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Association of activity and subsequent fertility of dairy cows after spontaneous estrus or timed artificial insemination

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ABSTRACT

The objective of this observational study was to evaluate the association between increased physical activity at first artificial insemination (AI) and subsequent pregnancy per AI (P/AI) in lactating Holstein cows following spontaneous estrus or a timed AI (TAI) protocol. We also wanted to identify factors associated with the intensity of activity increase (PA) captured by automated activity monitors (AAM) and fertility. Two experiments were conducted, in which cows either were inseminated based on the alert of the AAM system (AAM cows) or received TAI following a 7-d Ovsynch protocol (TAI cows) if not inseminated within a farm-specific period after calving. Experiment 1 included 2,698 AI services from AAM cows and 1,042 AI services from TAI cows equipped with the Smarttag Neck (Nedap Livestock Management) from a dairy farm in Slovakia (farm 1). In the second experiment, 6,517 AI services from AAM cows and 1,226 AI services from TAI cows fitted with Heatime (Heatime Pro; SCR Engineers Ltd.) from 8 dairy farms in Germany (farms 2–9) were included. Pregnancy diagnosis was performed on a weekly basis by transrectal ultrasound (farms 1, 3, 7, 8) or by transrectal palpation (farms 2, 4–6, 9). Estrous intensity was represented by the peak value of the change in activity. In experiment 1, PA was categorized into low (x-factor 0–20) and high (x-factor 21–100) PA, and in experiment 2 into low (activity change = 35–89) and high (activity change = 90–100) PA. In TAI cows from both experiments, PA was additionally categorized into cows with no AAM alert. Data were analyzed separately for AAM and TAI cows using multinomial logistic regression models for PA in TAI cows and logistic regression models for PA in AAM cows and P/AI in both groups. In experiment 1, P/AI of AAM cows was greater for AI services performed with conventional frozen semen (57.6%) compared with sexed semen (47.2%), whereas type of semen only tended to be associated with P/AI in TAI cows (54.4% conventional frozen semen vs. 48.9% sexed semen). In experiment 2, P/AI was greater for fresh semen (AAM cows: 44.4% vs. TAI cows: 44.2%) compared with conventional frozen semen (AAM cows: 37.6% vs. TAI cows: 34.6%). In both experiments, pregnancy outcomes were associated with PA. In experiment 1, AAM cows with high PA (55.1%) had greater P/AI than cows with low PA (49.8%). Within TAI cows, cows with no alert (38.8%) had reduced P/AI compared with cows with low (54.2%) or high PA (61.8%). In experiment 2, AAM cows with high PA (45.8%) had greater P/AI compared with cows with low PA (36.4%). Timed AI cows with no alert (27.4%) had decreased P/AI compared with cows with low (41.1%) or high (50.8%) PA. The greatest risk factors for high PA were parity (experiment 1) and season of AI (except for TAI cows from experiment 1). We conclude that high PA at the time of AI is associated with greater odds of pregnancy for both AAM and TAI cows. In both experiments, about 2 thirds of AAM cows (experiment 1: 69.9% and experiment 2: 70.7%) reached high PA, whereas only approximately one-third or less of TAI cows (experiment 1: 37.3% and experiment 2: 23.6%) showed high PA. Although we observed similar results using 2 different AAM systems for the most part, risk factors for high PA might differ between farms and insemination type (i.e., AAM vs. TAI).

Key words: estrous expression, automated activity monitor, timed artificial insemination, pregnancy per artificial insemination

INTRODUCTION

Automated activity monitoring (**AAM**) systems help to overcome the inefficiency of estrus detection based on visual observation by providing continuous monitoring of cows. Studies using modern neck-mounted AAM systems correctly identified more than 70% of preovulatory phases (Aungier et al., 2012; Valenza et al., 2012; LeRoy et al., 2018), but also recorded a varying number of false positive alerts ranging from 5 to 32% (Aungier et al., 2012; Valenza et al., 2012). Furthermore, AAM

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systems represent a practical tool for evaluation of individual cow estrous expression, such as duration (**DU**) and intensity (**PA**) of an estrus event. As both parameters were found to be strongly associated with the risk of pregnancy and embryonic development in lactating dairy cows, expression of estrus may be useful to predict fertility (Madureira et al., 2015a; Pereira et al., 2016; Tippenhauer et al., 2021a,b). A certain percentage of cows, however, will not be detected in estrus and consequently not inseminated most probably due to the inability of cows to display estrous behavior to be identified by AAM systems (Valenza et al., 2012; Stangaferro and Giordano, 2019). Risk factors, such as flooring (Britt et al., 1986), parity (López-Gatius et al., 2005; Burnett et al., 2017), heat stress (Polsky et al., 2017; Tippenhauer et al., 2021a), and milk yield (Rivera et al., 2010) have been identified to compromise estrous expression. Moreover, a considerable proportion of cows undergo silent ovulations or are anovular near the end of the voluntary waiting period (**VWP**; Bamber et al., 2009; Sauls et al., 2017). Stevenson et al. (2020) observed a strong association between transition cow health status and first ovulation risk and physical activity. Specifically, metritis, metabolic disorders, and mastitis have been shown to delay first postpartum ovulation and decrease physical activity postpartum.

Ovsynch protocols (Pursley et al., 1995), in contrast, allow timed artificial insemination (**TAI**) without the need for estrus detection. It has been shown, however, that cows expressing estrus at TAI have greater pregnancy per AI (**P/AI**) and reduced pregnancy loss (Pereira et al., 2016). The association between an increase in physical activity and subsequent pregnancy outcomes for synchronized cows, however, is not well researched. Enrolling cows fitted with a leg-mounted AAM system in a synchronization protocol, Madureira et al. (2019) found PA at TAI to be associated with greater ovulation rates, greater P/AI, and reduced pregnancy losses. Another study (Schweinzer et al., 2020) found only minor behavioral changes detected by an ear-tag based AAM system between cows inseminated based on spontaneous estrus and TAI. Furthermore, they found no association between intensity of behavioral changes and subsequent pregnancy outcomes in synchronized cows.

Considering the inconclusive findings, the objective of this observational study was to evaluate the association between PA at first AI and subsequent P/AI in lactating Holstein cows inseminated based on spontaneous estrus (**AAM cows**) or after a 7-d Ovsynch protocol (**TAI cows**). Moreover, we wanted to identify factors associated with PA captured by 2 different neck-mounted AAM systems and fertility. We hypothesized that (1) factors associated with PA and subsequent P/AI would be similar for AAM and TAI cows and (2) a greater PA would be associated with improved reproductive performance irrespective of insemination type (i.e., spontaneous estrus vs. TAI).

MATERIALS AND METHODS

This study was an observational, retrospective cohort study and comprised of 2 experiments with separate data sets based on 2 different AAM systems. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

Experiment 1

Animals, Housing, and Management. This experiment was conducted from January 2020 until September 2021 and included 3,858 AI services from a Slovakian commercial dairy farm, housing approximately 2,700 lactating cows (farm 1, Table 1). Inclusion criteria were cows past the VWP (Table 1), AI based on the alert of the AAM or after completion of a 7-d Ovsynch protocol, cows receiving first AI after calving, and cows eligible for insemination. Cows were housed in freestall barns 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) and was fed twice daily. Cows were milked twice daily having a milk yield of 11,500 kg per 305 d (Table 1). All cow-related events and procedures (e.g., estrus, AI, treatments, pregnancy outcomes) were entered into the farm management software DairyComp 305 (DC305, Valley Agriculture Software) by farm personnel.

Automated Activity Monitor. Twenty-one days before their first calving, all cows were equipped with the neck-mounted AAM Smarttag Neck (Nedap Livestock Management). This accelerometer recorded activity data in real time. The relative change in activity was measured 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 period of the day within 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 to 100 (0 = lowest, 100 = highest). 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 could be 0 (no estrus) or 1 (estrus). Onset of estrus was defined to occur at the first period with an x-factor exceeding the threshold. End of estrus was defined by the first instance at which the attention changed from 1 to 0. The intensity of increased activity was represented by the peak value of the x-factor within the observational period (i.e., 1 d before until 1 d after AI). The peak value was stratified into 10 equal categories and P/AI was calculated for each category to determine the threshold with the greatest decrease in P/AI. Based on this, PA was categorized into no alert (attention = 0), low (x-factor 0–20 index value), and high (x-factor 21–100 index value) PA. Estrous duration was defined as the interval (h) from onset to end of an estrus event. For each AI, PA and, in the case of an estrus event, DU were determined.

A file from the AAM system was generated on the farm computer including cow ID, lactation number, AI number, DIM at AI, and last calving date. In addition, raw activity data, the x-factor, and attention were exported for the years 2020 and 2021 as comma-separated values files. The files were processed with the data analysis and statistics library pandas (McKinney, 2010) to extract the desired AAM data for each cow 1 d before until 1 d post AI. The selected data included the following information for each cow: cow ID, lactation number, last insemination date, AI number, DIM at AI, raw activity data (peak value and hour of raw activity peak), x-factor (value and hour of peak), attention (x-factor and hour for start and end), and DU of each estrus event.

Reproductive Management. Lactating Holstein cows were inseminated based on the alert of the AAM system or received TAI after the 7-d Ovsynch protocol (Table 2). The VWP was 60 d (Table 1). On a daily basis, the AAM system generated a list of cows eligible for insemination based on the attention value. Estrus was verified by the AI technician via transrectal palpation considering a highly contractile uterus, visualization of clear, stringy vaginal discharge, or both. Cows with an AAM alert were inseminated on the same day. Cows that were not inseminated by 80 ± 3 DIM were enrolled into a 7-d Ovsynch protocol with a second $PGF_{2\alpha}$ treatment on d 8 (GnRH–7 d–PGF–1 d–PGF–32 h–GnRH–16 h–AI). Cows that expressed estrus at the time of the second GnRH treatment received GnRH and were inseminated at the same day. These cows were assigned to the TAI cows within the statistical analysis. Cows remained in the study until a confirmed pregnancy diagnosis after first insemination, which was performed on a weekly basis by transrectal ultrasound 38 ± 3 d after the first AI (Table 1). Pregnancy was based on visualization of an embryo with a heartbeat. All pregnancy diagnoses were conducted by trained farm personnel.

Inseminations were performed either with conventional frozen or sexed semen. The selection of cows for

Table 1. Descriptive information[1] of enrolled dairy farms from experiments 1 and 2

	Farm[2]								
Parameter	1	2	3	4	5	6	7	8	9
Average farm size	2,727	1,326	671	412	398	878	565	562	890
Average 305 d milk yield (kg)	11,500	10,824	10,114	9,337	11,900	11,428	9,049	10,371	10,520
Milking frequency per day	2	2	2	2	3	3	2	2	3
Voluntary waiting period (DIM)	60	50	60	50	70	60	40	40	40
Median DIM at first AI based on AAM[3]	70	64	83	89	99	86	56	57	60
Median DIM at first timed AI	93	94	115	—	102	120	84	83	88
21-d service rate (%)	65	57	41	40	52	43	60	55	48
Cows inseminated in estrus at first AI (%)	72	76	97	100	95	93	87	74	96
Overall conception rate (%)	49	20	44	43	42	33	37	32	35
21-d pregnancy rate[4] (%)	32	11	18	17	22	14	22	18	17
Day of pregnancy diagnosis after AI[5]	38 ± 3	38 ± 3	30 ± 3	38 ± 3	38 ± 3	43 ± 3	30 ± 3	30 ± 3	38 ± 3
Method of pregnancy diagnosis	Transrectal ultrasound	Transrectal palpation	Transrectal ultrasound	Transrectal palpation	Transrectal palpation	Transrectal palpation	Transrectal ultrasound	Transrectal ultrasound	Transrectal ultrasound

[1] Descriptive information for farms was obtained from the farm management software (experiment 1: DairyComp 305; Valley Agriculture Software; experiment 2: herdeW and herdeplus; dsp-Agrosoft GmbH).

[2] Farm 1 was part of experiment 1, farms 2 to 9 participated in experiment 2.

[3] All cows were equipped with neck-mounted automated activity monitors (AAM) for detection of estrus (farm 1: Smarttag Neck; Nedap Livestock Management; farms 2 to 9: Heatime; SCR Engineers Ltd.).

[4] The 21-d pregnancy rate was calculated using the individual farm voluntary waiting period.

[5] As pregnancy diagnosis was performed on a weekly basis on all farms, day of pregnancy diagnosis after AI is presented as ± 3 in d.

sexed semen was based on the pedigree information from a cow and the relative value, which is a computed item in DC305. It is a comparison of the cow's 305 mature equivalent milk with the herd average 305 mature equivalent milk.

Experiment 2

Animals, Housing, and Management. Experiment 2 included 9,525 AI services from 8 commercial dairy farms (farms 2 to 9) in northeastern Germany from July 2018 until July 2021. Inclusion criteria were a farm size >400 cows, the use of a neck-mounted AAM, cows past the VWP (Table 1), AI based on the alert of the AAM or after completion of a 7-d Ovsynch protocol, cows receiving first AI after calving, and eligible for insemination. Farm size ranged from approximately 400 to 1,300 cows per farm (Table 1). Cows were housed in freestall barns, had ad libitum access to feed and water and were fed twice daily a TMR diet consisting of corn silage and grass silage as forage with a corn and soybean meal-based concentrate. The TMR was formulated to meet or exceed the dietary requirements for dairy cows (NRC, 2001). Cows were milked twice or 3 times daily with a milk yield ranging from 9,049 to 11,900 kg per 305 d (Table 1). All cow related-events and procedures (e.g., estrus, AI, treatments, pregnancy outcomes) were entered into the farm management software (herdeW and herdeplus, respectively; dsp-Agrosoft GmbH) by farm personnel.

Automated Activity Monitor. All cows were fitted with a neck-mounted AAM (Heatime; SCR Engineers Ltd.) on the day of their first calving (farms 2, 3, 5, 7–9), at approximately 40 DIM (farm 4) or 14 d before their first calving (farm 6). The AAM was attached to the cows continuously (farms 2, 3, 5–9) or was removed at 65 d after AI when a cow was diagnosed pregnant (farm 4). Activity data of each individual cow was 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. Processing of the data were conducted as previously described (Tippenhauer et al., 2021a,b). The cow's raw activity data were converted by the accelerometer software into an activity change index by calculating 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). The period where the cow's activity change index value exceeded 35 was considered as an estrus event. Onset of estrus was defined as the first time a cow exceeded an activity change index value of 35. End of estrus was defined by the first instance at which the index value fell below 35 again. The intensity of estrus was represented by the peak value of the activity change index within the observational period (i.e., 1 d before until 1 d after AI). Estrous intensity was categorized into no alert (activity change = 0–34 index value), low PA (activity change = 35–89 index value), and high PA (activity change = 90–100 index value) according to previous studies (Tippenhauer et al., 2021a,b). Estrous duration was defined as the interval (h) from onset to end of an estrus event. For each AI, PA and, in the case of an estrus event, DU were determined.

For each cow on each farm, files from the SCR system were generated in XLSX format including cow ID, lactation number, last insemination date, AI number, DIM, last calving date, and record of raw activity, daily activity, and activity change within the past 7 d every 2 h. Using DataFlow II, these files were exported on a weekly basis for all cows that were inseminated within the last 7 d on each farm. The open-source Bovine Heat Detection and Analysis Tool (BovHEAT) was used to process all SCR exported XLSX files and to assemble a single result report XLSX file with the desired AAM data for each cow 1 d before until 1 d post AI (Plenio et al., 2021). 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, last insemination date, AI number, DIM at AI, raw activity data (peak value and hour), activity change (index value and hour for activity change start, peak, and end), and DU.

Reproductive Management. Lactating Holstein cows were inseminated based on the alert of the AAM system, after visual estrus detection or received TAI after hormonal intervention (i.e., 7-d Ovsynch protocol; Table 2). On a daily basis, lists of cows eligible for insemination with an activity alert were generated by the AAM. All farms set their threshold for an activity alert at an index of 35. Based on these lists, estrus was verified by the AI technician as described in experiment 1. Because the printing of these lists took place at fixed times depending on-farm, cows were inseminated at different intervals related to the AAM alert. Cows were inseminated once (farms 2, 4, 5, 9) or twice daily (farms 3, 6–8) following the a.m.–p.m. rule, with each cow receiving a single AI based on the AAM alert. Cows that were not inseminated by a farm-specific threshold of DIM were examined by the on-farm veterinarian (farms 3 and 9: 80 ± 3 DIM; farm 7: 70 ± 3 DIM; farm 4: 100 ± 3 DIM). Cows then were treated accordingly (i.e., PGF treatment in case of the presence of a corpus luteum) or enrolled into a standard 7-d Ovsynch protocol starting on a fixed day once a week (GnRH–7

Table 2. Descriptive information of characteristics between cows inseminated based on spontaneous estrus (AAM cows)[1] and cows receiving timed artificial insemination (TAI cows)[2] from farms from experiments 1 and 2

	Experiment 1		Experiment 2	
Parameter	AAM cows	TAI cows	AAM cows	TAI cows
n	2,698	1,042	6,517	1,226
Median DIM at first AI	70	93	70	98
Parity (no.)				
Primiparous	1,243	307	2,528	458
Multiparous	1,455	735	3,989	768
Mean cumulative milk yield within 100 DIM (kg)	3,833	4,018	3,873	3,944
Type of semen used for AI (%, no. of AI services)				
Conventional frozen	45.5 (1,227)	56.0 (584)	92.5 (6,031)	55.7 (683)
Sexed	54.5 (1,471)	44.0 (458)	—	—
Fresh	—	—	7.5 (486)	44.3 (543)
Duration of increased activity (h; mean ± SD)	17.68 ± 4.04	14.78 ± 4.50	14.00 ± 4.56	12.93 ± 4.59
Peak activity (index value; mean ± SD)	30.26 ± 15.59	19.00 ± 18.38	91.14 ± 14.34	38.15 ± 39.08
Estrous intensity[3] (%, no. of AI services)				
No AAM alert	—	41.2 (429)	—	65.1 (798)
Low	30.1 (812)	21.5 (224)	29.3 (1,910)	11.3 (139)
High	69.9 (1,886)	37.3 (389)	70.7 (4,607)	23.6 (289)
Pregnancy per AI[4] (%; no./no.)	50.4 (1,413/2,698)	51.6 (506/1,042)	41.0 (2,374/6,517)	39.3 (350/1,226)

[1] All cows were equipped with neck-mounted automated activity monitors (AAM) for detection of estrus (experiment 1: Smarttag Neck; Nedap Livestock Management; experiment 2: Heatime; SCR Engineers Ltd.).

[2] Cows not inseminated by a farm-specific threshold received timed AI using a 7-d Ovsynch protocol (experiment 1: treatment with a second prostaglandin F$_{2\alpha}$ treatment on d 8).

[3] Estrous intensity was categorized into no AAM alert (only for TAI cows), low (experiment 1: x-factor ≤20 index value; experiment 2: activity change 35–89 index value) and high (experiment 1: x-factor >20 index value; experiment 2: activity change 90–100 index value).

[4] In experiment 1, percentages for P/AI of AAM cows were derived using a logistic regression model including parity, type of semen, estrous intensity, and cumulative 100-d milk yield. Percentages for P/AI of TAI cows were derived using a logistic regression model including parity, type of semen, estrous intensity, and the interaction between estrous intensity and type of semen. In experiment 2, percentages for P/AI of AAM cows were derived using a logistic regression model including parity, season of AI, type of semen, and estrous intensity. Percentages for P/AI of TAI cows were derived from a logistic regression model including parity, season of AI, type of semen, and estrous intensity. In both experiments, cow was the experimental unit and lactation number was considered as a repeated measure. In addition, farm was considered a random effect in experiment 2.

d–PGF–56 h–GnRH–16 h–AI; DIM at enrollment farm 2: 80 ± 3 DIM; farms 5 and 8: 70 ± 3 DIM; farm 6: 90 ± 3 DIM). Cows that expressed estrus at the day of the second GnRH treatment received GnRH and were inseminated at the same day. These cows were assigned to the TAI cows within the statistical analysis. After receiving first insemination, cows remained in the study until a confirmed pregnancy diagnosis, which was performed on a weekly basis by transrectal palpation 38 ± 3 d after AI (farms 2, 4, 5, 9) and 43 ± 3 d after AI (farm 6), or by transrectal ultrasound at 30 ± 3 d after AI (farms 3, 7, 8; Table 1). For simplicity, the time at which pregnancy diagnosis was conducted will be referred to as 38 d after AI throughout the article. In case of transrectal palpation, pregnancy was based on a verified pregnancy diagnosis defined by the presence of uterine fluid, asymmetry of the uterine horns, and a positive fetal membrane slip. Nonpregnancy was based on absence of pregnancy at the day of examination or a re-insemination to an estrus before pregnancy diagnosis. Positive pregnancy diagnosis performed by ultrasound was based on visualization of an embryo with a heartbeat. All pregnancy diagnoses were conducted by a veterinarian or by trained farm personnel.

Farms predominantly performed AI using conventional frozen semen and fresh semen. The number of straws of fresh semen used depended on the farm, but was usually requested on a weekly basis for TAI cows. The remaining straws with fresh semen were used within 2 d after delivery for AAM cows.

Data Collection and Statistical Analyses

Cow ID, parity, calving date, milk yield for the first 100 DIM obtained from the monthly DHIA testing, and AI information [i.e., DIM, number of AI, insemination type (estrus = AAM cows vs. hormonal intervention = TAI cows), type of semen, pregnancy outcome] were obtained through the on-farm computer software (experiment 1: DC305; experiment 2: herdeW and herdeplus, respectively). All data were transferred to Microsoft Excel (Microsoft Corp.). Season of AI was categorized into winter (December 21 to March 20), spring (March 21 to June 20), summer (June 21 to September 22), and autumn (September 23 to December 20). For each of the 9 farms, cumulative milk yield within the first 100 DIM was classified into quartiles (**Q**) stratified by parity (Table 3). All statistical analyses were performed using SPSS for Windows (version 22.0; SPSS Inc.). The analysis for AAM and TAI cows was conducted in separate models because cows were not randomized to these treatments. For the analysis of PA in TAI cows, a multinomial logistic regression model was built. For the analysis of PA in AAM cows and P/AI in AAM and TAI cows, different logistic regression models using the GENLINMIXED procedure of SPSS were built. A Bonferroni adjustment was used to account for multiple comparisons. Variables were declared to be significant when $P < 0.05$. A statistical tendency was declared when $P \geq 0.05$ and $P \leq 0.10$.

Experiment 1

Cow was the experimental unit. Lactation number was considered as a repeated measure because some cows had 2 first AI events over consecutive lactations within the observation time. Model building was conducted as recommended by Dohoo et al. (2009), where each parameter was first analyzed separately in a univariable model. Only parameters resulting in univariable models with $P \leq 0.10$ were included in the final mixed model. Selection of the model that best fit the data was performed by using a backward stepwise elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model for PA included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), season of AI (winter, spring, summer, and autumn), and cumulative milk yield within 100 DIM (quartile (**Q**)1 to Q4). The initial model for P/AI included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), season of AI (winter, spring, summer, and autumn), type of semen (conventional frozen vs. sexed semen), PA [low vs. high (and vs. no alert in TAI cows)], and cumulative milk yield within 100 DIM (Q1 to Q4). Within the model for P/AI, we tested all biologically plausible 2-way interactions. Within AAM cows, there were no interactions between PA and parity ($P = 0.38$), PA and season ($P = 0.22$), PA and type of semen ($P = 0.55$), or PA and cumulative 100-d milk yield ($P = 0.13$) on P/AI. Within TAI cows, there were no interactions between PA and parity ($P = 0.83$), PA and season ($P = 0.32$), or PA and cumulative 100-d milk yield ($P = 0.39$) on P/AI. Therefore, these interactions were not included in the final statistical model. Regardless of the significance level, PA was forced to remain in the final model for P/AI. For AAM cows, the final model for the association between PA and factors that may affect PA therefore contained the following fixed effects: parity, season of AI, and cumulative 100-d milk yield. The final model for risk factors that may be associated with P/AI in AAM cows contained the following fixed effects: parity, type of semen, PA, and cumulative 100-d milk yield. For TAI cows, the final model for the association between PA and factors that may affect PA contained the following fixed effects: parity and cumulative 100-d milk yield. The final model for risk factors that may be associated with P/AI in TAI cows contained the following fixed effects: parity, type of semen, PA, and the interaction between PA and type of semen.

Experiment 2

Farm was considered a random effect. Cow was the experimental unit and nested within farm. Lactation number was considered as a repeated measure because some cows had 2 first AI events over consecutive lactations within the observation time. Model building was conducted as recommended by Dohoo et al. (2009), where each parameter was first analyzed separately in a univariable model. Only parameters resulting in univariable models with $P \leq 0.10$ were included in the final mixed model. Selection of the model that best fit the data was performed by using a backward stepwise elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model for PA included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), season of AI (winter, spring, summer, and autumn), and cumulative milk yield within 100 DIM (Q1 to Q4). The initial model for P/AI included the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), season of AI (winter, spring, summer, and autumn), type of semen (fresh vs. conventional frozen semen), PA [low vs. high (and vs. no alert in TAI cows)], and cumulative milk yield within 100 DIM (Q1 to Q4). Within the model for P/AI, we tested all biologically plausible 2-way interactions. Within AAM cows, there were no interactions between PA and parity ($P = 0.70$), PA and season ($P = 0.21$), PA and type of semen ($P = 0.78$), or PA and cumulative 100-d milk yield ($P = 0.16$) on P/AI. Within TAI cows, there were no interactions between PA and parity ($P = 0.49$), PA and season ($P = 0.70$), PA and type of semen ($P = 0.30$), or PA and cumulative 100-d milk yield

Table 3. Quartiles (Q1–4) of cumulative milk yield within the first 100 DIM (kg) from enrolled farms stratified by farm and parity

Quartile	Farm[1]								
	1	2	3	4	5	6	7	8	9
Primiparous cows									
Q1	≤2,930	≤3,208	≤3,051	≤2,712	≤3,380	≤3,203	≤2,533	≤3,407	≤3,122
Q2	2,931–3,260	3,209–3,531	3,052–3,358	2,713–2,947	3,381–3,732	3,204–3,546	2,534–2,824	3,408–3,691	3,123–3,536
Q3	3,261–3,540	3,532–3,828	3,359–3,632	2,948–3,224	3,733–3,964	3,547–3,789	2,825–3,162	3,692–3,971	3,537–3,809
Q4	>3,540	>3,828	>3,632	>3,224	>3,964	>3,789	>3,162	>3,971	>3,809
Multiparous cows									
Q1	≤3,970	≤3,997	≤3,894	≤3,423	≤4,211	≤4,262	≤3,466	≤3,632	≤3,980
Q2	3,971–4,360	3,998–4,418	3,895–4,361	3,424–3,877	4,212–4,699	4,263–4,704	3,467–3,864	3,633–4,059	3,981–4,460
Q3	4,361–4,720	4,419–4,826	4,362–4,759	3,878–4,264	4,700–5,098	4,705–5,048	3,865–4,235	4,060–4,389	4,461–4,846
Q4	>4,720	>4,826	>4,759	>4,264	>5,098	>5,048	>4,235	>4,389	>4,846

[1] Farm 1 was part of experiment 1, farms 2 to 9 participated in experiment 2.

($P = 0.13$) on P/AI. Therefore, these interactions were not included in the final statistical model. Regardless of the significance level, PA was forced to remain in the final model for P/AI. For AAM cows, the final model for the association between PA and factors that may affect PA therefore contained the following fixed effect: season of AI. The final model for risk factors that may be associated with P/AI in AAM cows contained the following fixed effects: parity, season of AI, type of semen, and PA. For TAI cows, the final model for the association between PA and factors that may affect PA contained the following fixed effect: season of AI. The final model for risk factors that may be associated with P/AI in TAI cows contained the following fixed effects: parity, season of AI, type of semen, and PA.

RESULTS

Experiment 1

After exclusion of 118 AI services from the total of 3,858 AI services (3.1%) due to AI based on visual observation of estrus (i.e., AAM cows with no activity alert; n = 37) or culling before a pregnancy diagnosis (n = 35) or before 100 DIM (n = 46), 3,740 AI services representing 1,550 primiparous and 2,190 multiparous cows were included in the final statistical analyses. Of these AI services, 2,698 were from AAM cows and 1,042 from TAI cows. Descriptive information of characteristics between AAM and TAI cows is depicted in Table 2.

AAM Cows: Factors Associated with Increased Physical Activity. The mean (± SD of the mean) DU was 17.68 ± 4.04 h, and the mean PA was 30.26 ± 15.59 index value. Peak of x-factor in increments of 10 is depicted in Figure 1. Within AAM cows, 30.1% had low and 69.9% had high PA. Intensity of increased activity was associated ($P < 0.01$) with parity. Compared with primiparous cows, multiparous cows were more likely to have an estrus event with high PA [OR = 1.4 (95% CI 1.3–1.6)]. Season of AI was associated ($P = 0.01$) with the risk of low or high PA. The risk of high PA was similar in winter and spring, but lower in summer [OR = 0.74 (95% CI = 0.6–0.9); $P < 0.01$]. There tended to be a greater risk for high PA in winter compared with autumn ($P = 0.10$) and for high PA in autumn compared with summer ($P = 0.07$). There was an association ($P = 0.03$) between cumulative 100-d milk yield and PA. Cows with a Q1 100-d milk yield had a lower risk for high PA compared with cows with a Q3 [OR = 1.2 (95% CI = 1.1–1.4); $P = 0.01$] or a Q4 [OR = 1.2 (95% CI = 1.1–1.5); $P = 0.01$] 100-d milk yield.

AAM Cows: Risk factors for P/AI. Overall P/AI was 52.4%. Primiparous cows (59.1 ± 1.3%) had

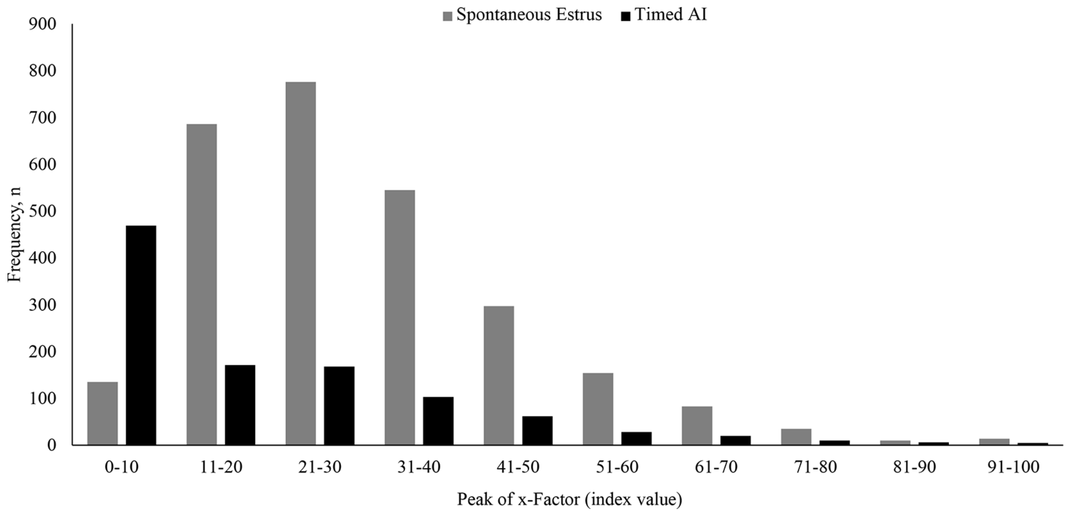

Figure 1. Frequency distribution of AI services in experiment 1 according to estrous intensity and insemination type (i.e., spontaneous estrus vs. timed AI) using a neck-mounted accelerometer system for detection of estrus (Smarttag Neck; Nedap Livestock Management). Estrous intensity was represented by the peak index value of the x-factor within the observational period (i.e., 1 d before until 1 d after AI).

greater P/AI compared with multiparous cows (45.7 ± 1.3%; $P < 0.01$). There was no association between season and P/AI ($P = 0.19$), as cows had similar P/AI in winter (53.1 ± 1.6%), spring (52.9 ± 1.7%), summer (48.0 ± 2.3%), and autumn (54.5 ± 2.4%). A total of 1,471 cows were inseminated with sexed semen, whereas 1,227 cows received AI with conventional frozen semen. Cows inseminated with sexed semen (47.2 ± 1.3%) had decreased P/AI ($P < 0.01$) compared with cows inseminated with conventional frozen semen (57.6 ± 1.3%). Intensity of increased activity was associated ($P = 0.01$) with P/AI. Cows with high PA (55.1 ± 1.1%) had greater P/AI than cows with low PA (49.8 ± 1.6%). Cumulative milk yield within 100 DIM tended ($P = 0.09$) to be associated with P/AI. Cows with a Q1 100-d milk yield (56.4 ± 1.8%) had greater P/AI compared with cows with a Q4 (50.0 ± 1.9%; $P = 0.02$) 100-d milk yield and tended to have greater P/AI compared with Q2 (51.2 ± 1.8%; $P = 0.05$) or Q3 cows (52.1 ± 1.8%; $P = 0.10$).

TAI Cows: Factors Associated with Increased Physical Activity. Overall, 41.2% (429/1,042) of TAI cows were inseminated without an AAM alert. For AI services associated with an AAM alert, the mean DU was 14.78 ± 4.50 h. Mean PA was 19.00 ± 18.38 index value. Peak of x-factor in increments of 10 is depicted in Figure 1. Out of the group of cows with AAM alerts, 21.5 and 37.3% cows had low or high PA, respectively. Intensity of increased activity was associated ($P < 0.01$) with parity. Compared with multiparous cows, primiparous cows had a lower risk for no alert [OR = 0.5 (95% CI = 0.4–0.7)] compared with high PA, whereas both primiparous and multiparous cows had similar odds for low and high PA [OR = 0.9 (95% CI = 0.6–1.2)]. There was no association between season and PA ($P = 0.43$). Intensity of increased activity was associated ($P < 0.01$) with cumulative 100-d milk yield. Cows with a Q4 100-d milk yield were more likely to have high PA and less likely [OR = 0.5 (95% CI = 0.4–0.8)] to have no alert at timed AI compared with cows with a Q1 or a Q2 100-d milk yield.

TAI Cows: Risk factors for P/AI. Overall P/AI was 51.6%. Primiparous cows (57.0 ± 2.3%) had greater P/AI compared with multiparous cows (46.2 ± 1.6%; $P = 0.01$). Season of AI was not associated ($P = 0.19$) with P/AI, as cows had similar P/AI in winter (52.1 ± 2.7%), spring (49.5 ± 2.8%), summer (43.0 ± 3.3%), and autumn (46.8 ± 4.0%). A total of 458 cows were inseminated with sexed semen, whereas 584 cows received AI with conventional frozen semen. Cows inseminated with sexed semen (48.9 ± 2.0%) tended to have decreased P/AI ($P = 0.09$) compared with cows inseminated with conventional frozen semen (54.4 ± 1.9%). Intensity of increased activity was associated ($P < 0.01$) with P/AI. Cows with no AAM alert (38.8 ± 2.1%) had reduced P/AI compared with cows with low PA (54.2 ± 2.7%; $P < 0.01$) or high PA (61.8 ± 2.0%; $P < 0.01$). Compared with cows with low PA, P/AI tended ($P = 0.07$) to be increased for cows with high PA. There tended to be an interaction between PA and type of semen ($P = 0.07$), with greater P/AI for high PA cows when AI services were performed with conventional frozen semen (69.3 ± 2.6%; $P = 0.01$) compared with sexed semen (53.8 ± 2.9%). Cumulative milk yield within 100 DIM was not associated ($P = 0.17$) with P/AI (Q1: 53.0 ± 2.8% vs. Q2: 56.1 ± 2.7% vs. Q3: 48.2 ± 2.8% vs. Q4: 49.4 ± 2.7%).

Experiment 2

Overall, activity information from 9,525 AI services were included in this experiment. After exclusion of 1,782 AI services (18.7%) due to hormone treatments unrelated to a 7-d Ovsynch protocol (e.g., single injection of PGF to induce estrus; n = 651), AI based on visual observation of estrus (i.e., AAM cows with no activity alert; n = 336), use of sexed semen (n = 249), and culling before a pregnancy diagnosis (n = 148) or before 100 DIM (n = 398), 7,743 AI services (primiparous cows: 2,986; multiparous cows: 4,757) were available for final statistical analyses. Of these AI services, 6,517 were from AAM cows and 1,226 from TAI cows (Table 2).

AAM Cows: Factors Associated with Increased Physical Activity. The mean DU was 14.00 ± 4.56 h. Mean PA was 91.14 ± 14.3 index value (Figure 2). Within AAM cows, 29.3% had low and 70.7% had high PA (Table 2). Intensity of increased activity was not associated ($P = 0.49$) with parity. Season of AI was associated ($P < 0.01$) with the risk of low or high PA. Odds of high PA were similar in autumn and winter, but high PA was less likely in spring [OR = 0.8 (95% CI = 0.7–1.0)] or summer [OR = 0.6 (95% CI = 0.5–0.6)]. There was no association ($P = 0.38$) between cumulative 100-d milk yield and PA.

AAM Cows: Risk factors for P/AI. Overall P/AI was 41.0%. Primiparous cows (45.6 ± 3.7%) had greater P/AI than multiparous cows (36.5 ± 3.4%; $P < 0.01$). Pregnancy per AI was greater in winter (44.5 ± 3.8%; $P < 0.01$), spring (39.7 ± 3.7%; $P = 0.04$), and autumn (44.2 ± 3.8%; $P < 0.01$) than in summer (35.8 ± 3.6%). Cows inseminated in spring had decreased P/AI compared with cows inseminated in winter ($P = 0.01$) and autumn ($P = 0.02$). A total of 6,031 AI services were performed with conventional frozen semen, whereas 486 cows received AI with fresh semen. Cows inseminated with fresh semen (44.4 ± 4.2%) had greater P/AI ($P = 0.01$) compared with cows inseminated with conventional frozen semen (37.6 ± 3.3%). Estrous intensity was associated ($P < 0.01$) with P/AI, as cows with high PA (45.8 ± 3.7%) had greater P/AI compared with cows with low PA (36.4 ± 3.5%). Cumulative milk yield within 100 DIM was not associated ($P = 0.45$) with P/AI. Cows within Q1 100-d milk yield (38.8 ± 3.2%) had similar P/AI compared with cows with Q2 (38.1 ± 3.2%), Q3 (40.3 ± 3.3%), and Q4 (40.6 ± 3.3%) 100-d milk yield.

TAI Cows: Factors Associated with Increased Physical Activity. Overall, 65.1% of TAI cows were inseminated without an AAM alert. The mean DU was 12.93 ± 4.59 h, whereas the mean PA was 38.2 ± 39.1 index value (Figure 2). Out of the group of cows with AAM alerts, 11.3 and 23.6% cows had low or high PA, respectively (Table 2). Intensity of increased activity was not associated ($P = 0.47$) with parity. Season of AI was associated ($P = 0.03$) with the increase in activity, as compared with high PA odds of an estrus event with low PA were more likely in summer [OR = 1.8 (95% CI 1.0–3.3)] and spring [OR = 1.9 (95% CI = 1.1–3.3)] than in autumn. Compared with high PA, odds for no alert were greater in summer [OR = 1.6 (95% CI = 1.1–2.4)] than in autumn. In spring, low PA was more likely than having no alert at timed AI [OR = 1.7 (95% CI = 1.0–2.9)]. There was no association ($P = 0.52$) between cumulative 100-d milk yield and PA.

TAI Cows: Risk factors for P/AI. Overall P/AI was 39.3%. Primiparous cows (45.7 ± 5.6%) had greater P/AI than multiparous cows (33.3 ± 4.8%; $P < 0.01$). Season of AI tended ($P = 0.05$) to be associated with P/AI, with greater P/AI in autumn (44.9 ± 6.1%; $P = 0.01$), and a tendency for greater P/AI in spring (40.5 ± 5.7%; $P = 0.06$), and winter (39.9 ± 5.6%; $P = 0.09$) than in summer (32.4 ± 5.3%). A total of 683 AI services were performed with conventional frozen semen, whereas 543 cows received AI with fresh semen. Cows inseminated with fresh semen (44.2 ± 5.8%) had greater P/AI ($P = 0.01$) compared with cows inseminated with conventional frozen semen (34.6 ± 4.8%). Estrous intensity was associated ($P < 0.01$) with P/AI, as cows with low PA (41.1 ± 6.5%) achieved greater P/AI compared with cows with no alert (27.4 ± 4.3%; $P = 0.01$). Cows with high PA (50.8 ± 5.8%) had greater P/AI ($P < 0.01$) compared with cows with no alert and tended ($P = 0.07$) to have greater P/AI compared with low PA cows. Cumulative milk yield within 100 DIM was not associated ($P = 0.26$) with P/AI. Cows within Q1 100-d milk yield (27.0 ± 3.9%) had similar P/AI compared with cows with Q2 (34.2 ± 4.4%), Q3 (33.7 ± 4.2%), and Q4 (32.0 ± 4.1%).

DISCUSSION

The objective of this observational study was to evaluate the association between increased physical activity detected by neck-mounted AAM systems at first AI and subsequent P/AI in lactating Holstein cows following spontaneous estrus or after a 7-d Ovsynch protocol. Furthermore, we wanted to identify factors associated with activity increase and fertility. We expected a greater PA to be associated with improved

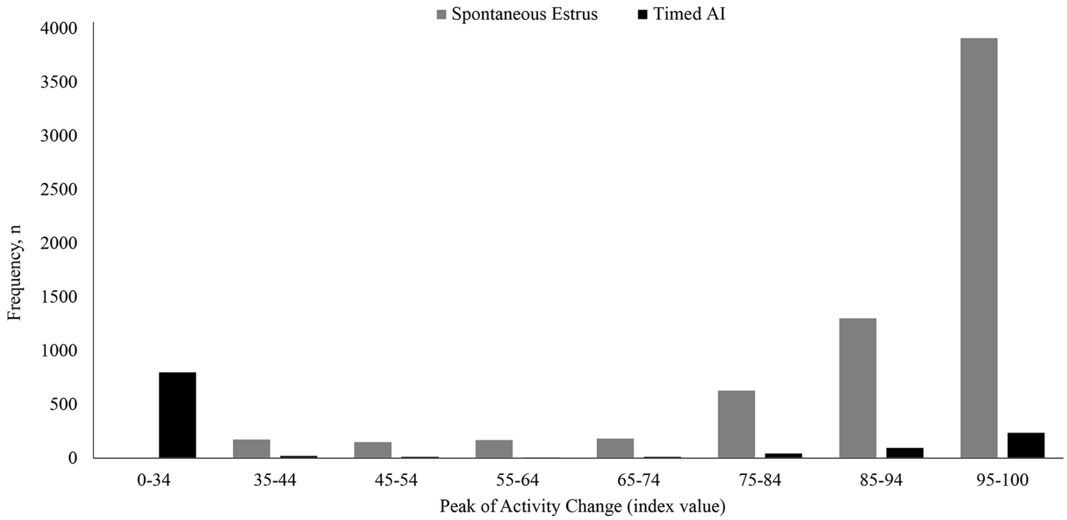

Figure 2. Frequency distribution of AI services in experiment 2 according to estrous intensity and insemination type (i.e., spontaneous estrus vs. timed AI) using a neck-mounted accelerometer system for detection of estrus (Heatime; SCR Engineers Ltd.). Estrous intensity was represented by the peak index value of the activity change index within the observational period (i.e., 1 d before until 1 d after AI).

reproductive performance irrespective of insemination type (i.e., AAM vs. TAI).

Irrespective of insemination type, cows with high PA had greater P/AI compared with cows with low PA. In addition, TAI cows with low or high PA had greater P/AI compared with TAI cows with no AAM alert. The positive association between reproductive performance and high PA in dairy cows has already been demonstrated earlier (Polsky et al., 2017; Burnett et al., 2018; Madureira et al., 2019). In a recent study (Tippenhauer et al., 2021a) we noted 1.35 greater odds of pregnancy during lactation for cows with high PA compared with cows with low PA measured by a neck-mounted AAM system. Several factors seem to influence the association between estrous expression and reproductive performance. It has been shown that cows with low PA had a greater risk for irregular timing of ovulation and ovulation failure both at spontaneous estrus and TAI (Silper et al., 2017; Burnett et al., 2018; Madureira et al., 2019). Despite the key role of estradiol (**E2**) during fertilization and early embryonic development (Buhi, 2002; Galvão et al., 2004), there exist no consistent study results on the association between preovulatory follicle diameter and E2 concentration on PA (Cerri et al., 2004; Aungier et al., 2015; Madureira et al., 2015a). The latter 2 studies demonstrated greater E2 concentrations in cows with high PA compared with cows with low PA. Progesterone (**P4**) concentrations before luteolysis could also be associated with high PA, as cows with high PA at TAI had greater P4 concentrations 4 d before TAI and greater P/AI compared with cows with low or no PA (Madureira et al., 2021). This was also illustrated by 2 studies, where supplementation of P4 in TAI protocols increased the proportion of cows inseminated in estrus at TAI, highlighting P4 as an important primer for the responsiveness of the hypothalamus to E2 (Rhodes et al., 2002; Bisinotto et al., 2015). Furthermore, a positive association between high PA and low P4 concentrations at AI and greater P4 concentrations at d 7, 14, and 21 after AI have been shown (Madureira et al., 2015b; Madureira et al., 2021). Greater P4 concentrations post AI were reported to have favorable effects on fertility of beef cows, such as receptivity of the endometrium and maternal recognition of pregnancy, embryo development and survival (Davoodi et al., 2016; Forde and Lonergan, 2017; Cooke et al., 2019).

The large proportion of TAI cows without estrous expression in our study (i.e., 41.2% in experiment 1 and 65.1% in experiment 2) may be caused by many reasons, such as a lack of proper synchronization, inadequate duration of exposure to or concentration of E2 before induction of ovulation with GnRH, or increased P4 concentration at the time of AI. Already marginally elevated P4 concentrations at the time of AI have been demonstrated to block the effects of E2 in the region of the hypothalamus responsible for estrous behavior (Allrich, 1994; Woelders et al., 2014; Pereira et al., 2016). At the same time, incomplete luteolysis has been observed in 12 to 21% of cows treated with a single PGF dose in a 7-d Ovsynch protocol (Brusveen et al., 2009; Carvalho et al., 2015; Wiltbank et al., 2015). While it is not valid to directly compare the 2 data sets due to the use of different sensor technology and no controlled study design, it is noteworthy that the percentage of TAI cows with estrous expression (58.8%) in experiment 1 (Ovsynch with an additional PGF treatment on d 8) was greater than in experiment 2 (34.9%; standard Ovsynch with 1 PGF treatment). This observation could be explained by a greater risk for luteolysis when cows were treated with a second PGF dose on d 8 (Wiltbank et al., 2015; Borchardt et al., 2018a; Tippenhauer et al., 2021c). As ultrasound examinations of cow's ovaries or collection of blood samples were not feasible due to our study design, this possible explanation, however, remains speculative. Furthermore, Perry et al. (2014) observed greater circulating E2 concentrations in TAI cows expressing estrus compared with TAI cows without estrous expression. Another possibility might be that TAI cows without estrous expression could have achieved a GnRH-induced surge of the luteinizing hormone sufficient to trigger ovulation but not behavioral estrus as circulating E2 concentrations were still too low to induce estrous behavior of sufficient magnitude to generate an AAM alert (Reames et al., 2011; Rodrigues et al., 2013; Perry et al., 2014).

About 2 thirds of AAM cows (experiment 1: 69.9%; experiment 2: 70.7%) had high PA, whereas this percentage was considerably lower in TAI cows (experiment 1: 37.3%; experiment 2: 23.6%). Percentage of AAM cows with high PA from this study is similar to previous studies using neck- or leg-mounted AAM (Reith et al., 2014: 76.5%; Tippenhauer et al., 2021a: 73.5%), but greater compared with other studies (Madureira et al., 2015a: 37.4%; Polsky et al., 2017: 27.3%; Madureira et al., 2019: 46.6%). Divergent results might be explained by many causes, such as different housing or environmental conditions, health status of cows or varying thresholds for low or high PA. The finding of TAI cows having a great risk for low PA in our study is noteworthy, as estrous intensity at AI was highly associated with reproductive performance in TAI cows in both experiments. During a Heatsynch protocol (d-10 GnRH, d-3 PGF, d-2 estradiol cypionate, d-0 TAI), greater P/AI was described for cows with estrous expression compared with cows without estrous expression (Cerri et al., 2004; Galvão et al., 2004). There is one study (Madureira et al., 2019) assessing the association between the intensity of estrous expression measured with a leg-mounted AAM system and subsequent ability to sustain pregnancy for TAI cows. A strong increase (i.e., $\geq$ 300% relative increase) in physical activity around TAI was associated with greater ovulation rates, greater P/AI, and reduced pregnancy loss. Contrary to their findings, behavioral patterns detected by an ear-tagged AAM did not differ between cows receiving AI based on spontaneous estrus or TAI (Schweinzer et al., 2020). In addition, the authors detected similar behavioral patterns between AAM or TAI cows that became pregnant. Overall, direct comparison of studies is difficult, as Schweinzer et al. (2020) only evaluated a limited sample size and an Ovsynch protocol including a combination of GnRH analog and PGF, whereas Madureira et al. (2019) used a combination of a GnRH analog and estradiol cypionate to induce ovulation. The additional estradiol cypionate treatment might have caused more cows to express estrus after the induction of luteolysis and considerably increased circulating E2 concentrations (Stevenson et al., 2004; Sauls et al., 2017). Overall, previous research as well as our results provide evidence that PA at the end of a TAI protocol could be used for decision-making on dairy farms. Therefore, future research should focus on the activity level at the end of a TAI protocol to identify cows with greater PA for selection of sexed semen or embryo transfer (Madureira et al., 2022). Another promising approach includes the effect of a GnRH treatment at the time of AI on PA and P/AI, as Burnett et al. (2022) reported greater P/AI for cows with low PA after receiving a single GnRH treatment. Nevertheless, improvement in P/AI could not be explained by greater ovulation rates, which is why the authors suspected another mechanism (e.g., ovulation timing, luteinizing hormone pulsatility, improved subsequent luteal function) behind this phenomenon.

In experiment 1, AAM cows had decreased P/AI (−10.4 percentage points) and TAI cows tended to have decreased P/AI (−5.5 percentage points) when inseminated with sexed semen compared with cows inseminated with conventional frozen semen in each group. This is similar to previous studies demonstrating decreased P/AI and increased pregnancy loss after the use of sexed semen compared with conventional frozen semen (Karakaya et al., 2014; Karakaya-Bilen et al., 2019; Diniz et al., 2021). Some researchers noted improved P/AI for sexed semen by postponing AI to 80 to 100 DIM and excluding cows with previous postpartum diseases such as metritis, mastitis, and metabolic disorders (DeJarnette et al., 2008; Schenk et al., 2009). As we conducted a large observational study, we were not able to collect disease history, although we only included first postpartum AI, thus AI services at similar DIM as suggested by the authors from the latter studies (Table 1). In experiment 2, cows inseminated with fresh semen had increased P/AI (AAM cows: +6.8 percentage points, TAI cows: +9.6 percentage points) compared with AI services performed with conventional frozen semen. Contrary to this, previous studies observed similar P/AI for fresh and conventional frozen semen in beef cows allocated to a TAI protocol (Bucher et al., 2009), in grazing dairy cows in Ireland (Murphy et al., 2017), and in Holstein dairy cows enrolled to a 7-d Ovsynch protocol (Borchardt et al., 2018b). Collectively, studies evaluating reproductive performance of different types of semen are difficult to compare due to divergent sperm numbers per straw, semen diluents, storage time, temperature of semen, and insemination type.

For inseminations conducted in the summer, we observed a decreased risk for high PA (cows from experiment 2, AAM cows from experiment 1) as well as a reduced risk for high P/AI (cows from experiment 2), which is in agreement with other studies (Polsky et al., 2017; Schüller et al., 2017; Tippenhauer et al., 2021a,b). Heat stress is known to reduce E2 and increase P4 concentrations in cows leading to ovulations without estrous expression (Wilson et al., 1998; De Rensis and Scaramuzzi, 2003; Schüller et al., 2017). Furthermore, heat stress was reported to impair the uterine environment and quality of the oocyte, thereby inhibiting embryonic development and increasing the risk of early embryonic death (Roth et al., 2000; De Rensis and Scaramuzzi, 2003). It has been suggested to enroll cows to TAI protocols (Cartmill et al., 2001) to overcome the detrimental effects of heat stress on reproductive outcomes. There was limited evidence in experiment 1, however, that the seasonal effect might be minimized by enrollment of cows into a TAI protocol, as TAI cows had similar P/AI throughout the year. Controversially, however, we also observed no association between season of AI and AAM cows from experiment 1, leading us to speculate that farm-individual heat abatement strategies (e.g., facility design and ventilation) are more essential to maintain high fertility during the warmer months.

Regarding parity, we obtained different results depending on the experiment and insemination type. Whereas parity was not associated with PA in all cows from experiment 2, within AAM cows from experiment 1 multiparous cows were more likely (OR = 1.4) to have low than high PA compared with primiparous cows. Within TAI cows from experiment 1, however, primiparous and multiparous cow had similar risk for reaching low and high PA, but primiparous cows were less likely to have no alert at timed AI. Using AAM systems, greater PA for primiparous cows has already been found by others (Madureira et al., 2015a; Burnett et al., 2018). In accordance with our results from experiment 2, however, some studies did not observe an association between parity and the risk for high PA (Løvendahl and Chagunda, 2010; Tippenhauer et al., 2021a). Inconsistencies among studies may be the result of different AAM systems, housing systems, and divergent thresholds for high and low PA. Nevertheless, there is universal agreement that fertility is greater for primiparous compared with multiparous cows. Level of milk production was found to be associated with lower circulating steroid concentrations (Sartori et al., 2004). In addition, Bruinjé et al. (2019) described a faster increase in milk P4 concentration post-AI in primiparous compared with multiparous cows, whereas a lower milk P4 beyond 10 d post-AI was associated with decreased P/AI.

In the present study, 100-d cumulative milk yield was not associated with PA or P/AI, except for PA of AAM and TAI cows from experiment 1 and P/AI of AAM cows from experiment 1. Previous research has been controversial, as some authors did not find an association (Polsky et al., 2017; Burnett et al., 2018), whereas others found milk yield to either positively or negatively affect PA and/or P/AI (Adewuyi et al., 2006; Valenza et al., 2012; Tippenhauer et al., 2021b). High-yielding cows have an increased feed intake and a greater metabolic clearance of E2, making it more difficult to identify these cows in estrus (Sangsritavong et al., 2002; Sartori et al., 2004). In addition, genetic and nutritional effects, such as a negative energy balance, might interact with the effect of milk yield. Cows experiencing a more pronounced negative energy balance have reduced pulsatility of luteinizing hormone, thereby decreasing the synthesis of E2 by the preovulatory follicle (Butler, 2003; Grimard et al., 2006). Cows with a higher milk yield, however, were not necessarily those mostly associated with a severe energy deficit or the greatest loss of body condition score (Lucy, 2001).

One limitation of our observational study design was that cows were not randomized based on insemination type (AAM vs. TAI). As a consequence, we were not able to directly compare PA and pregnancy outcomes between AAM and TAI cows. Furthermore, we did not include information about cow's genetic merit or health status, the latter because it is challenging to find consistent disease definitions across farms (Espadamala et al., 2016). Enrollment of a total of 9 commercial farms, however, improved external validity in contrast to a more controlled study design on a single farm. Although we could not conduct a combined analysis of risk factors as AAM systems differed between both experiments, for the most part similar results were portrayed for both types of accelerometers, suggesting that results are repeatable on other farms with different environmental conditions.

CONCLUSIONS

Results from the present study underline the importance of high estrous intensity (i.e., PA) at the time of AI to optimize reproductive performance in dairy cows. For both cows receiving AI based on spontaneous estrus and cows enrolled in an Ovsynch protocol, P/AI was strongly associated with the intensity of increased physical activity at AI. This underlines the potential of

AAM systems to serve as a predictive tool. Identification of cows with greater PA at TAI might assist in decision-making on dairy farms, such as the selection for cows receiving sexed semen. Because a great proportion of TAI cows had no or only low estrous expression at AI, future studies should aim to evaluate environmental and cow individual risk factors to increase the percentage of TAI cows with high PA, thereby optimizing reproductive performance.

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