selected categorical risk factors in the transition period and estrous characteristics (i.e., DIM, DU, PA) of the first postpartum estrus event, 3 linear regression models were built using the GENLINMIXED procedure of SPSS. Model building was conducted as recommended by Dohoo et al. (2009), in which each variable was first analyzed separately in a univariable model using the GENLINMIXED procedures described. Only variables resulting in univariable models with $P \leq 0.10$ were included in the final mixed models. Selection of the model that most appropriately 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; quartiles (**Q**) for 305-d milk yield based on the second test day (Q1 = 5,160–10,570 kg, Q2 = 10,576–11,570 kg, Q3 = 11,580–12,510 kg, Q4 = 12,520–16,280 kg); number of calves; dystocia; stillbirth; season of calving; individual transition cow diseases (i.e., RP, PM, subclinical ketosis, milk fever, clinical mastitis, DA); and the number of transition cow diseases.

To evaluate the association between selected categorical risk factors in the transition period and the probability of anestrus from d 7 until d 60, a logistic regression model was built using the GENLINMIXED procedure in SPSS. The outcome of interest was anestrus. The initial model contained the same variables as previously described.

To evaluate the association between estrous expression detected by an AAM system within 60 DIM and reproductive performance of lactating Holstein cows, 2 linear regression models (DU and estrous intensity at first AI) and a logistic regression model (P/AI at first AI) were built using the GENLINMIXED procedure

Table 1. Number of estrus events[1] detected by an automated activity monitoring system[2] from d 7 until d 60 postpartum for 3,750 Holstein cows from 1 farm

Number of estrus events	All cows		Primiparous cows		Multiparous cows	
	n	%	n	%	n	%
0	778	20.8	273	17.5[a]	505	23.1[b]
1	1,419	37.8	615	39.3[a]	804	36.8[a]
≥2	1,553	41.4	675	43.2[a]	878	40.1[a]
Total	3,750		1,563		2,187	

[a,b]Percentages with different superscripts differ within a column ($P < 0.05$).

[1]An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm.

[2]Smarttag Neck, Nedap Livestock Management.

of SPSS. Parity was considered as a repeated measure because some cows had more than one calving within the observation period. Model building was conducted as described. The initial models included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous); year of AI; season of AI (winter = December 1–February 28, spring = March 1–May 31, summer = June 1–August 31, or autumn = September 1–November 30); estrous activity within 60 DIM (Estrus0 vs. Estrus1 vs. Estrus2+); and AI code at first AI (estrus vs. hormonal intervention). Type of semen (conventional vs. sexed semen) was considered in the logistic model for P/AI.

Cox proportional hazards were used to model the time to event outcomes (i.e., time to first AI, time to pregnancy). Cows were censored from the study if they were culled before first insemination or pregnancy diagnosis or at the end of the observation period. The variables of parity, year of calving, season of calving, and estrous activity within 60 DIM were tested as risk factors. The proportional hazard assumption was checked using Schoenfeld residuals.

The frequency distribution of estrus events from d 7 until d 60 postpartum was tested among parities using a chi-squared test. The same procedure was used for cows receiving AI upon heat detection at first AI among different categories of estrus events from d 7 until d 60 postpartum.

To account for multiple comparisons, the P-value was adjusted using a Bonferroni correction. Variables were declared to be significant when $P \leq 0.05$. A tendency was declared when P-value was between 0.05 and ≤0.10.

RESULTS

Descriptive Statistics

Overall, 3,750 cows were included in the final statistical analyses. Out of these, 20.8% (778/3,750 cows) were anestrous cows (Table 1). Parity ($P = 0.001$) was associated with the frequency of estrus events from d 7 until d 60 postpartum, as a greater proportion of multiparous cows (23.1%; 505/2,187) were found to be anestrous compared with primiparous cows (17.5%; 273/1,563).

For Estrus1 cows, the median DIM for the first estrus event was 35 (Figure 1). A difference was observed ($P < 0.001$) between primiparous (37 DIM) and multiparous (33 DIM) cows.

For Estrus2+ cows, the interval between the first 2 estrus events is depicted in Figure 2. Of these cows, 52.9% (822/1,553 cows) had an interestrus interval between 18 to 24 d. Additionally, a difference was noted ($P = 0.001$) between primiparous (58.5%; 395/675) and multiparous (48.6%; 427/878) cows. The characteristics of the first postpartum estrus event are summarized in Table 2. This includes the univariate associations between DIM, DU, and PA at the first postpartum estrus event, the prevalence of anestrus, and selected categorical risk factors.

Risk Factors for Anestrus

The prevalence of anestrus was associated with parity ($P < 0.001$), twins ($P = 0.029$), dystocia ($P = 0.004$), stillbirth ($P = 0.003$), RP ($P < 0.001$), PM ($P < 0.001$), subclinical ketosis ($P < 0.001$), number of transition cow disorders ($P < 0.001$), and the season of calving ($P < 0.001$). Cows delivering a singleton had a reduced prevalence for anestrus (20.5%) compared with cows with twins (29.6%). Cows with dystocia had a greater probability of anestrus (26.8%) than cows with eutocia (20.1%). Cows with a stillbirth had an increased probability for anestrus (31.6%) compared with cows with living calves (20.4%). The prevalence of anestrus was greater in cows with RP (40.2% affected vs. 20.0% unaffected), metritis (30.3% affected vs. 19.6% unaffected), and subclinical ketosis (26.7%

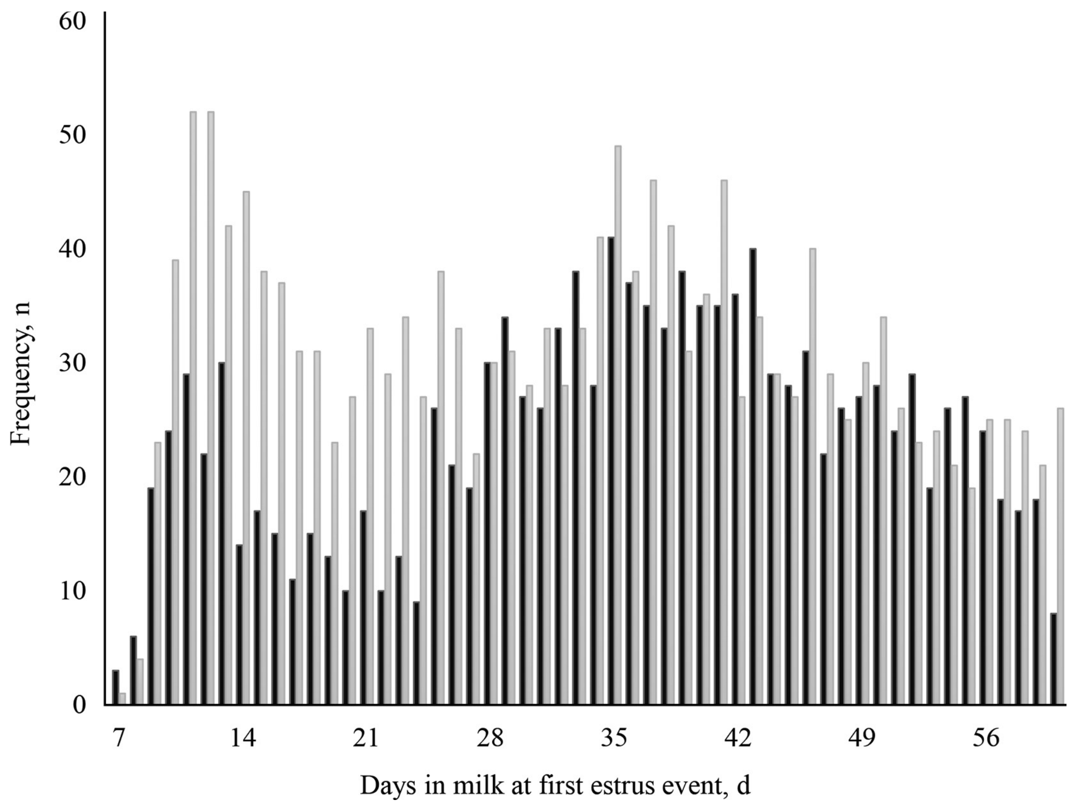

Figure 1. Frequency distribution of DIM at first estrus event for 2,972 cows (black bars = primiparous cows, n = 1,290; gray bars = multiparous cows, n = 1,682) from 1 commercial dairy farm. Cows were equipped with a neck-mounted activity monitoring system (Smarttag Neck, Nedap Livestock Management). An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm.

affected vs. 18.6% unaffected). Cows unaffected by a transition cow disorder had a reduced probability of anestrus (17.8%) compared with cows affected by 1 disease (23.4%) or 2 or more diseases (32.5%). For season of calving, the prevalence of anestrus was 25.5% (723/971 cows) in spring, 23.9% (709/932 cows) in summer, 14.1% (581/676 cows) in autumn, and 18.1% (959/1,171 cows) in winter. Spring and summer differed significantly from autumn and winter.

Results from the final logistic regression model for the association of selected risk factors in the transition period and the probability of anestrus are summarized in Table 3.

Estrous Expression at First Postpartum AI

Overall, 73.0% (2,738/3,750) of cows received first postpartum AI upon heat detection. Among Estrus0 cows, 55.0% (428/778) received first postpartum AI upon heat detection. Among cows in Estrus1 and Estrus2+, 74.1% (1,052/1,419) and 81.0% (1,258/1,553) received first postpartum AI upon heat detection, respectively. More cows in Estrus1 and Estrus2+ received first postpartum AI upon heat detection compared with Estrus0 ($P = 0.001$).

Given that 574 cows had no AAM alert at first AI as they received TAI, data on DU at first postpartum AI was only available for 3,176 cows. The mean (± SEM) DU at first AI was 11.4 ± 0.07 h. Duration of estrous activity at first AI was associated with season of AI ($P = 0.001$), AI code ($P = 0.001$), and estrous expression from d 7 until d 60 postpartum ($P = 0.001$). Parity did not affect DU ($P = 0.124$); however, a significant interaction between parity and estrous expression ($P = 0.049$) was found. Overall, Estrus0 cows had the shortest DU (9.4 ± 0.18 h) compared with cows in Estrus1 (10.5 ± 0.13 h; $P = 0.001$) and Estrus2+ (11.4 ± 0.12 h; $P = 0.001$) at the estrus event associated with the first postpartum AI. Cows in Estrus2+ had a longer DU at first postpartum AI compared with cows in Estrus1 ($P = 0.001$). Estrous duration was reduced in summer (9.6 ± 0.15 h) compared with spring (10.3 ± 0.14 h; $P = 0.001$), autumn (10.8 ± 0.16 h; $P = 0.003$), and winter (11.1 ± 0.17 h; $P = 0.001$). Cows with spontaneous estrus (11.4 ± 0.08 h) at first AI had longer DU ($P = 0.001$) compared with cows receiving

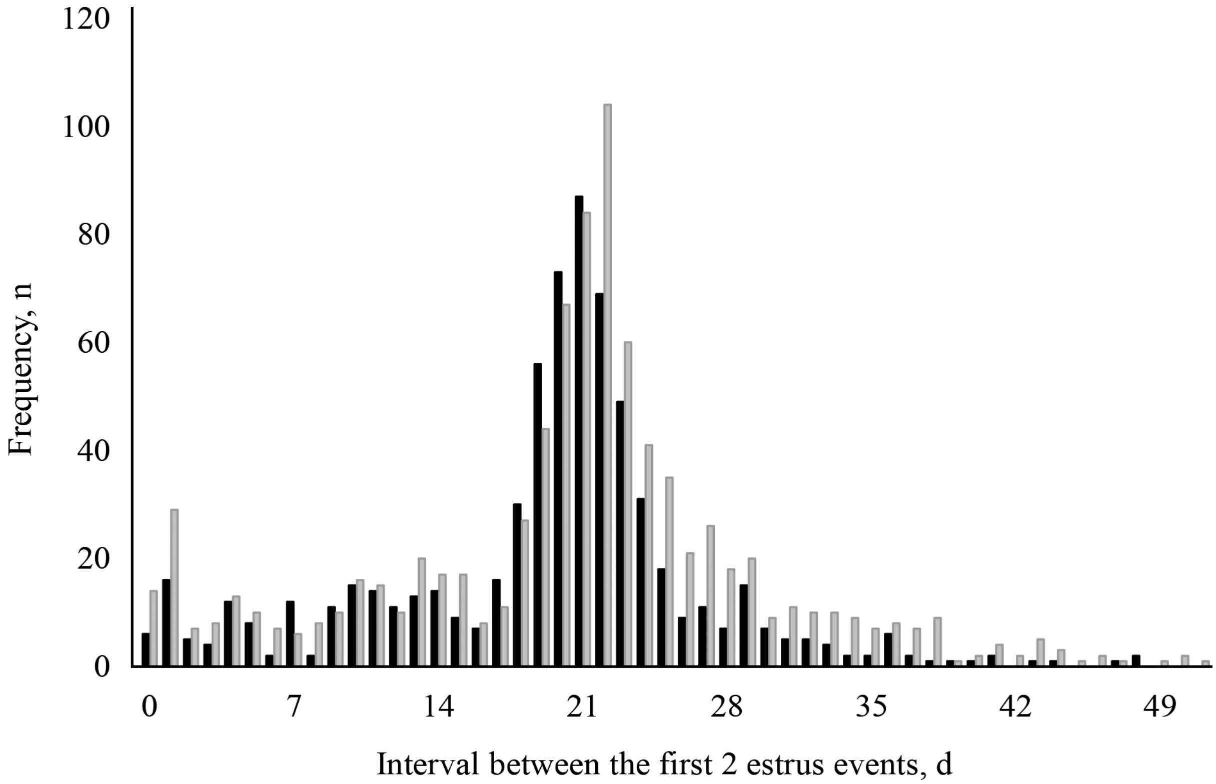

Figure 2. Frequency distribution of the interval between the first 2 estrus events from d 7 until d 60 postpartum for 1,553 cows (black bars = primiparous cows, n = 675; gray bars = multiparous cows, n = 878) from 1 commercial dairy farm. Cows were equipped with a neck-mounted activity monitoring system (Smarttag Neck, Nedap Livestock Management). An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm.

TAI (9.4 ± 0.17 h). Within Estrus0 cows, a difference ($P = 0.019$) in DU between primiparous (9.8 ± 0.27 h) and multiparous cows (8.9 ± 0.22 h) was observed. No differences between primiparous and multiparous cows were noted within cows displaying 1 ($P = 0.744$) and 2 or more ($P = 0.399$) estrus events from d 7 until d 60.

Estrous intensity at first postpartum AI was associated with parity ($P = 0.001$), season of AI ($P = 0.001$), AI code ($P = 0.001$), and estrous expression from d 7 until d 60 postpartum ($P = 0.001$). Primiparous cows had greater PA compared with multiparous cows (90.7 ± 1.1 vs. 66.5 ± 0.8 index value). Estrous intensity was reduced in summer (74.0 ± 1.2 index value) and autumn (73.9 ± 1.3 index value) compared with winter (82.6 ± 1.4 index value) and spring (83.9 ± 1.1 index value). Estrous intensity was reduced in cows receiving TAI (65.4 ± 1.2 index value) compared with cows inseminated based on spontaneous estrus (91.9 ± 0.7 index value). A linear response was observed in PA with Estrus0 cows showing reduced PA (72.3 ± 1.4 index value), Estrus1 cows having medium PA (78.9 ± 1.0 index value), and Estrus2+ cows having the greatest PA (84.7 ± 1.0 index value).

Pregnancy Per AI at First Postpartum AI

At d 39 after first AI, overall P/AI was 48.6% (1,823/3,750). Pregnancy per AI at first postpartum AI was associated with parity ($P = 0.001$), year of AI ($P = 0.005$), season of AI ($P = 0.001$), type of semen ($P = 0.001$), and estrous expression from d 7 until d 60 postpartum ($P = 0.001$). Primiparous cows (58.7%) had greater P/AI compared with multiparous cows (40.5%; $P = 0.001$). Pregnancy per AI was reduced in 2021 (46.8%) compared with 2020 (52.3%). Pregnancy per AI was reduced in autumn (31.5%) compared with summer (42.2%; $P = 0.027$), spring (53.2%; $P = 0.002$), and winter (56.1%; $P = 0.017$). Autumn was used as the referend. Cows receiving sexed semen (42.1%) had reduced P/AI compared with conventional semen (57.1%; $P = 0.001$). For Estrus0, Estrus1, and Estrus2+ cows, P/AI was 42.5%, 50.9%, and 55.4%, respectively. Within primiparous cows, P/AI in Estrus0 cows (53.7%) was reduced compared with Estrus1 cows (61.6%; $P = 0.050$) and a tendency toward a difference in P/AI between Estrus0 and Estrus2+ cows (60.7%; $P = 0.080$) was observed, as shown in Figure 3. Within

Table 2. Univariate associations between DIM, estrous duration, and estrous intensity at the first postpartum estrus event, the prevalence of anestrus,[1] and selected categorical risk factors from 3,750 Holstein cows equipped with a neck-mounted activity sensor system[2]

Risk factor	N (n)[3]	First postpartum estrus event[4] DIM at first estrus event	Estrous duration, h	Estrous intensity, index value	Prevalence of anestrus,[5] %
Parity	3,750				
Primiparous cows	1,563	35.6 ± 0.38[a]	8.7 ± 0.11	23.2 ± 0.42[a]	17.5[a]
Multiparous cows	2,187	32.7 ± 0.36[b]	8.5 ± 0.10	21.5 ± 0.37[b]	23.1[b]
305-d milk yield quartile[6]	3,750 (2,972)				
Q1	942 (765)	33.8 ± 0.51	8.6 ± 0.14	22.7 ± 0.56	18.8
Q2	940 (748)	33.8 ± 0.52	8.6 ± 0.14	21.8 ± 0.56	20.4
Q3	936 (737)	33.6 ± 0.54	8.7 ± 0.14	22.4 ± 0.56	21.3
Q4	932 (722)	34.6 ± 0.54	8.4 ± 0.14	22.1 ± 0.56	22.5
Number of calves					
Singleton	3,652 (2,903)	33.9 ± 0.27	8.6 ± 0.07	22.3 ± 0.28	20.5[a]
Twins	98 (69)	36.9 ± 1.63	8.2 ± 0.45	20.7 ± 1.69	29.6[b]
Dystocia[7]					
No	3,370 (2,694)	33.9 ± 0.28	8.6 ± 0.07	22.3 ± 0.29	20.1[a]
Yes	380 (278)	34.8 ± 0.83	8.6 ± 0.23	22.3 ± 0.89	26.8[b]
Stillbirth					
No	3,633 (2,892)	33.9 ± 0.27	8.6 ± 0.07	22.4 ± 0.28[a]	20.4[a]
Yes	117 (80)	33.1 ± 1.55	8.2 ± 0.41	19.1 ± 1.45[b]	31.6[b]
Retained placenta[8]	3,738				
No	3,606 (2,883)	33.9 ± 0.27	8.6 ± 0.07	22.2 ± 0.28	20.0[a]
Yes	132 (79)	35.6 ± 1.59	8.5 ± 0.44	24.2 ± 2.12	40.2[b]
Puerperal metritis[9]					
No	3,360 (2,700)	33.7 ± 0.28[a]	8.6 ± 0.07	22.2 ± 0.29	19.6[a]
Yes	390 (272)	36.6 ± 0.86[b]	8.5 ± 0.24	22.5 ± 0.96	30.3[b]
Subclinical ketosis[10]					
No	2,737 (2,229)	33.9 ± 0.30	8.6 ± 0.08	22.5 ± 0.33	18.6[a]
Yes	1,013 (743)	33.9 ± 0.53	8.4 ± 0.14	21.7 ± 0.54	26.7[b]
Clinical milk fever[11]					
No	3,731 (2,959)	33.9 ± 0.27	8.6 ± 0.07[a]	22.3 ± 0.28	20.7
Yes	19 (13)	34.4 ± 4.29	6.9 ± 0.67[b]	24.8 ± 5.74	31.6
Clinical mastitis[12]					
No	3,346 (2,651)	33.9 ± 0.28	8.6 ± 0.07	22.5 ± 0.29[a]	20.8
Yes	404 (321)	33.5 ± 0.79	8.5 ± 0.23	19.9 ± 0.81[b]	20.5
Displaced abomasum[13]					
No	3,733 (2,958)	33.9 ± 0.27	8.6 ± 0.07	22.2 ± 0.28	20.8
Yes	14 (12)	31.2 ± 4.02	9.2 ± 1.24	30.2 ± 7.52	14.3
Number of transition cow disorders					
0	2,322 (1,908)	33.8 ± 0.33	8.7 ± 0.09	22.5 ± 0.35	17.8[a]
1	1,102 (844)	33.8 ± 0.49	8.5 ± 0.13	21.9 ± 0.51	23.4[b]
≥2	326 (220)	35.6 ± 0.98	8.3 ± 0.27	21.3 ± 1.07	32.5[c]

Continued

multiparous cows, P/AI differed ($P < 0.050$) for Estrus0, Estrus1, and Estrus2+ cows (32.0%, 40.0%, and 50.0%, respectively). An interaction between parity and number of estrus events from d 7 until d 60 ($P = 0.015$) was observed. A tendency was observed between type of semen and number of estrus events from d 7 until d 60 ($P = 0.100$) on P/AI. For cows receiving conventional semen, P/AI for Estrus0 cows was reduced (53.1%, $P = 0.026$) compared with Estrus2+ cows (61.0%). For cows receiving sexed semen, P/AI for Estrus0 cows was reduced (32.5%; $P = 0.001$) compared with Estrus1 (44.6%) and Estrus2+ (49.7%) cows. Pregnancy per AI did not differ between cows receiving AI upon spontaneous estrus compared with cows receiving TAI ($P = 0.698$).

Time to First AI

Median DIM to first AI were 84, 74, and 74 for cows in Estrus0, Estrus1, and Estrus2+, respectively. Time to first AI was associated with estrous expression from d 7 until d 60 postpartum ($P = 0.001$; Figure 4) and parity ($P = 0.001$). Compared with Estrus0 cows, cows in Estrus1 [hazard ratio (**HR**) = 1.43; $P = 0.001$] and Estrus2+ (HR = 1.62; $P = 0.001$) had an increased hazard of being inseminated within 100 DIM. Compared with Estrus2+ cows, Estrus1 cows had a reduced hazard of being inseminated within 100 DIM (HR = 0.89; $P = 0.001$). Multiparous cows had a reduced hazard (HR = 0.76; $P = 0.001$) of being inseminated within 100 DIM compared with primiparous cows.

Table 2 (Continued). Univariate associations between DIM, estrous duration, and estrous intensity at the first postpartum estrus event, the prevalence of anestrus,[1] and selected categorical risk factors from 3,750 Holstein cows equipped with a neck-mounted activity sensor system[2]

Risk factor	N (n)[3]	First postpartum estrus event[4] DIM at first estrus event	Estrous duration, h	Estrous intensity, index value	Prevalence of anestrus,[5] %
Fresh season[14]					
Spring	971 (723)	35.8 ± 0.54[a]	8.6 ± 0.14[ab]	22.2 ± 0.54[ab]	25.5[a]
Summer	932 (709)	33.7 ± 0.53[b]	8.1 ± 0.14[a]	20.3 ± 0.54[a]	23.9[a]
Autumn	676 (581)	30.2 ± 0.58[c]	8.8 ± 0.17[b]	22.2 ± 0.64[ab]	14.1[b]
Winter	1,171 (959)	34.9 ± 0.46[ab]	8.8 ± 0.12[b]	23.8 ± 0.52[b]	18.1[b]

[a–c]Within a column and risk factor, proportions with different superscripts differ ($P < 0.05$).

[1]Cows with no estrus alarm from d 7 until d 60 were defined as anestrous.

[2]Smarttag Neck, Nedap Livestock Management.

[3]N = total number of observed cows; n = number of observed cows with an estrus alarm within d 7 and 60.

[4]Least squares means for DIM, estrous duration, and estrous intensity at the first postpartum estrus event among levels of risk factors using ANOVA. For DIM, the day when the maximum x-factor was recorded was used. Estrous duration was defined as the interval from onset to end of an estrus event. Estrous intensity was defined by the intensity of increased activity represented by the peak value of the x-factor within the observational period (i.e., DU).

[5]Least squares means for probability of anestrus among risk factors using logistic regression.

[6]Milk yield was projected based on the second test day including a mature-equivalent factor (Q1 = 5,160–10,570 kg; Q2 = 10,576–11,570 kg; Q3 = 11,580–12,510 kg; Q4 = 12,520–16,280 kg).

[7]Dystocia was defined as a calving of 3 or greater. Calving ease was defined as 0 = physiological calving; 1 = calving assisted by 1 person; 2 = calving assisted by 2 people; 3 = calving assisted by 3 people; 4 = calving assisted by more than 3 people).

[8]Retained placenta was defined as cows whose fetal membranes were not expulsed until 24 h after calving.

[9]Puerperal metritis was defined as an animal having an abnormally enlarged uterus and a fetid, watery, red-brown uterine discharge, associated with a sign of systemic illness such as decreased milk yield, dullness, or other signs of toxemia, and a rectal body temperature >39.5°C within 21 d after parturition.

[10]Subclinical ketosis was defined as cows with blood BHB ≥1.2 mmol/L.

[11]Clinical milk fever was defined as a multiparous cow showing recumbency and paresis.

[12]Clinical mastitis was defined by visible signs of inflammation in an affected mammary gland such as redness, swelling, pain, or heat, and an altered milk secretion, such as the appearance of clots, flakes, discoloration, or abnormal consistency by 60 DIM.

[13]Displaced abomasum was defined as cows in which percussion of the left flank resulted in tympanic resonance detected using a stethoscope.

[14]Fresh season was defined as the month of calving and categorized into spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February).

Time to Pregnancy

Median DIM to pregnancy were 121, 96, and 92 for cows in Estrus0, Estrus1, and Estrus2+, respectively. Time to pregnancy was associated with estrous expression from d 7 until d 60 postpartum ($P = 0.001$; Figure 5) and parity ($P = 0.001$). Compared with Estrus0 cows, Estrus1 cows (HR = 1.24; $P = 0.001$) and Estrus2+ cows (HR = 1.46; $P = 0.001$) had an increased hazard of becoming pregnant within 200 DIM. Compared with Estrus2+ cows, cows in Estrus1 had a reduced hazard of becoming pregnant within 200 DIM (HR = 0.85; $P = 0.001$). Multiparous cows had a reduced hazard (HR = 0.54; $P = 0.001$) of becoming pregnant within 200 DIM compared with primiparous cows.

DISCUSSION

The objective of this study was to describe the association of transition cow health and estrous expression detected by an AAM system within 60 DIM on reproductive performance of lactating dairy cows. Our results suggest that cows with no estrus event detected by an AAM from d 7 until d 60 postpartum had reduced DU at first postpartum AI and a greater chance of receiving TAI. Moreover, cows displaying at least 1 estrus event from d 7 until d 60 postpartum had improved reproductive performance, as shown by greater P/AI at first AI, especially in multiparous cows. Cows in Estrus1 and Estrus2+ also had an increased hazard of being inseminated within 100 DIM and becoming pregnant within 200 DIM. In addition, we wanted to evaluate risk factors for anestrus within the VWP. Multiparous cows had a greater chance for anestrus. Cows with stillbirth, RP, PM, or subclinical ketosis were more likely to be in anestrus within the VWP. Our study was conducted on a single herd, so the external validation is limited.

The overall prevalence of anestrus was 20.8% in our study. Studies that also used an AAM system to evaluate the prevalence of anestrus observed a greater number of anestrous cows than in our study (Borchardt

Table 3. Final logistic regression model using the GENLINMIXED procedure in SPSS (version 28.0, IBM Corp.) for association between risk factors in the transition period and the probability of anestrus[1] from 3,750 Holstein cows equipped with a neck-mounted activity sensor system[2]

Variable	Estimate	SE[3]	Odds ratio (OR)	95% CI for OR Lower	95% CI for OR Upper	P-value
Intercept	−0.97	0.18	0.38	0.27	0.54	0.001
Parity						
Primiparous cows	Referent					
Multiparous cows	0.34	0.11	1.41	1.14	1.75	0.002
Stillbirth						
No	Referent					
Yes	0.57	0.27	1.76	1.04	2.98	0.034
Retained placenta[4]						
No	Referent					
Yes	0.78	0.26	2.19	1.31	3.67	0.003
Puerperal metritis[5]						
No	Referent					
Yes	0.39	0.17	1.48	1.07	2.05	0.019
Subclinical ketosis[6]						
No	Referent					
Yes	0.41	0.12	1.51	1.20	1.90	0.001
Fresh season[7]						
Spring	Referent					
Summer	−0.20	0.14	0.82	0.63	1.07	0.151
Autumn	−0.96	0.17	0.38	0.27	0.53	0.001
Winter	−0.58	0.14	0.56	0.43	0.73	0.001

[1]Cows having no estrus alarm from d 7 until d 60 were defined as anestrous.

[2]Smarttag Neck, Nedap Livestock Management.

[3]SE = standard error of the estimate.

[4]Retained placenta was defined as cows whose fetal membranes were not expulsed until 24 h after calving.

[5]Puerperal metritis was defined as an animal having an abnormally enlarged uterus and a fetid, watery, red-brown uterine discharge, associated with a sign of systemic illness such as decreased milk yield, dullness, or other signs of toxemia, and a rectal body temperature >39.5°C within 21 d after parturition.

[6]Subclinical ketosis was defined as cows with blood BHB ≥1.2 mmol/L.

[7]Fresh season was defined as month of calving and categorized into 1 (spring = March, April, May), 2 (summer = June, July, August), 3 (autumn = September, October, November), and 4 (winter = December, January, February).

et al., 2021: 52.1%, 5 herds, 7–40 DIM; Chebel and Veronese, 2020: 52.7%, 1 herd, within 62 DIM). Parity was associated with the prevalence of anestrus (multiparous cows: 23.1% vs. primiparous cows: 17.5%). These results correspond with the findings of Bruinjé et al. (2021c; multiparous cows: 37% vs. primiparous cows: 25%), whereas other studies have shown either no parity association (Borchardt et al., 2021) or a greater prevalence of anestrus in primiparous cows (Bamber et al., 2009; Vercouteren et al., 2015).

In our study, median DIM until first estrus event was 35, which is similar to another study using a neck-mounted AAM system (Løvendahl and Chagunda, 2010; 33 DIM). Based on the interestrus interval from d 7 until d 60 postpartum in our study, 52.9% (822/1,553 cows) had an interestrus interval between 18 and 24 d. This proportion of cows showing a regular cycle length was greater compared with a previous study from our group (Borchardt et al., 2021: 24%), in which the majority of cows (43.6%; 340/780 cows) had a short interestrus interval (i.e., <18 d). The reason for this difference remains speculative, but might be associated with different AAM systems, observational periods, and observational groups.

Inflammatory and metabolic diseases are major factors that underlie anovulatory conditions in dairy cows (Santos et al., 2016). Cows with RP, PM, or subclinical ketosis had a greater chance for anestrus compared with healthy cows. This is in agreement with a study by Santos et al. (2010) in which they compiled data from 8 experiments representing 5,719 cows from 7 farms. All cows were evaluated for cyclicity at 65 d postpartum by sequential P4 analysis in plasma 12 to 14 d apart. Calving-related disorders [e.g., dystocia, twins, stillbirth, and RP; odds ratio (**OR**) = 0.52] and diseases affecting the reproductive tract (i.e., metritis, OR = 0.37; clinical endometritis, OR = 0.51) were the major contributors for depressed cyclicity at d 65 postpartum. Cows with clinical ketosis tended to have reduced odds (OR = 0.71) for cyclicity. Another study

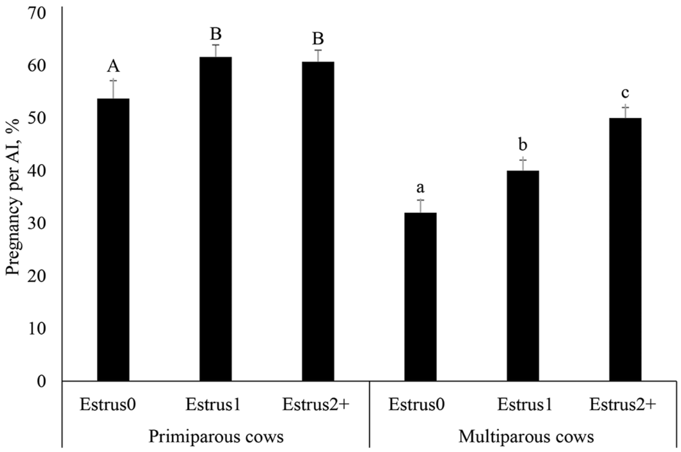

Figure 3. Association between estrous expression from d 7 until d 60 postpartum on pregnancy per AI (%; ± SEM) at first postpartum AI using a neck-mounted activity monitoring system (Smarttag Neck, Nedap Livestock Management). An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm. Cows were classified according to the number of estrus events from d 7 until d 60 postpartum into 3 categories: (1) no estrus event (Estrus0), (2) 1 estrus event (Estrus1), and (3) 2 or more estrus events (Estrus2+). Bars with different uppercase letters denote tendencies ($P \geq 0.05$ and $P < 0.1$); bars with different lowercase letters denote significant differences ($P < 0.05$) within a parity group.

in grazing dairy cows revealed similar findings (Ribeiro et al., 2013). In this particular study, cows with metritis, respiratory problems, and indigestion increased the odds of cows remaining anovular by d 49 postpartum by 2.4-, 4.1-, and 4.2-fold, respectively. It is interesting to note that disease and anovulation had additive effects depressing P/AI in dairy cows, thereby suggesting that some of the mechanisms might be independent and complementary relative to the lesions that impair establishment and maintenance of pregnancy in cattle (Ribeiro et al., 2016).

Cows in Estrus2+ had a greater likelihood of conceiving at the first postpartum AI (55.4%) compared with cows in Estrus0 (42.5%) and Estrus1 (50.9%). Similarly, cows with 2 or more estrus events from d 7 until d 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%; $P = 0.039$) in another recent study from our group (Borchardt et al., 2021). Studies using either milk (Walsh et al., 2007) or blood P4 concentration (Galvão et al., 2009) to determine estrus cyclicity in the early postpartum period also found anovular cows had reduced odds of conceiving at the first postpartum AI. In our study, the association of estrus cyclicity and P/AI at first postpartum AI was even more pronounced

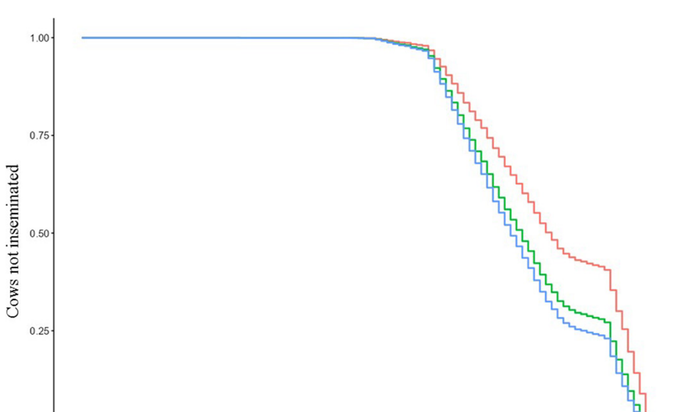

Figure 4. Kaplan-Meier survival analysis illustrating the association of estrous expression from d 7 until d 60 postpartum with time to first insemination in 3,750 cows. Cows were equipped with a neck-mounted activity monitoring system (Smarttag Neck, Nedap Livestock Management). An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm. Cows were classified according to the number of estrus events from d 7 until d 60 postpartum into 3 categories: (1) no estrus event (Estrus0; red line; n = 778); (2) 1 estrus event (Estrus1; green line; n = 1,419); and (3) 2 or more estrus events (Estrus2+; blue line; n = 1,553). Median DIM at first insemination were 84, 74, and 74 for cows in Estrus0, Estrus1, and Estrus2+, respectively.

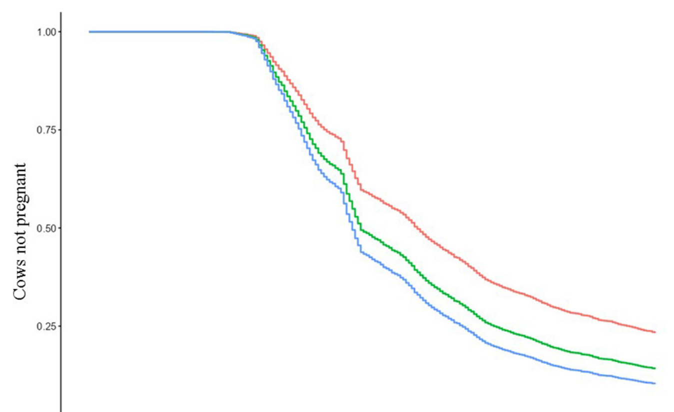

Figure 5. Kaplan-Meier survival analysis illustrating the association of estrous expression from d 7 until d 60 postpartum with time to pregnancy in 3,750 cows. Cows were equipped with a neck-mounted activity monitoring system (Smarttag Neck, Nedap Livestock Management). An estrus event was defined as an attention change from 0 to 1 for more than 2 time periods (5 h) based on a proprietary algorithm. Cows were classified according to the number of estrus events from d 7 until d 60 postpartum into 3 categories: (1) no estrus event (Estrus0; red line; n = 778); (2) 1 estrus event (Estrus1; green line; n = 1,419); and (3) 2 or more estrus events (Estrus2+; blue line; n = 1,553). Median DIM at pregnancy were 121, 96, and 92 for cows in Estrus0, Estrus1, and Estrus2+, respectively.

in multiparous cows compared with primiparous cows, indicating that resumption of cyclicity within the VWP seems to be especially important for multiparous cows. This corresponds with the results from Bruinjé et al. (2021a). To the best of our knowledge, our study is the first to show an association between early resumption of estrus cyclicity and P/AI for cows receiving sexed semen. The use of sexed semen in lactating Holstein cows is becoming more popular (Lauber et al., 2020). Our results suggest that activity data in early lactation can be used to optimize reproductive strategy by detecting cows with early resumption and a targeted use of sexed semen in these cows.

Considering the characteristics of an estrus event at first postpartum AI, we observed that Estrus0 cows displayed shorter DU and were less likely to have high PA. This is consistent with a previous study from our group (Borchardt et al., 2021).

Postpartum estrous activity was also associated with time to first AI in our study. Estrus0 cows had a reduced hazard of being inseminated until 100 DIM. Median DIM to first insemination were 84, 74, and 74 for cows in Estrus0, Estrus1, and Estrus2+, respectively. This is in agreement with a previous observational study in which a different AAM system (Heatime Pro; SCR Engineers Ltd.) was used (Borchardt et al., 2021). Here, median DIM to first insemination were 70, 59, and 58 for cows in Estrus0, Estrus1, and Estrus2+, respectively. Results from these 2 studies using AAM systems correspond with studies in which milk P4 or blood P4 were used to detect estrous activity (Walsh et al., 2007; Galvão et al., 2009). In the study using milk P4 profiles (Walsh et al., 2007), median days to first insemination were 72 and 80 for ovular and anovular cows, respectively. In the study from Galvão et al. (2009), a Presynch-Ovsynch protocol with insemination after estrus detection was used to facilitate first postpartum TAI. Evaluating blood P4 profiles, median days to first insemination were 71, 76, and 96 for cows cycling at 21 d, 49 d, and anovular cows, respectively. When comparing different studies regarding the effect of anovulation on insemination risk, however, one has to be careful, as interventions with TAI protocols might confound the effects.

Using time to pregnancy as an important measure of reproductive performance (Lean et al., 2016), cows displaying estrous activity from d 7 until d 60 postpartum had an increased hazard of conceiving until 200 DIM compared with Estrus0 cows. Median time to pregnancy was 121, 96, and 92 d for cows in Estrus0, Estrus1, and Estrus2+, respectively. This is in agreement with a previous study using a similar study design but a different AAM system (Borchardt et al., 2021). Here, median time to pregnancy was 127, 112, and 103 d for cows in Estrus0, Estrus1, and Estrus2+, respectively. The results from these 2 studies using AAM technology correspond with 3 other studies using either milk (Walsh et al., 2007) or blood P4 profiles to identify anovular cows (Galvão et al., 2009; Dubuc et al., 2012). Overall, this observational study provides evidence that displaying estrous activity in the early postpartum period may be beneficial for subsequent reproductive performance.

Using AAM data might be an opportunity for the dairy industry to improve reproductive performance, optimize herd-management practices, and increase profitability. This concept has been described as targeted reproductive management (Giordano et al., 2022). Major steps in the development and implementation of targeted reproductive management programs for dairy cattle include the identification and validation of robust predictors of reproductive outcomes and cow performance. Furthermore, these reproductive management strategies for optimizing outcomes of interest for subgroups of cows need an on-farm evaluation. Results from this study again highlight the potential of using AAM data within the VWP as a proxy for resumption of cyclicity. Using a neck-mounted AAM system, we observed inferior reproductive performance for cows without estrous expression from d 7 until d 60 postpartum compared with cows displaying estrous activity. Future studies need to evaluate management strategies to improve the reproductive performance in anestrous cows. One possibility might be to select cows based on estrous expression within the VWP, such as enrollment of anestrus cows in a TAI protocol (e.g., Double-Ovsynch protocol or an Ovsynch including a P4 supplementation), whereas cows with estrous activity should be inseminated based on estrus detection after the VWP. Further, our results suggest that estrous expression within the VWP seems to be more important for AI services performed with sexed semen compared with conventional semen. Particularly in primiparous cows with higher fertility and more advanced genetics compared with multiparous cows, the use of sexed semen is becoming more established (Lauber et al., 2020). With the aid of activity data, sexed semen could be used in a more targeted way, such as for cows displaying estrous activity within the VWP, to optimize reproductive performance. However, this assumption needs to be investigated in additional trials involving a greater number of commercial herds.

CONCLUSIONS

Results from the present study provide further evidence that early postpartum estrous expression influences fertility in lactating dairy cows. Cows with no estrous expression detected by an AAM system from d

7 until d 60 postpartum had inferior reproductive performance compared with cows that displayed estrous activity. Cows with stillbirth, RP, metritis, and subclinical ketosis were more likely to be anestrous within the VWP. Future studies should address intervention strategies in these cows to improve their reproductive performance.

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REFERENCES

Bamber, R. L., G. E. Shook, M. C. Wiltbank, J. E. P. Santos, and P. M. Fricke. 2009. Genetic parameters for anovulation and pregnancy loss in dairy cattle. J. Dairy Sci. 92:5739–5753. https://doi.org/10.3168/jds.2009-2226.

Borchardt, S., C. M. Tippenhauer, J.-L. Plenio, A. Bartel, A. M. L. Madureira, R. L. A. Cerri, and W. Heuwieser. 2021. Association of estrous expression detected by an automated activity monitoring system within 40 days in milk and reproductive performance of lactating Holstein cows. J. Dairy Sci. 104:9195–9204. https://doi.org/10.3168/jds.2020-19705.

Bruinjé, T. C., M. G. Colazo, M. Gobikrushanth, and D. J. Ambrose. 2017. Relationships among early postpartum luteal activity, parity, and insemination outcomes based on in-line milk progesterone profiles in Canadian Holstein cows. Theriogenology 100:32–41. https://doi.org/10.1016/j.theriogenology.2017.05.021.

Bruinjé, T. C., E. I. Morrison, D. Renaud, E. S. Ribeiro, and S. J. LeBlanc. 2021a. Impacts of transition health on reproductive performance based on detection of estrus in dairy cows. J. Dairy Sci. 104(Suppl. 1):281. (Abstr.)

Bruinjé, T. C., E. I. Morrison, D. Renaud, E. S. Ribeiro, and S. J. LeBlanc. 2021b. Impact of systemic and uterine inflammation on luteal function in postpartum dairy cows. J. Dairy Sci. 104(Suppl. 1):282. (Abstr.)

Bruinjé, T. C., E. I. Morrison, R. C. Serrenho, D. Renaud, E. S. Ribeiro, and S. J. LeBlanc. 2021c. Association between transition health and estrus detection by activity monitors in dairy cows. J. Dairy Sci. 104(Suppl. 1):183. (Abstr.)

Chebel, R. C., and A. Veronese. 2020. Associations between genomic merit for daughter pregnancy rate of Holstein cows and metabolites postpartum and estrus characteristics. J. Dairy Sci. 103:10754–10768. https://doi.org/10.3168/jds.2020-18207.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. VER Inc.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2012. Risk factors and effects of postpartum anovulation in dairy cows. J. Dairy Sci. 95:1845–1854. https://doi.org/10.3168/jds.2011-4781.

Galvão, K. N., M. Frajblat, W. R. Butler, S. B. Brittin, C. L. Guard, and R. O. Gilbert. 2009. Effect of early postpartum ovulation on fertility in dairy cows. Reprod. Domest. Anim. 45:e207–e211. https://doi.org/10.1111/j.1439-0531.2009.01517.x.

Galvão, K., M. Frajblat, W. Butler, S. Brittin, C. Guard, and R. Gilbert. 2010. Effect of early postpartum ovulation on fertility in dairy cows. Reprod. Domest. Anim. 45:e207–e211. https://doi.org/10.1111/j.1439-0531.2009.01517.x.

Giordano, J. O., E. M. Sitko, C. Rial, M. M. Pérez, and G. E. Granados. 2022. Symposium review: Use of multiple biological, management, and performance data for the design of targeted reproductive management strategies for dairy cows. J. Dairy Sci. 105:4669–4678. https://doi.org/10.3168/jds.2021-21476.

Grymer, J., and K. E. Sterner. 1982. Percutaneous fixation of left displaced abomasum, using a bar suture. J. Am. Vet. Med. Assoc. 180:1458–1461.

Guterbock, W. M. 2004. Diagnosis and treatment programs for fresh cows. Vet. Clin. North. Am. Food Anim. Pract. 20:605–626. https://doi.org/10.1016/j.cvfa.2004.06.002.

Lauber, M. R., B. McMullen, J. J. Parrish, and P. M. Fricke. 2020. Short communication: Effect of timing of induction of ovulation relative to timed artificial insemination using sexed semen on pregnancy outcomes in primiparous Holstein cows. J. Dairy Sci. 103:10856–10861. https://doi.org/10.3168/jds.2020-18836.

Lean, I. J., M. C. Lucy, J. P. McNamara, B. J. Bradford, E. Block, J. M. Thomson, J. M. Morton, P. Celi, A. R. Rabiee, J. E. P. Santos, W. W. Thatcher, and S. J. LeBlanc. 2016. Invited review: Recommendations for reporting intervention studies on reproductive performance in dairy cattle: Improving design, analysis, and interpretation of research on reproduction. J. Dairy Sci. 99:1–17. https://doi.org/10.3168/jds.2015-9445.

Løvendahl, P., and M. G. G. Chagunda. 2010. On the use of physical activity monitoring for estrus detection in dairy cows. J. Dairy Sci. 93:249–259. https://doi.org/10.3168/jds.2008-1721.

McArt, J. A., D. V. Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198:560–570. https://doi.org/10.1016/j.tvjl.2013.08.011.

NRC. 2001. Chapter 9, pages 184–205 in Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press.

Nordlund, K. V., and N. B. Cook. 2004. Using herd records to monitor transition cow survival, productivity, and health. Vet. Clin. North. Am. Food Anim. Pract. 20:627–649. https://doi.org/10.1016/j.cvfa.2004.06.012.

Overton, M. W., and V. E. Cabrera. 2017. Monitoring and quantifying the value of change in reproductive performance. Pages 549–564 in Large Dairy Herd Management. D. K. Beede, ed. American Dairy Science Association.

Ribeiro, E. S., G. Gomes, L. F. Greco, R. L. A. Cerri, A. Vieira-Neto, P. L. J. Monteiro Jr., F. S. Lima, R. S. Bisinotto, W. W. Thatcher, and J. E. P. Santos. 2016. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J. Dairy Sci. 99:2201–2220. https://doi.org/10.3168/jds.2015-10337.

Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J. Dairy Sci. 96:5682–5697. https://doi.org/10.3168/jds.2012-6335.

Roelofs, J. B., F. J. C. M. van Eerdenburg, N. M. Soede, and B. Kemp. 2005. Pedometer readings for estrous detection and as predictor for time of ovulation in dairy cattle. Theriogenology 64:1690–1703. https://doi.org/10.1016/j.theriogenology.2005.04.004.

Roelofs, J. B., and E. Van Erp-Van der Kooij. 2015. Estrus detection tools and their applicability in cattle: Recent and perspectival situation. Anim. Reprod. Sci. 12:498–504.

Santos, J. E., R. S. Bisinotto, E. S. Ribeiro, F. S. Lima, L. F. Greco, C. R. Staples, and W. W. Thatcher. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. Soc. Reprod. Fertil. Suppl. 67:387–403. https://doi.org/10.7313/upo9781907284991.030.

Santos, J. E. P., R. S. Bisinotto, and E. S. Ribeiro. 2016. Mechanisms underlying reduced fertility in anovular dairy cows. Theriogenology 86:254–262. https://doi.org/10.1016/j.theriogenology.2016.04.038.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sá Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207–221. https://doi.org/10.1016/j.anireprosci.2008.01.014.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530. https://doi.org/10.1016/j.theriogenology.2005.08.021.

Stevenson, J. S., S. Banuelos, and L. G. D. Mendonça. 2020. Transition dairy cow health is associated with first postpartum ovulation risk, metabolic status, milk production, rumination, and physical activity. J. Dairy Sci. 103:9573–9586. https://doi.org/10.3168/jds.2020-18636.

Tippenhauer, C. M., J.-L. Plenio, W. Heuwieser, and S. Borchardt. 2023. Association of activity and subsequent fertility of dairy cows after spontaneous estrus or timed artificial insemination. J. Dairy Sci. 106:4291–4305. https://doi.org/10.3168/jds.2022-22057.

Vasquez, A. K., D. V. Nydam, M. B. Capel, S. Eicker, and P. D. Virkler. 2017. Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd. J. Dairy Sci. 100:2992–3003. https://doi.org/10.3168/jds.2016-11614.

Vercouteren, M. M. A. A., J. H. J. Bittar, P. J. Pinedo, C. A. Risco, J. E. P. Santos, A. Vieira-Neto, and K. N. Galvão. 2015. Factors associated with early cyclicity in postpartum dairy cows. J. Dairy Sci. 98:229–239. https://doi.org/10.3168/jds.2014-8460.

Walsh, R. B., D. F. Kelton, T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2007. Prevalence and risk factors for postpartum anovulatory condition in dairy cows. J. Dairy Sci. 90:315–324. https://doi.org/10.3168/jds.S0022-0302(07)72632-2.

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