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Original Research Article

# Tail chalk improved detection of true estrus alerts from an automated activity monitor system in a cohort study

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ABSTRACT

This study aimed to assess how two estrus detection tools, an automated activity monitor (AAM) and tail chalk (TC), were associated with true estrus events in lactating Holstein cows. Cows with days in milk ≥5 were monitored twice daily for estrus alerts, identified by the AAM and a TC score of 3 (<50 % of chalk remaining). Transrectal ultrasonography was performed on the day of alert to confirm estrus (cows with a follicle ≥10 mm and corpus luteum ≤24 mm). For AAM-identified events, peak activity and duration of estrus were measured. Mixed logistic and linear regression models were used to assess associations for dichotomous and continuous outcomes, respectively. A total of 1714 events were recorded, of which 1098 from 371 cows were considered in true estrus (AAM only: n = 629; TC only: n = 86; AAM + TC: n = 383). Cows with both AAM and TC alerts had higher odds of true estrus compared to those with only AAM alerts (OR = 4.24; 95 %CI = 3.05–5.87) or only TC alerts (OR = 17.70; 95 %CI = 11.90–26.34). Cows having AAM and TC alert had greater peak activity index (AAM only = 221.64 ± 7.07; AAM + TC = 323.89 ± 9.13) and longer duration of estrus (AAM only = 8.37 ± 0.27 h; AAM + TC = 13.17 ± 0.33 h) compared to cows with AAM alerts only. In conclusion, TC could be used as a marker of high-intensity estrus and, when combined with an AAM, improves identification of true estrus in dairy cows.

## 1. Introduction

Suboptimal reproductive performance is a major contributor to economic losses in dairy farming. Studies conducted in Europe and North America have consistently linked poor reproductive outcomes with reduced cow lifespan and significant financial losses in dairy systems [1–3]. One of the first steps toward achieving a successful pregnancy in cows is the timely and accurate detection of estrus, a behavioural indicator that precedes ovulation [4]. Effective estrus detection is crucial for reproductive management on dairy farms, as it is associated with improved insemination rates, optimized calving intervals, and increased pregnancy outcomes [5]. However, despite its critical role in reproductive success, estrus detection remains a challenging and labor-intensive task. The variable nature of estrus behaviour, combined with increasing herd sizes and labor constraints on modern dairy farms, often makes consistent and accurate detection difficult to achieve through visual observation alone.

Over the past several decades, various estrus detection tools have been developed and integrated into dairy farm operations, primarily relying on identifying behavioural changes associated with estrus [4,6]. For example, one of the most well-established indicators of estrus behaviour is standing to be mounted, which can be monitored cost-effectively by applying chalk to the cow's tail base before the expected estrus period [5,7]. The chalk is removed by friction when the cow is mounted by other cows, providing a visual indicator of estrus activity [5,7]. In addition to mounting behaviour, secondary indicators, such as increased physical activity [8], can be monitored using automated activity monitors (AAM). These wearable sensors detect locomotion changes linked to estrus, reducing reliance on direct visual observation [4]. In fact, estrus detection aids have significantly contributed to improving reproductive performance on dairy farms by offering valuable support to dairy producers, and by enabling the

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possibility of targeted reproductive management protocols [9].

Despite its advancements, one significant challenge when using estrus detection tools is the occurrence of false positive alerts. Studies have shown that the rate of false positive estrus alerts can reach up to 29 % on dairy farms [10–13]. These inaccuracies may arise from behaviours such as cows licking off tail chalk [14] or the misclassification of activity changes when cows become lame [15]. One potential solution could be the integration of multiple estrus-related behaviours (e.g., mounting behaviour and increased physical activity) into detection systems. In fact, studies have suggested that combining tools with visual observation of estrus can improve the accuracy of true estrus detection [11,16,17]. However, this approach does not alleviate the time demands associated with visual observation. Integrating cost-effective visual markers, such as TC, with an AAM could improve the accuracy of estrus detection while minimizing the need for continuous visual monitoring on dairy farms. Although previous studies have compared the use of TC and AAM for estrus detection [11,13,18], limited research has evaluated their combined use in the same animal [18], particularly during spontaneous estrus, which may better represent the underlying reproductive physiology of the modern lactating dairy cow.

The primary objective of this cohort study was to assess how two estrus detection tools (AAM and TC), used separately and in combination, were associated with true estrus events in spontaneous episodes of estrus from lactating Holstein cows. Our secondary objectives were to explore additional associations with true estrus, considering different factors such as estrus characteristics (peak and duration), milk production and peri-estrus ovarian structures dynamics.

## 2. Materials and methods

This cohort study was conducted between July 2018 and November 2019 at the University of British Columbia's Dairy Education and Research Centre, in Agassiz. All experimental procedures were approved by the Animal Care Committee of the University of British Columbia following the protocol #A18-0315.

### 2.1. Animals, housing, and management

During the study period, postpartum Holstein cows (≥5 days in milk; DIM) were enrolled weekly and were followed until they became pregnant, resulting in a total enrollment of 386 cows. From these cows, a total of 1714 spontaneous estrus events were recorded, including 531 events from primiparous and 1183 from multiparous. Cows were housed in sand-bedded free-stalls with concrete flooring, headlock system, fans, manure scraper and was not equipped with automated brushes. Milking was performed twice daily (05:00 and 15:00 h) in a conventional milking parlor (Boumatic Dairy Equipment). Daily milk production was recorded by the parlor system throughout the experiment period. Cows were fed twice daily with total mixed ration (TMR) formulated to meet or exceed the requirements of a lactating 700 kg Holstein cow producing 45 kg/day of 3.5 % fat corrected milk [19]. Water and TMR were available for *ad libitum* intake.

### 2.2. Estrus detection

Lactating cows had TC (Paintstik Livestock Marker, All-Weather) applied at the base of their tail at enrollment. Chalk was applied as a 10 cm horizontal stripe (approximately 4 cm wide) along the dorsal surface of the first coccygeal vertebra, in a single, uniform stroke. Tail chalk scores were monitored twice daily (07:00 and 15:00 h) and classified according to the chalk removal. Scores were classified as follows: (1) no chalk removal: cows had 100 % of the chalk remaining on the base of their tail; (2) poor chalk removal: cows had more than 50 % of the chalk remaining on the base of their tail; and (3) significant chalk removal: cows had less than 50 % of the chalk remaining on the base of their tail. Tail chalk was reapplied at the base of the tail 36 h after an estrus event. A fresh application of chalk was reapplied every week to cows with no chalk removal (score 1), ensuring the chalk remained effective for detection. For cows showing poor chalk removal (score 2), chalk was reapplied on the base of the tail 36 h after score detection if chalk removal remained unchanged. Cows showing significant chalk removal (score 3) were considered to have an estrus alert.

Cows were monitored continuously by an AAM (Afi Pedometer Plus Tag, AfiMilk) placed on the back leg above the distal expansion of the metatarsal bone two weeks prior to the expected calving date and remained on the animals throughout their entire time within the herd. Activity data was recorded hourly by the system throughout the experiment period. The AAM system was checked twice daily (07:00 and 15:00 h) for estrus alerts. The threshold for an alert of estrus, set by the system, was based on the comparison between the baseline and the current physical activity for each cow within each 1 h block.

### 2.3. Estrus, pregnancy, and health monitoring

When either an AAM or TC alert was identified, the ovaries of the cows were scanned using a portable ultrasound (US) scanner with 7.5-MHz linear-array transducer (Ibex Pro; E.I. Medical Imaging). During the US exams, follicles and corpus lutea (CL) were identified and their diameters were measured. Cows with a pre-ovulatory follicle ≥10 mm and a CL ≤ 24 mm were considered to have a true estrus alert. For true estrus alert, ovulation of the pre-ovulatory follicle was monitored 24 h and 48 h post estrus by assessing its disappearance by US. This was also confirmed by US at 7 days post-alert by the appearance of a new CL. If a new alert occurred ≤7 days after a confirmed true estrus, and the cow appeared to have ovulated from the previous alert (evidenced by follicle disappearance and a growing new CL), but the new CL had not yet reached 24 mm, the new alert was not considered a true estrus event. Body condition, scored on a 5-point scale from thin (1) to obese (5; [20], and lameness, scored on a 5-point scale from sound (1) to severely lame (5; [21]), were also assessed at the beginning of each estrus.

Artificial insemination (AI) was performed for a total of 647 estrus events from 312 lactating Holstein cows that were considered to have a true estrus alert. Insemination followed the AM/PM rule according to the farm reproductive management and was performed either after 60 DIM when the average milk production was below 50 kg/day, or after 80 DIM when the average milk production was above 50 kg/day. Pregnancy diagnosis was conducted 35 ± 7 days post-AI by US and cows were considered pregnant when an embryonic vesicle with a viable embryo (heartbeat) was detected. All cows with an AAM and/or TC alert were eligible for re-enrollment.

### 2.4. Statistical analysis

A sample size of 245 estrus events per type of alert (AAM, TC, or both) was calculated to identify a minimum difference of 16 % in the detection of true estrus events between tools, with a SD of 40.2, 1.14 adjustment factor for confounders, 95 % confidence and 80 % power. The sample size was further adjusted for clustering (n = 5 estrus events per cow). Estimates were based on the findings of Holman et al. [11] which evaluated similar tools measuring mounting behaviour and step counts for estrus detection.

Statistical analyses were conducted using SAS Studio (version 3.8, SAS Institute Inc.). Descriptive analyses were performed, and continuous variables were visually examined for normality and potential outliers (PROC UNIVARIATE). Estrus events were dichotomized as true estrus (1) and false alerts (0). Estrus detection tools were used to categorize alerts as: AAM and TC alerts (AAM + TC), AAM alerts only (AAM only), or TC alerts only (TC only). Tail chalk scores were used to dichotomize estrus events as TC alert (when the outcome was score 3) and no TC alert (when outcome was score 1 or 2). Daily milk production from the 3 days before an estrus event was used to calculate the daily average milk production for each estrus event. Days in milk was used to categorize cows into early lactation (<60 DIM), mid-lactation (60–120 DIM), and late lactation (>120 DIM). Parity was used to classify animals into primiparous (parity = 1) or multiparous (parity >1).

The peak in physical activity was defined as the maximum heat index (heat indicator; AfiActII, Afimilk) during an estrus episode. The duration of estrus was defined as the sum of hours from the beginning to the end of an estrus event identified by the AAM system. Cows with 2 consecutive episodes of AAM estrus within a 4-h interval had their activity data combined into 1 event of estrus. When 2 consecutive estrus events occurred within a 5- to 23-h interval, the event with the shortest duration was removed from the analysis.

For all models, the estrus event was considered the experimental unit, and a clustering structure was used (cow as random intercept). Intra-class correlation coefficients (ICCs) were calculated to quantify the proportion of total variance attributable to the clustering structure (Table 1). For linear models, ICCs were estimated by dividing the variance of the random effect by the total variance. For logistic models, the latent variable method was used, assuming a residual variance of $\pi^2/3$ [22]. Parity, DIM, body condition and lameness scores were tested as possible confounders and kept in the models if $P \leq 0.20$ [22].

The binary outcomes (true estrus, TC alert, ovulation and pregnancy) were analyzed using mixed logistic regression models and the continuous outcomes (peak of activity, duration of estrus and pre-ovulatory follicle size) using mixed linear regression models (PROC GLIMMIX and PROC MIXED, respectively; Table 1). Briefly, models using true estrus as the outcome variable included all data, but only true estrus events were included for all other models (Table 1). Estrus events with only TC alert did not have data on AAM characteristics (peak and duration) and were not included in models where these characteristics were considered as an exposure or an outcome. Finally, models for pregnancy included only true estrus events followed by AI (Table 1).

For logistic regression models, the linear relationship between the numeric variables and the log odds of the outcome were assessed graphically (PROC SGPLOT). For linear regression models, homoscedasticity and normality of the residuals were assessed graphically using residual plots. Effect plots for the logistic regression models assessing the association between estrus behaviour (peak and duration) and the probability of tail chalk alerts were generated using the PROC PLM procedure in SAS. In contrast, the logistic regression model evaluating the probability of true estrus events and the linear regression models examining the relationship between estrus behaviour (peak and duration) and alert type were plotted using ggplot2 in RStudio (version 2023.12.0). Results from logistic regression models are presented as odds ratios (OR) with 95 % confidence interval (CI). Results were considered statistically significant when $P \leq 0.05$ and those between $0.05 > P \leq 0.10$ were considered as a tendency.

## 3. Results

A total of 1714 spontaneous episodes of estrus from 386 lactating Holstein cows were recorded over the 16-month study period. Of these, 531 events were from primiparous and 1183 from multiparous cows. Distribution of estrus events by detection method was as follows: AAM only: n = 996; TC only: n = 279; AAM + TC: n = 439. A total of 1098 estrus episodes (64 %) from 371 cows were considered true estrus events. Among these, 351 events originated from primiparous cows and 747 from multiparous cows. The proportion of true estrus events by detection method was as follows: AAM only with 63 % (629 of 996); TC only with 32 % (86 of 279) and AAM + TC with 87 % (383 of 439). On average, cows had 3.1 true estrus events (SD = 1.9; median = 3; range = 1 to 10) and 2.4 false alerts (SD = 1.6; median = 2; range = 1 to 8) during the study period. Among estrus events classified as false alerts, nine (from eight cows) occurred ≤7 d after a previous confirmed estrus, with evidence of prior ovulation and a developing CL that had not yet reached 24 mm. Milk production data was missing for 21 true events and AAM data was missing for 103 true events due to unrecorded data or sensor malfunction. These events were excluded from statistical models (Table 1). Across models, ICCs for the random intercepts averaged 0.15,

**Table 1**
Model information for the different outcomes and exposures assessed in mixed regression models using estrus event as the unit of interest and a clustering structure (cow as random intercept). Cow parity, days in milk (DIM), body condition score (BCS) and lameness scores were tested as possible confounders and kept in the models if $P \leq 0.20$ [22].

| Outcome ~ | Exposure | Model type | Included data | n events | Confounders | ICC (cow) |
|---|---|---|---|---|---|---|
| True estrus ~ | Type of alert | Logistic regression | All | 1714 | Parity, DIM | 0.06 |
| ~ | Milk production | | | 1693 | Parity, DIM | 0.04 |
| Tail chalk alert ~ | Peak | Logistic regression | True estrus | 909 | Parity, BCS | 0.03 |
| ~ | Duration | | | 909 | Parity, BCS | 0.02 |
| ~ | Milk production | | | 1077 | Parity, BCS | 0.03 |
| Peak of activity ~ | Milk production | Linear regression | True estrus | 909 | Parity, DIM | 0.11 |
| ~ | Type of alert | | | 909 | Parity | 0.15 |
| ~ | Pre-ovulatory follicle size | | | 909 | Parity, milk | 0.12 |
| Duration ~ | Milk production | Linear regression | True estrus | 909 | Parity, DIM, BCS | 0.14 |
| ~ | Type of alert | | | 909 | Parity | 0.16 |
| ~ | Pre-ovulatory follicle size | | | 909 | Parity, milk | 0.14 |
| Pre-ovulatory follicle size ~ | Type of alert | Linear regression | True estrus | 1077 | Parity, BCS | 0.34 |
| ~ | Milk production | | | 1077 | Parity | 0.37 |
| Ovulation ~ | Type of alert | Logistic regression | True estrus | 1077 | Parity, milk, BCS | 0.37 |
| ~ | Milk production | | | 1077 | Parity | 0.34 |
| ~ | Peak | | | 909 | Parity, milk | 0.36 |
| ~ | Duration | | | 909 | Parity, milk | 0.35 |
| Pregnancy ~ | Type of alert | Logistic regression | True estrus followed by AI | 647 | DIM, parity | 0.02 |
| ~ | Milk production | | | 647 | DIM, parity | 0.03 |
| ~ | Peak | | | 502 | DIM, parity | 0.02 |
| ~ | Duration | | | 502 | DIM, parity | 0.04 |

True estrus: estrus alerts when cows had a pre-ovulatory follicle ≥10 mm and a CL ≤ 24 mm, or were not in alert ≤7 days after a confirmed true estrus when ovulation from the prior alert was evident. Type of alert: AAM and TC alerts (AAM + TC), AAM alerts only (AAM only), or TC alerts only (TC only). Tail chalk alert: TC alert (when the outcome was score 3) and no TC alert (when outcome was score 1 or 2). Milk production: daily average milk production from 3 d before estrus for each estrus event. DIM: Cows were categorize into early lactation (<60 DIM), mid-lactation (60–120 DIM), and late lactation (>120 DIM). Parity: primiparous (parity = 1) or multiparous (parity >1). ICC: intra-class correlation coefficients.

ranging from very low (0.02) to moderate (0.37). To maintain consistency and more accurately reflect the repeated-measures design, the clustering structure was retained in all models.

At the time of estrus, cows were, on average, 83.2 ± 47.9 DIM (median = 77; range = 6 to 380) and of parity 2.5 ± 1.5 (median = 2, range = 1 to 8). The average daily milk production from 3 days before estrus events was 40.8 ± 10.5 kg (median = 40.6, range = 24.0–76.5 kg). Among all recorded estrus events, 40.8 % occurred in animals with a body condition score (BCS) greater than 2.75 (range: 3.0–3.5), 31.8 % in those with a BCS of 2.75, and 27.4 % in those with a BCS below 2.75 (range: 2.25–2.5). Additionally, 78.4 % of estrus events were observed in animals with a lameness score of 1 or 2, while 21.6 % occurred in those with a score of 3 or 4. The average peak and duration of estrus for all true estrus events were 242.9 ± 157.6 activity index and 9.8 ± 5.3 h, respectively.

### 3.1. True estrus events

Cows that alerted in both estrus detection tools (AAM + TC) had 4.24 times greater odds of a true estrus event when compared with cows with an alert in the AAM only (95 % CI = 3.05–5.87; $P < 0.01$; Fig. 1). Similarly, cows that alerted in both estrus detection tools (AAM + TC) had 17.70 times greater odds of true estrus event when compared to cows with a TC alert only (95 % CI = 11.90–26.34; $P < 0.01$; Fig. 1). In addition, cows with a TC alert only had lower odds of true estrus alert when compared to cows alerting only on the AAM system (OR = 0.25; 95 % CI = 0.18–0.33; $P < 0.01$; Fig. 1). Milk production did not affect the odds of having a true estrus alert by TC and/or by the AAM system (OR = 1.00; 95 % CI = 0.99–1.01; $P = 0.64$). Similarly, the odds of having a true estrus alert were not significantly influenced by BCS or lameness score ($P > 0.05$). However, cow parity and DIM had a significant effect irrespective of the estrus detection tool used. Multiparous cows tended to have lower odds of a true estrus alert compared to primiparous cows (OR = 0.79; 95 % CI: 0.59–1.04; $P = 0.09$). The odds of a true estrus alert was 1.9 times higher (95 % CI: 1.4–2.5; $P < 0.0001$) in cows in early lactation compared with those in mid-lactation. Additionally, cows in late lactation had lower odds of receiving a true estrus alert compared with mid-lactation cows (OR = 0.6; 95 % CI: 0.45–0.80; $P < 0.0001$).

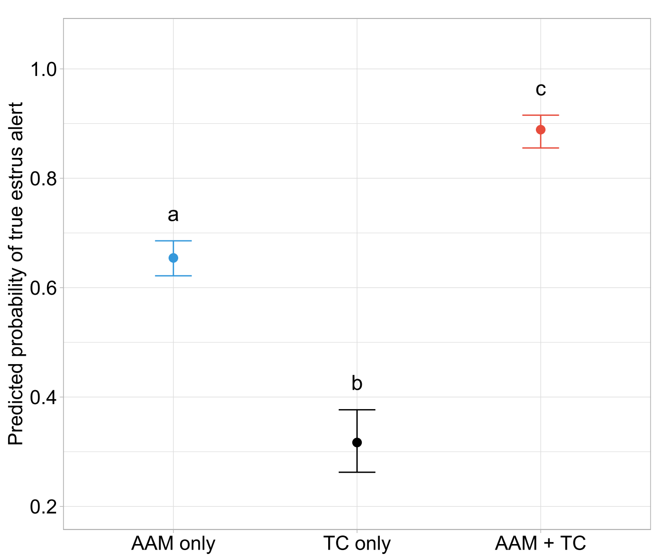


**Fig. 1.** Predicted probability (±CI) of true estrus alert in lactating Holstein cows from a logistic regression model assessing the association between estrus alert type and the likelihood of a true estrus alert. Tail chalk (TC only), automated activity monitor (AAM only), or both (AAM + TC). Parity and DIM were included in the model as significant confounders ($P \leq 0.20$). Predicted probabilities were 0.89 (95 % CI: 0.86–0.92) for AAM + TC, 0.65 (95 % CI: 0.62–0.69) for AAM only, and 0.32 (95 % CI: 0.26–0.38) for TC only. Different superscript letters indicate significant differences among alert types ($P < 0.01$).

### 3.2. Type of alert

The type of alert was associated with the characteristics of estrus measured by the AAM. Cows that alerted in both estrus detection tools (AAM + TC) had longer duration of estrus (Fig. 2A; AAM only = 8.37 ± 0.27 h; AAM + TC = 13.17 ± 0.33 h; $P < 0.01$) and greater peak activity index (Fig. 2B; AAM only = 221.64 ± 7.07; AAM + TC = 323.89 ± 9.13 index; $P < 0.001$) when compared to cows having an AAM alert only. Multiparous cows had a lower peak of activity index (multiparous = 260.22 ± 7.7; primiparous = 322.89 ± 10.3; $P < 0.01$) and shorter duration of estrus (multiparous = 11.12 ± 0.23 h; primiparous = 12.85 ± 0.31 h; $P < 0.01$) compared to primiparous cows. The peak activity and duration of true estrus events were not associated with the average milk production from 3 d before estrus ($P > 0.05$).

The odds of having a TC alert during a true estrus event identified by the AAM system increased by 1.42 for each hour increase in the duration of estrus (95 % CI = 1.04–1.95; $P < 0.01$; Fig. 3A) and by 1.36 for each 10-point index increase in peak of activity (95 % CI = 1.04–1.9; $P < 0.01$; Fig. 3B). Neither DIM nor lameness score were associated with the odds of having a TC alert ($P > 0.05$). In contrast, parity and BCS were considered as potential confounders ($P \leq 0.20$). Multiparous cows had numerically higher odds of having a TC alert compared to primiparous cows (OR = 1.19; 95 % CI = 0.86–1.65; $P = 0.18$). Cows with a BCS <2.75 (range: 2.25–2.5) had numerically lower odds of a TC alert compared to those with a BCS ≥2.75 (range = 2.75–3.5; OR = 0.86, 95 % CI = 0.57–1.03; $P = 0.20$).

### 3.3. Preovulatory follicle and ovulation

Cows that alerted in both estrus detection tools (AAM + TC) had greater diameter of the dominant follicle (DF) at estrus compared with cows alerting in the AAM or TC only (AAM only = 20.1 ± 0.3; TC only = 21.2 ± 0.8; AAM + TC = 22.05 ± 0.3 mm; $P = 0.008$). The diameter of the DF was associated with both the peak of activity and duration of estrus, as measured by the AAM. For each 1 mm increase in DF diameter there was a 2.83-unit increase in the peak activity ($P = 0.005$). Similarly, for each 1 mm increase in DF diameter there was a 0.1-h increase in the duration of estrus ($P < 0.001$). The diameter of the DF increased 1.0 mm (95 % CI = 0.4–1.2; $P = 0.002$) per 10-L increase in daily milk production. Lameness score and DIM were not associated with DF diameter ($P > 0.20$). Multiparous cows had greater DF diameter than primiparous cows (multiparous: 21.2 ± 0.3 mm; primiparous: 19.4 ± 0.4 mm; $P = 0.003$). Body condition was retained in the model as a potential confounder ($P \leq 0.20$), as cows with a BCS between 2.25 and 2.5 had a numerically lower mean DF diameter (20.5 ± 0.5 mm), followed by those with a BCS of 2.75 (21.4 ± 0.3 mm), and cows with a BCS between 3.0 and 3.5 (21.8 ± 0.4 mm; $P = 0.12$).

Cows alerting in both tools (AAM + TC) had greater odds of ovulation compared to cows alerting in the AAM only (OR = 2.84; 95 % CI = 1.55–5.32; $P = 0.002$) or TC only (OR = 3.87; 95 % CI = 1.47–10.17; $P = 0.002$). The odds of ovulation increased by 1.14 for each 1-h increase in the duration of estrus (95 % CI = 1.09–1.20; $P < 0.001$) and by 1.06 for each 10-point index increase at the peak of activity (95 % CI = 1.03–1.08; $P < 0.01$). Furthermore, the odds of ovulation decreased as the daily milk production increased ($OR_{per\ 10\text{-}L}$ = 0.7; 95 % CI = 0.49–0.90; $P = 0.01$). Lameness score and DIM were not associated with the odds of ovulation ($P > 0.20$). However, multiparous cows tended to have lower odds of ovulation compared to primiparous (OR = 0.69; 95 % CI = 0.53–1.13; $P = 0.09$).

### 3.4. Pregnancy per AI

Artificial insemination was performed for a total of 647 estrus events from 312 lactating Holstein cows (AAM only: n = 338, TC only: n = 45,

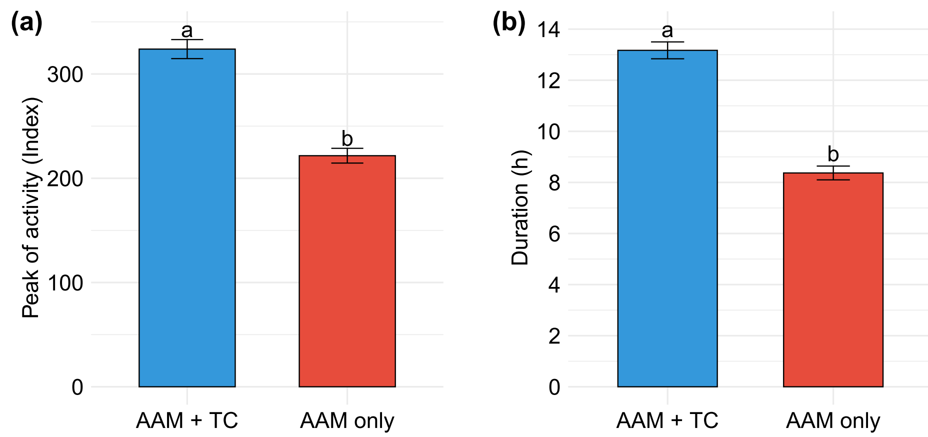


**Fig. 2.** Marginal means (±SE) from mixed linear regression models summarizing the association between the (A) duration of estrus and (B) peak of activity with the type of alert (AAM only or AAM + TC). Parity was included in the model as a significant confounder ($P \leq 0.20$). Marginal means (±SE) were 8.37 ± 0.27 h (AAM only) and 13.17 ± 0.33 h (AAM + TC) for duration, and 221.64 ± 7.07 and 323.89 ± 9.13 for peak activity index, respectively. Different superscript letters indicate significant differences among alert types ($P < 0.01$).

and AAM + TC: n = 264) and pregnancy was confirmed in 266 of them (AAM only: 37 %, n = 126; TC only: 44 %, n = 20; and AAM + TC: 45 %, n = 120). The odds of pregnancy was lower for cows that did not have a TC alert compared to cows having a TC alert (OR = 0.69; 95 % CI = 0.50–0.96; $P = 0.04$), but there was no association of AAM alerts with pregnancy outcomes (OR = 1.17; 95 % CI = 0.63–2.18; $P = 0.61$). The odds of pregnancy were not affected by the peak and duration of estrus (peak: OR = 1.00, 95 % CI = 0.99–1.00, $P = 0.50$; duration: OR = 1.03, 95 % CI = 0.97–1.05, $P = 0.12$) or by milk production ($OR_{per\ 10\text{-}L} = 1.00$; 95 % CI = 0.98–1.02; $P = 0.88$). Similarly, BCS and lameness score were not associated with the odds of pregnancy ($P > 0.20$). However, multiparous cows had lower odds of pregnancy compared to primiparous (OR = 0.66; 95 % CI = 0.43–0.99, $P = 0.05$). Days in milk was considered as a potential confounder ($P \leq 0.20$) as cows in late lactation had numerically lower odds of pregnancy compared to those in mid lactation (OR = 0.55; 95 % CI = 0.32–0.94; $P = 0.11$).

## 4. Discussion

Our findings suggest that using multiple tools to identify cows in estrus can improve the detection of true estrus events in lactating dairy cows. Specifically, cows identified in estrus by both an AAM and TC were more likely to have true estrus events than cows with a TC or AAM alert only. Furthermore, cows with only an AAM alert were more likely to have a true estrus than cows with a TC alert only. This study also showed that cows with both AAM and TC alerts had a higher peak activity index and longer estrus duration compared with cows with only an AAM alert. Moreover, cows that received alerts from both tools (AAM + TC) had greater DF diameter and higher odds of ovulation than cows with only a TC or AAM alert. Finally, TC alerts, whether or not accompanied by an AAM alert, were associated with greater odds of pregnancy in cows with true estrus events. Milk production was not associated with the multiple outcomes measured (true estrus, type of alerts, estrus behaviour, or pregnancy per AI), except for the diameter of the DF and the odds of ovulation.

Cows with both an AAM and a TC alert had greater odds of having a true estrus event when compared with cows that had only a TC or only an AAM alert. Enhancing the accuracy of estrus detection tools may benefit reproductive outcomes in dairy herds by increasing the likelihood of successful pregnancy [4]. In addition, improving detection of true estrus events may help mitigate the high rate of false-positive estrus alerts generated by sensor technologies [10–13]. However, while our results suggest that combining detection methods improved detection of true estrus, relying solely on both alerts may not be practical. This approach could result in missed true estrus events, as 36.6 % (629 of 1714) of the true estrus alerts in this study were detected only by the AAM system. Consequently, the overall impact of integrating these two tools on reproductive indices in the dairy industry remains uncertain. Our study demonstrates that cows having both an AAM and TC alert had greater peak of activity and longer duration of estrus when compared to cows having only an AAM alert. Given that increased estrus intensity and duration have been positively associated with fertility outcomes [23,24], perhaps TC could be used as a potential marker for high-fertility estrus events in lactating dairy cows. For example, TC alerts could be helpful if they supported targeted reproductive management strategies which use more expensive technologies such as embryo transfer or high merit semen choice. In the long term, TC data could also help refine the algorithms and thresholds of AAM systems, ultimately enhancing the accuracy of pedometers in detecting true estrus events in lactating dairy cows.

We did not find an association between the odds of pregnancy and the occurrence, peak, or the duration of estrus measured by the AAM system. However, TC alert was associated with greater odds of pregnancy than no TC alert. Positive associations between greater intensity of estrus expression, measured by AAM [24,25] or tail-head devices [23,26] and pregnancy per AI were reported in several studies. For instance, Burnett et al. [25] demonstrated that estrus events with greater peak of activity and longer duration had greater pregnancy per AI than those with lower peak activity or short duration. When using a tail-head device for detection of estrus, Pereira et al. [23] demonstrated greater pregnancy results at 30 and 60 days post estrus in cows displaying standing to be mounted behaviour when compared to cows not displaying standing estrus. Considering positive associations between estrus expression and fertility reported in previous studies, we expected increased odds of pregnancy in cows displaying estrus measured by AAM and TC. However, because the number of inseminated estrus events in our study was relatively modest (n = 647 estrus events), it is possible that we had limited statistical power to detect differences in the effect of estrus expression measured by AAM on pregnancy per AI. An alternative explanation could be that mounting behaviour, considered the gold standard for estrus detection [7], more accurately reflects both the observable reproductive behaviour and the underlying reproductive physiology, compared to activity-based metrics recorded by AAMs. Although AAMs can effectively detect a broad range of estrus-related behaviours, this wide range may reduce their specificity, as not all recorded activities are equally indicative of true estrus. Still, our results should be interpreted with caution. In a real farm scenario, if all cows receiving estrus alerts had been inseminated (i.e., not confirmed in estrus via ultrasonography), the association between TC alerts and pregnancy outcomes could have been different as there was a high rate

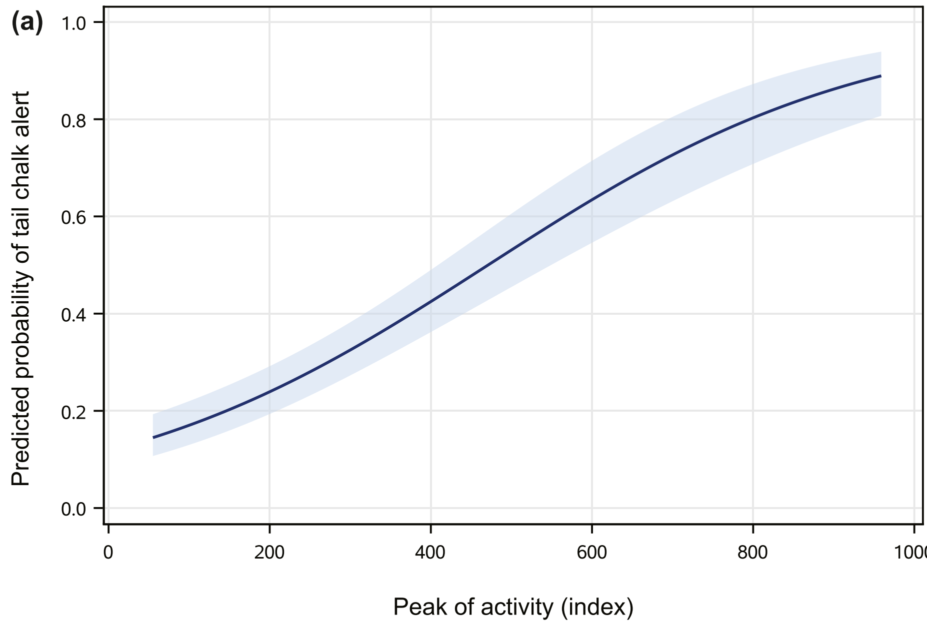


**Fig. 3.** Predicted probabilities (±CI) from logistic regression models displaying the association between (A) duration of estrus and (B) peak of activity with the odds of having a tail chalk alert in cows detected in spontaneous estrus using an automated activity monitor. Parity and BCS were included in the model as significant confounders ($P \leq 0.20$). Odds of a tail chalk alert increased by 1.42 for each hour increase in the duration of estrus (95 % CI: 1.04–1.95; $P < 0.01$) and by 1.36 for each 10-point index increase in peak of activity (95 % CI: 1.04–1.95; $P < 0.01$).

of false-positive alerts from the TC only group.

True estrus events that had an AAM and TC alert had greater diameter of the DF at enrollment compared with those with only an AAM or TC alert. In addition, we found that the diameter of the DF was positively associated with both the peak of activity and the duration of estrus as measured by the AAM system. These findings appear to contrast with previous studies reporting no association between DF diameter and the intensity of estrus behaviour in lactating cows [24,27,28]. While it is well established that the hormonal profile of cows expressing estrus behaviour is characterized by low concentrations of progesterone and increased concentrations of estradiol produced by the DF [29], the relationship between DF diameter, estradiol production, and behavioural estrus is not completely understood. Studies evaluating the association between estradiol and estrus behaviour [30,31] as well as between the DF diameter and estradiol production [32] have yielded discrepant results. Furthermore, because our study did not evaluate hormone concentrations, we cannot determine whether the observed link between DF diameter and estrus behaviour in our dataset was mediated by estradiol. This represents a key limitation in interpreting the biological mechanisms underlying our findings, and further research incorporating hormonal measurements is needed to clarify these relationships. Nevertheless, we observed that the characteristics of true estrus (measured by TC, AAM, or both) were associated with greater odds of the DF ovulation. Similar to our study, other experiments from our research laboratory [24,25] have shown an association between the occurrence and intensity of estrus behaviour with the odds of ovulation in lactating dairy cows. While it is possible that cows that exhibit more intense estrus behaviour may be more capable of activating the hypothalamic mechanisms that trigger ovulation of the DF, the exact mechanisms underlying this relationship remain unclear. Again, although estradiol is a plausible intermediary linking estrus expression to the ovulatory cascade, the relationship between estrus intensity and circulating estradiol concentrations has been inconsistent across studies [30,31], and our study did not evaluate estradiol to assess this association within our dataset. Further research involving detailed hormonal profiling is needed to clarify the biological basis of the association between estrus behaviour and ovulation in lactating dairy cows.

In this study, the occurrence or intensity of estrus behaviour (measured by TC, AAM or both) were not associated with the level of milk production from 3 days before estrus. Although several earlier [33–35] and recent [36–38] studies have evaluated the association between the level of milk production and the occurrence or intensity of estrus behaviour in dairy cows, these studies have not yielded consistent findings. To the best of our knowledge, this is the first study evaluating the association between the level of milk production from 3 days before estrus and the display of estrus behaviour using two different estrus detection tools, separately and in combination, in spontaneous episodes of estrus from lactating Holstein cows. Increased milk production has been linked with increased liver blood flow and steroid metabolism resulting in low progesterone and estradiol concentrations in the circulation [39], which could affect reproductive performance. However, despite these physiological effects, our results suggested that milk production is not associated with the detection of estrus by either TC or AAM, or with the intensity of estrus expression, measured by an AAM, in spontaneous episodes of estrus. To date, the definitive mechanism regulating the intensity of estrus behaviour in dairy cows is still not fully understood [40]. Moreover, the extent to which the milk production by itself can affect the occurrence and the intensity of estrus behaviour in a healthy and well-managed high milk producer may have been overstated [41]. For example, maintaining proper energy balance in the postpartum period may support ovarian activity and estrus expression in high-producing dairy cows. This can be achieved through nutritional strategies that minimize postpartum BCS loss and help sustain key metabolic hormones such as insulin, glucose, and IGF-I [42]. In fact, adequate IGF-I levels may not only contribute to follicular development, but may also modulate estradiol action in the hypothalamus, thereby influencing the regulation of estrus behaviour [43,44]. Beyond hormonal regulation, environmental factors such as slippery or dirty flooring have also been shown to reduce the expression of estrus behaviour in lactating cows [7]. In addition, the use of true spontaneous estrus, reducing the confounding effect of hormonal interventions and false alerts, as well as the use of milk data closer to estrus events may have contributed to the results found in our study. Measuring milk production during the 3 days immediately preceding estrus may offer a more relevant indicator of the cow's physiological and metabolic status at the time of estrus expression, reducing the potential confounding effects associated with lactation stage or prior fluctuations in milk production.

We found that as average milk production increased, the diameter of the DF also increased, while the odds of ovulation decreased. Similarly to estrus behaviour, previous studies examining the relationship between milk yield, DF diameter, and ovulation in lactating dairy cows have reported inconsistent results [25,36,45,46]. Studies demonstrated no differences in the diameter of the DF between high and low producers [24,28] or greater diameter of follicles for high producing cows [36]. Likewise, studies have shown no associations between milk production and ovulation failure [25] or negative associations in the ovulation timing postpartum [36]. In the study by Lopez et al. [36], high producing dairy cows were reported to have greater diameter of the DF, however, lower estradiol concentration in the circulation when compared to low producers. Larger DF in high milk producers may be associated with an extended growth period, likely due to increased liver blood flow and steroid metabolism [36,39]. Perhaps this may represent a compensatory response, in which greater follicular growth is required to produce sufficient estradiol to reach the circulating concentrations necessary to induce the GnRH and LH surges. However, this compensatory mechanism may occasionally fail to adequately trigger these hormonal surges, ultimately reducing the likelihood of ovulation. Yet, as our study did not assess estradiol or LH concentrations, we cannot confirm that hormonal differences account for the observed associations in DF diameter and ovulation between high and low milk producers.

One potential limitation of our study is that we did not classify estrus alerts as true estrus events if they occurred within 7 days of a previous confirmed event, ovulation appeared to have occurred during that prior event, and the developing CL had not yet reached 24 mm. This approach may have led to the misclassification of legitimate very short cycles. However, as only nine estrus alerts (from eight cows) met these criteria, very short cycles represented only a small proportion of the estrus events analyzed in our study. Moreover, relatively low prevalence of short cycles in lactating cows has been documented in the literature. A recent study by Denis-Robichaud et al. [47] reported that only 12 % of estrous cycles were short (<17 days). Interestingly, that study also reported that prolonged luteal phases (regardless of embryonic loss) may be more prevalent in lactating cows, with 19 % of estrous cycles classified as long (24–30 days) and 38 % as very long (≥31 days). Taken together, the small number of very short cycles in our data and their low reported prevalence suggest that their exclusion likely did not introduce meaningful bias into our study.

## 5. Conclusion

We found that the use of AAM and TC together increased the odds of identifying true estrus events within spontaneous episodes of estrus from lactating Holstein cows. Moreover, cows which had an AAM alert that were also alerted with TC had greater estrus expression, greater DF diameter and higher odds of ovulation than cows with only an AAM alert. We did not, however, find associations between milk production and true estrus events, estrus behaviour, or pregnancy. In conclusion, TC could be used as a marker of high-intensity estrus and, when combined with AAM, improves the detection of true estrus events in lactating dairy cows.

## CRediT authorship contribution statement

**J.C.S. Marques:** Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **T.A. Burnett:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis. **J. Denis-Robichaud:** Writing – review & editing, Visualization, Validation, Supervision, Formal analysis. **A.M.L. Madureira:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **W.A. Gomes:** Validation, Resources, Project administration, Methodology. **A.M. Bega:** Resources, Project administration, Methodology, Data curation. **A. Moore:** Visualization, Resources, Methodology, Investigation, Data curation. **R.S. Conceicao:** Visualization, Resources, Project administration, Methodology, Data curation. **S. Moore:** Visualization, Validation, Methodology. **Ronaldo L. A. Cerri:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

[1] Meadows C, Rajala-Schultz PJ, Frazer GS. A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. J Dairy Sci 2005;88:1244–54. https://doi.org/10.3168/jds.S0022-0302(05)72791-0.
[2] Inchaisri C, Jorritsma R, Vos PLAM, van der Weijden GC, Hogeveen H. Economic consequences of reproductive performance in dairy cattle. Theriogenology 2010;74:835–46. https://doi.org/10.1016/j.theriogenology.2010.04.008.
[3] De Vries A, Marcondes MI. Review: overview of factors affecting productive lifespan of dairy cows. Animal 2020;14:s155–64. https://doi.org/10.1017/S1751731119003264.
[4] Roelofs JB, van Erp-van der Kooij E. Estrus detection tools and their applicability in cattle: recent and perspectival situation. Anim Reprod 2015;12(3):498–504.
[5] Firk R, Stamer E, Junge W, Krieter J. Automation of oestrus detection in dairy cows: a review. Livest Prod Sci 2002;75:219–32. https://doi.org/10.1016/S0301-6226(01)00323-2.
[6] Giordano JO, Sitko EM, Rial C, Pérez MM, Granados GE. Symposium review: use of multiple biological, management, and performance data for the design of targeted reproductive management strategies for dairy cows. J Dairy Sci 2022;105:4669–78. https://doi.org/10.3168/jds.2021-21476.
[7] Roelofs J, López-Gatius F, Hunter RHF, van Eerdenburg FJCM, Hanzen Ch. When is a cow in estrus? Clinical and practical aspects. Theriogenology 2010;74:327–44. https://doi.org/10.1016/j.theriogenology.2010.02.016.
[8] Jónsson R, Blanke M, Poulsen NK, Caponetti F, Højsgaard S. Oestrus detection in dairy cows from activity and lying data using on-line individual models. Comput Electron Agric 2011;76:6–15. https://doi.org/10.1016/j.compag.2010.12.014.
[9] Cerri RLA, Burnett TA, Madureira AML, Silper BF, Denis-Robichaud J, LeBlanc S, et al. Symposium review: linking activity-sensor data and physiology to improve dairy cow fertility. J Dairy Sci 2021;104:1220–31. https://doi.org/10.3168/jds.2019-17893.
[10] Ranasinghe RMSBK, Nakao T, Yamada K, Koike K. Silent ovulation, based on walking activity and milk progesterone concentrations, in Holstein cows housed in a free-stall barn. Theriogenology 2010;73:942–9. https://doi.org/10.1016/j.theriogenology.2009.11.021.
[11] Holman A, Thompson J, Routly JE, Cameron J, Jones DN, Grove-White D, et al. Comparison of oestrus detection methods in dairy cattle. Vet Rec 2011;169. https://doi.org/10.1136/vr.d2344. 47–47.
[12] Aungier SPM, Roche JF, Sheehy M, Crowe MA. Effects of management and health on the use of activity monitoring for estrus detection in dairy cows. J Dairy Sci 2012;95:2452–66. https://doi.org/10.3168/jds.2011-4653.
[13] Chanvallon A, Coyral-Castel S, Gatien J, Lamy J-M, Ribaud D, Allain C, et al. Comparison of three devices for the automated detection of estrus in dairy cows. Theriogenology 2014;82:734–41. https://doi.org/10.1016/j.theriogenology.2014.06.010.
[14] Skenandore CS, Cardoso FC. The effect of tail paint formulation and heifer behavior on estrus detection. Int J Vet Sci Med 2017;5:113–20. https://doi.org/10.1016/j.ijvsm.2017.08.001.
[15] LeRoy CNS, Walton JS, LeBlanc SJ. Estrous detection intensity and accuracy and optimal timing of insemination with automated activity monitors for dairy cows. J Dairy Sci 2018;101:1638–47. https://doi.org/10.3168/jds.2017-13505.
[16] Palmer MA, Olmos G, Boyle LA, Mee JF. Estrus detection and estrus characteristics in housed and pastured Holstein–Friesian cows. Theriogenology 2010;74:255–64. https://doi.org/10.1016/j.theriogenology.2010.02.009.
[17] Peralta OA, Pearson RE, Nebel RL. Comparison of three estrus detection systems during summer in a large commercial dairy herd. Anim Reprod Sci 2005;87:59–72. https://doi.org/10.1016/j.anireprosci.2004.10.003.
[18] Cavalieri J, Eagles V, Ryan M, Macmillan K. Comparison of four methods for detection of oestrus in dairy cows with resynchronised oestrous cycles. Aust Vet J 2003;81:422–5. https://doi.org/10.1111/j.1751-0813.2003.tb11553.x.
[19] National Research Council. Nutrient requirements of dairy cattle. 7th rev ed. Washington (DC): National Academies Press; 2001.
[20] Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. J Dairy Sci 1989;72:68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.
[21] Flower FC, Weary DM. Effect of hoof pathologies on subjective assessments of dairy cow gait. J Dairy Sci 2006;89:139–46. https://doi.org/10.3168/jds.S0022-0302(06)72077-X.
[22] Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. second ed. VER Inc.; 2009. Charlottetown (PE).
[23] Pereira MHC, Wiltbank MC, Vasconcelos JLM. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. J Dairy Sci 2016;99:2237–47. https://doi.org/10.3168/jds.2015-9903.
[24] Madureira AML, Polsky LB, Burnett TA, Silper BF, Soriano S, Sica AF, et al. Intensity of estrus following an estradiol-progesterone-based ovulation synchronization protocol influences fertility outcomes. J Dairy Sci 2019;102:3598–608. https://doi.org/10.3168/jds.2018-15129.
[25] Burnett TA, Polsky L, Kaur M, Cerri RLA. Effect of estrous expression on timing and failure of ovulation of Holstein dairy cows using automated activity monitors. J Dairy Sci 2018;101:11310–20. https://doi.org/10.3168/jds.2018-15151.
[26] DeJarnette JM, Salverson RR, Marshall CE. Incidence of premature estrus in lactating dairy cows and conception rates to standing estrus or fixed-time inseminations after synchronization using GnRH and PGF2α. Anim Reprod Sci 2001;67:27–35. https://doi.org/10.1016/s0378-4320(01)00107-5.
[27] Van Eerdenburg FJCM, Karthaus D, Taverne MAM, Mercis I, Szenci O. The relationship between estrous behavioral score and time of ovulation in dairy cattle. J Dairy Sci 2002;85:1150–6. https://doi.org/10.3168/jds.S0022-0302(02)74177-5.
[28] Madureira AML, Silper BF, Burnett TA, Polsky L, Cruppe LH, Veira DM, et al. Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. J Dairy Sci 2015;98:7003–14. https://doi.org/10.3168/jds.2015-9672.
[29] Dieleman SJ, Bevers MM, Van Tol HTM, Willemse AH. Peripheral plasma concentrations of oestradiol, progesterone, cortisol, LH and prolactin during the oestrous cycle in the cow, with emphasis on the peri-oestrous period. Anim Reprod Sci 1986;10:275–92. https://doi.org/10.1016/0378-4320(86)90003-5.
[30] Silper BF, Madureira AML, Kaur M, Burnett TA, Cerri RLA. Short communication: Comparison of estrus characteristics in Holstein heifers by 2 activity monitoring systems. J Dairy Sci 2015;98:3158–65. https://doi.org/10.3168/jds.2014-9185.
[31] Aungier SPM, Roche JF, Duffy P, Scully S, Crowe MA. The relationship between activity clusters detected by an automatic activity monitor and endocrine changes during the periestrous period in lactating dairy cows. J Dairy Sci 2015;98:1666–84. https://doi.org/10.3168/jds.2013-7405.
[32] Perry GA, Swanson OL, Larimore EL, Perry BL, Djira GD, Cushman RA. Relationship of follicle size and concentrations of estradiol among cows exhibiting or not exhibiting estrus during a fixed-time AI protocol. Domest Anim Endocrinol 2014;48:15–20. https://doi.org/10.1016/j.domaniend.2014.02.001.
[33] Fonseca FA, Britt JH, McDaniel BT, Wilk JC, Rakes AH. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J Dairy Sci 1983;66:1128–47. https://doi.org/10.3168/jds.S0022-0302(83)81910-9.
[34] Harrison RO, Young JW, Freeman AE, Ford SP. Effects of lactational level on reactivation of ovarian function, and interval from parturition to first visual oestrus and conception in high-producing holstein cows. Anim Sci 1989;49:23–8. https://doi.org/10.1017/S0003356100004219.
[35] Harrison RO, Ford SP, Young JW, Conley AJ, Freeman AE. Increased milk production versus reproductive and energy status of high producing dairy cows. J Dairy Sci 1990;73:2749–58. https://doi.org/10.3168/jds.S0022-0302(90)78960-6.
[36] Lopez H, Satter LD, Wiltbank MC. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 2004;81:209–23. https://doi.org/10.1016/j.anireprosci.2003.10.009.
[37] López-Gatius F, Santolaria P, Mundet I, Yániz JL. Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology 2005;63:1419–29. https://doi.org/10.1016/j.theriogenology.2004.07.007.
[38] Marques O, Veronese A, Merenda VR, Bisinotto RS, Chebel RC. Effect of estrous detection strategy on pregnancy outcomes of lactating Holstein cows receiving artificial insemination and embryo transfer. J Dairy Sci 2020;103:6635–46. https://doi.org/10.3168/jds.2019-17892.
[39] Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J Dairy Sci 2002;85:2831–42. https://doi.org/10.3168/jds.S0022-0302(02)74370-1.
[40] Woelders H, van der Lende T, Kommadath A, te Pas MFW, Smits MA, Kaal LMTE. Central genomic regulation of the expression of oestrous behaviour in dairy cows: a review. Animal 2014;8:754–64. https://doi.org/10.1017/S1751731114000342.
[41] Berry DP, Friggens NC, Lucy M, Roche JR. Milk production and fertility in cattle. Annu Rev Anim Biosci 2016;4:269–90. https://doi.org/10.1146/annurev-animal-021815-111406.
[42] Nigussie T. A review on the role of energy balance on reproduction of dairy cow. DRT 2018;1:1–9. https://doi.org/10.24966/DRT-9315/100003.
[43] Mendez P, Wandosell F, Garcia-Segura LM. Cross-talk between estrogen receptors and insulin-like growth factor-I receptor in the brain: cellular and molecular mechanisms. Front Neuroendocrinol 2006;27:391–403. https://doi.org/10.1016/j.yfrne.2006.09.001.
[44] Boer HMT, Veerkamp RF, Beerda B, Woelders H. Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions. Animal 2010;4:446–53. https://doi.org/10.1017/S1751731109991169.
[45] Desaulniers DM, Lussier JG, Goff AK, Bousquet D, Guilbault LA. Follicular development and reproductive endocrinology during a synchronized estrous cycle in heifers and mature cows displaying contrasting superovulatory responses. Domest Anim Endocrinol 1995;12:117–31. https://doi.org/10.1016/0739-7240(94)00014-R.
[46] Wolfenson D, Inbar G, Roth Z, Kaim M, Bloch A, Braw-Tal R. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. Theriogenology 2004;62:1042–55. https://doi.org/10.1016/j.theriogenology.2003.12.020.
[47] Denis-Robichaud J, Oliveira AP, Sica A, Soriano S, Araújo RL, Pereira MHC, et al. Is prolonged luteal phase a problem in lactating Holstein cows? J Dairy Sci 2024;107:8582–91. https://doi.org/10.3168/jds.2024-24792.