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# Performance and optimization of an ear tag automated activity monitor for estrus prediction in dairy heifers☆

K. Macmillan [a], M. Gobikrushanth [b], G. Plastow [a], M.G. Colazo [c, *]

[a] Livestock Gentec, University of Alberta, Edmonton, AB, T6G 2C8, Canada
[b] Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, S7N 5B4, Canada
[c] Livestock Systems Section, Alberta Agriculture and Forestry, Edmonton, AB, T6H 5T6, Canada

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ABSTRACT

The objectives of this study were to evaluate the performance of the SCR eSense ear tag automated activity monitor (AAM) to detect estrus behavior in Holstein heifers and to determine the optimal time from estrus alert to artificial insemination (AI) using sex-sorted or conventional semen. In total, 281 heifers were fitted with the AAM once eligible for breeding (>13.5 m of age). For the first AI, estrus was synchronized using 500 μg of cloprostenol (PGF), given 14 d apart, and heifers were given estrus detection patches (Estrotect™) after the second PGF. Heifers were inseminated at randomly attributed times after high activity alert from the AAM system or if the estrus patch had ≥ 50% colour change. Most heifers received sex-sorted semen for the first AI and conventional semen for subsequent inseminations. Pregnancy diagnosis was performed at 30 d post AI and heifers had four opportunities to become pregnant. In a subset of heifers (n = 149), ovaries were scanned every 12 h from the time of AI until ovulation (OV). The system recorded a heat index (measure of estrus strength), maximum activity change, maximum rumination change and duration of high activity. The sensitivity was 91.0%, with a false positive and false negative rate of 8.0%, and the positive predictive value to detect true estrus events was 83.5%. Pregnancy per AI to first AI was 67.6% and 97.9% of heifers become pregnant after four inseminations. Most false positive estrus events had a heat index < 45 and a rumination change < -20, while false negative events had a rumination change ≥ -20. Odds of pregnancy was not associated with any estrus characteristics measured by the system. However, pre-ovulatory follicle diameter had a weak correlation (r < 0.25) with all estrus characteristics. The average (range) interval of onset of high activity, peak activity and end of high activity to OV was 28 h (16–46 h), 22 h (10–40 h) and 16 h (0–36 h), respectively. For conventional semen, each hour increase in interval from activity onset or peak activity to AI reduced the predicted probability of pregnancy by 3.8 and 4.2%, respectively. For sex-sorted semen, the relationship between activity onset or peak activity to AI and predicted probability of pregnancy was quadratic, but not significant. Overall, the SCR eSense ear tag AAM performed well and strategies to identify false positive and false negative estrus events, along with optimization of timing of AI, should further improve performance in Holstein heifers.



## 1. Introduction

In a survey of Canadian dairy farms, 66% of respondents ranked reproduction as one of the top three challenges and 46% of respondents indicated that their targets for herd reproductive performance were not met [1]. Approximately 69% of Canadian farms are using visual observation, 9% are using automated activity monitors (AAM), 7% are using timed-AI (TAI) and 9% a combination of methods for estrus detection and insemination in heifers [2]. Each of these methods comes with their own pros and cons; visual estrus detection is low cost, but can result in low estrus detection rates [3], TAI removes the need for estrus detection, but requires strict adherence to a hormone schedule [4], and AAM can remove the labor required for visual estrus detection, but requires a large

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* Corresponding author. 7000 – 113 Street NW Edmonton, Alberta, T6H 5T6, Canada.

*E-mail address:* marcos.colazo@gov.ab.ca (M.G. Colazo).

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up-front investment [5]. The raising of replacement heifers is the second largest contributor to annual operating expenses, with the largest expenses being feeding cost and labour [6]. Therefore, reducing age a first calving and increasing efficiency can reduce rearing costs. As a method to reduce the labour required for estrus detection and improve estrus detection rate, the use of AAM in heifer reproduction programs may benefit dairy producers.

While numerous studies have looked at AAM in lactating cows, there is a lack of studies evaluating performance of AAM and determining the optimization of AAM performance in heifers. In addition, there are no publications evaluating a newer ear tag AAM from SCR by Allflex. The eSense ear tag AAM records activity and rumination change, providing data to producers on estrus and health alerts. Estrus detection rates (71–88%) and conception rates (31–35%) have been variable in cows [7–9] subjected to AAM, however, there is evidence of improved performance in primiparous compared with multiparous cows. Recently, Burnett et al. [10,11] reported increased peak activity, duration of high activity and conception rates in primiparous compared with multiparous cows. Therefore, performance between cows and heifers likely differs as well. To that end, previous studies have observed a higher activity peak and longer duration of high activity in heifers compared with cows [12,13].

When using either TAI or visual estrus detection, insemination of conventional semen within 36 h prior to ovulation (OV) in dairy heifers resulted in acceptable pregnancy rates [14]. LeRoy et al. [7] reported AI within 8 h of onset of high activity increased odds of pregnancy in primiparous cows, whereas odds of pregnancy for multiparous cows did not differ between 0 and 24 h after the onset of high activity determined by AAM. Conversely, Stevenson et al. [8] indicated that insemination of primiparous cows 13–16 h after onset of activity and insemination of multiparous cows within 12 h after onset of activity improved conception rates. For use of sex-sorted semen in TAI or visual estrus detection programs, insemination within 12 h of OV is recommended in heifers [14]. When using AAM, Bombardelli et al. [15] reported that AI from 13 to 41 h after onset of high activity and within 20 h after peak activity increased odds of pregnancy in Jersey cows. The ideal interval from onset of high activity to AI may differ in heifers compared with cows and the ideal interval to AI with conventional or sex-sorted semen may also differ.

The objectives of this study were to evaluate the performance of the SCR eSense ear tag AAM to predict estrus behavior in Holstein heifers and to determine the optimal time from estrus alert to AI for both sex-sorted and conventional semen. We hypothesized that, due to an anticipated longer duration of high activity, delaying insemination of heifers with conventional semen after onset of high activity will improve odds of pregnancy. Additionally, delaying insemination with sex-sorted semen beyond that of conventional semen will further improve odds of pregnancy.

## 2. Materials and methods

This study was conducted on a commercial dairy farm located near Wetaskiwin, Alberta, Canada (53°02′54.0"N, 113°19′28.0"W) from October 2018 to August 2019. All experimental procedures used in this study were approved by the University of Alberta Animal Care Committee (AUP00002800) and conducted according to the guidelines of the Canadian Council of Animal Care [16].

### 2.1. Animals and management

Holstein dairy heifers were enrolled onto the study once they reached 13.5 mo of age. A total of 281 heifers were enrolled to achieve over 460 estrus events and 400 inseminations, which was required to detect a 10% difference in pregnancy per AI. Heifers were housed in a bedded pack facility, bedded with chopped straw and had free access to fresh water and an outdoor area. A total mixed ration (TMR) was fed once daily at approximately 11:00 with automated feed pushing done every 2 h. The TMR was balanced by a professional nutritionist according to the guidelines of the National Research Council Committee on Animal Nutrition [17]. The main ingredients included barley silage (5.0 kg), haylage (4.0 kg), barley grain (1.0 kg) and straw (1.0 kg), grass hay (1.0 kg), brewery yeast (5.0 kg) and grain (4.5 kg), and a vitamin/mineral supplement. Once heifers were 13.5 mo of age, groups of 20–25 animals were moved into the breeding pen (567 $m^2$) for the first insemination and then moved into another pen (1134 $m^2$) for subsequent inseminations. Both pens were housed in the same covered bedded pack facility with access to outdoor dirt pens.

### 2.2. Reproductive protocol for artificial insemination (AI)

Once heifers were eligible for breeding, the group was fitted with an ear tag AAM (eSense, SCR by Allflex, Netanya, Israel). Ear tags were placed to give at least 5 d baseline period before beginning the synchronization protocol. All heifers were examined by transrectal ultrasonography using a MicroMAXX color Doppler scanner equipped with a multifrequency 5–10 MHz linear transducer (SonoSite, Bothell, WS, USA) to determine ovarian cyclicity status (presence of corpus luteum) and normalcy of the reproductive tract. For the first AI, all heifers were subjected to estrus synchronization using two treatments of 500 μg im of cloprostenol (PGF; Bioestrovet; Vetoquinol N.-A Inc., Lavaltrie, QC, Canada) given 14 d apart. After the second PGF, heifers were also given an estrus detection patch (Estrotect™ Rockway Inc., Spring Valley, WI, USA) to aid in the identification of true estrus events, which were checked thrice daily. All heifers were randomly assigned a time for AI based on the high activity alert generated from the AAM and the interval from start of estrus to planned AI. The goal was to achieve approximately equal numbers into the interval categories of < 8 h (21.8%), 8–16 h (32.6%), 16–24 h (28.7%) and > 24 h (16.8%); however, inseminations were only done during the day, which increased the proportion of heifers in the middle categories. Most heifers received sex-sorted semen for the first AI and conventional semen for subsequent AIs. Heifers returning to estrus after AI were re-bred based on high activity alert from the system. All estrus events detected by AAM, starting when heifers were brought into the breeding pen until diagnosis of pregnancy, were confirmed using the estrus detection patches and/or by transrectal ultrasonography (lack of a corpus luteum > 20 mm; presence of uterine fluid and tone).

### 2.3. Determination of ovulation (OV) and pregnancy

In a subset of heifers (n = 149) ovaries were scanned every 12 h using transrectal ultrasonography starting at the time of AI until OV. Ovulation was determined by the disappearance of the dominant follicle ≥10 mm in diameter between consecutive scannings. The timing of OV was considered to happen 6 h before the scan that confirmed the disappearance of the dominant follicle. Pregnancy diagnosis was performed at 30 d post-AI and non-pregnant heifers were treated with PGF. Heifers had four opportunities to become pregnant to AI before moving them to the bull pen.

### 2.4. Data collection

For each insemination, the timing of AI, sire and type of semen (sex-sorted or conventional) were recorded. In addition, pregnancy diagnosis and timing of OV (in the subset of heifers) were recorded for each insemination. The functioning of the ear tag AAM is similar to that of the SCR neck mounted AAM, as described by Stangaferro et al. [18]. In short raw activity and rumination data were recorded in minutes per 2 h interval as an arbitrary number determined by a 3-axis accelerometer that records the speed and angle of head movements. Data was transmitted from the tag to the antennae in the office every 20 min where the system software reported change in activity and rumination from baseline, as well as generated a heat and heath index (0–100 arbitrary units) using a series of internal algorithms which are proprietary to SCR. The AAM system recorded the date and time of onset of high activity, which was when activity change surpassed 25 units greater than the baseline activity, as recommended by the manufacturer. The system also recorded the date and time of peak activity and the end of high activity, the heat index (a measure of estrus strength), the change in time spent ruminating, the duration of high activity and the interval from the last estrus event. A cumulative heat index was also calculated, in which the heat indices reported for each 2-h period over the entire estrus event were combined. After confirmation of each estrus event using the estrus patches and/or ultrasonography of the ovaries and uterus, the event was categorized as a true, false positive (the system recorded a heat event which did not occur) or false negative estrus event (the system missed a heat that was confirmed by the patches and/or ultrasonography). For all inseminations based on true estrus events detected by AAM, the interval in hours between onset of high activity, peak activity, end of high activity and AI were determined. For the first AI, the interval in hours from onset of high activity, peak activity and end of high activity to estrus patch activation were also determined. Finally, for the subset of heifers with a recorded time of OV, the interval in hours between onset of high activity, peak activity, end of high activity and AI to OV were determined.

### 2.5. Statistical analysis

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The descriptive statistics and normality for age, number of times bred, and estrus interval variables like onset of high activity to AI, peak activity to AI, end of high activity to AI, onset of high activity to OV, peak activity to OV, end of high activity to OV, AI to OV, onset of high activity to activated patch, and peak activity to activated patch, were all determined using the UNIVARIATE procedure.

The differences in heat index maximum, activity change maximum, rumination change minimum, and duration of high activity between pregnant and non-pregnant heifers were compared by analysis of variance (ANOVA) method, by semen type, using the MIXED procedure. As none of the aforementioned parameters differed between pregnant and non-pregnant heifers by semen type, the semen type was excluded from the final model and the differences in least square means were tested between pregnant and non-pregnant heifers using the PDIFF multiple means comparison test. Data from 6 heifers that did not become pregnant after four inseminations were removed from further analysis, leaving a total of 275 heifers (394 AI).

The association between pre-ovulatory follicle diameter and heat index maximum, activity change maximum, duration of high activity, and cumulative heat index was tested by Pearson correlation of coefficient using the CORR procedure.

The relationship between onset of high activity to AI, peak activity to AI or end of high activity to AI (predictor continuous variable) and pregnancy outcome (binomial outcome) was evaluated by logistic regression analysis, separately for conventional and sex-sorted semen type, using the LOGISTIC procedure. Although both linear and quadratic models were initially tested for both conventional and sex-sorted semen type, the final model for conventional semen was linear and for sex-sorted semen was quadratic. The predicted probability for pregnancy against onset of high activity to AI, peak activity to AI or end of high activity to AI were plotted using scatter plot for both conventional and sex-sorted semen. In the analysis of estrus interval parameters, a total of 6 outlying values from 3 heifers were removed. Significant differences were reported if $P \leq 0.05$ and considered to be a tendency if $P > 0.05$ and $\leq 0.10$.

## 3. Results

### 3.1. Validation of the eSense ear tag monitors in heifers

In total, 281 heifers were used for the validation of the ear tag activity monitoring system. This resulted in a total of 468 estrus events and 418 AI, 257 with sex-sorted semen and 161 with conventional semen. Including all AI, the pregnancy per AI to first AI was 67.6% and the total percentage of heifers pregnant after four inseminations was 97.9%. The pregnancy per AI using conventional semen was 67.5% and the pregnancy per AI using sex-sorted semen was 63.3%. Overall, the sensitivity (ability to correctly identify true positive heat events) was 91.0% with 8.0% false positive estrus alerts and 8.0% false negative estrus alerts. The positive predictive value (true estrus events/all estrus events) of the system was 83.5%. In total, 21 heifers were bred based on Estrotec patches (false negative heats), with 19 becoming pregnant. Comparison of the activity monitor measures between true positive, false positive and false negative estrus are shown in Fig. 1. Between true positive and false positive events, the heat index, duration of high activity and cumulative heat index were all greater ($P < 0.001$) for true positive heats. Between true positive, false positive and false negative events, activity change was greatest ($P < 0.001$) in true positive estrus and lowest in false negative estrus, with false positive estrus intermediate. On the other hand, rumination change was greatest ($P < 0.001$) in true positive estrus and lowest in false positive estrus, with false negative estrus being intermediate. In total, 85% of false

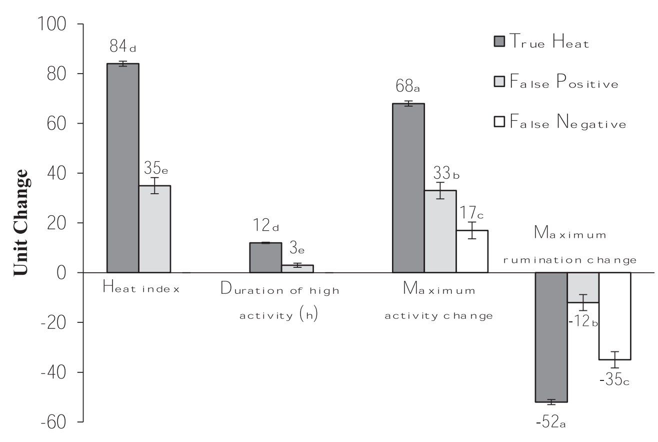


**Fig. 1.** Comparison of heat characteristics (heat index, heat length, maximum activity change and rumination change) between different heat categories (true positive, false positive and false negative). A true positive heat is a heat event that is correctly identified by the SCR eSense ear tag activity monitor, while a false positive is incorrectly identifying an event as a heat and a false negative is missing a true heat event. A heat event was confirmed using transrectal ultrasonography or with an Estrotect heat detection patch. The SCR system measures heat index (heat strength), a maximum change in activity and rumination from baseline during a heat event and the duration of heat, starting with activity change surpassing a threshold of 25 units until consistent activity change below the threshold. [a,b,c] Different superscripts within the same heat characteristic differed ($P < 0.05$). [d,e] Different superscripts within the same heat characteristic differed ($P < 0.001$).

positive events had a heat index < 45 and a rumination change < -20, while 82% of false negative events had a rumination change ≥ -20 combined with a positive activity change.

### 3.2. Optimization of eSense ear tag monitor performance in heifers

Descriptive data on age, number of AI and estrus intervals (onset, peak and end) to AI, OV and patch activation are shown in Table 1. There was no difference in any estrus characteristics (heat index, activity change, rumination change and duration of high activity) between heifers that were pregnant and non-pregnant to AI, for all heifers and for heifers inseminated using either conventional or sex-sorted semen (Table 2).

In the subset of heifers for which OV was determined (n = 149), there was significant positive correlation between pre-ovulatory follicle diameter and heat index ($P < 0.01$; Fig. 2a), activity change ($P = 0.01$; Fig. 2b), rumination change ($P < 0.01$; Fig. 2c) and duration of high activity ($P = 0.05$; Fig. 2d). However, only weak correlations were observed (r = 0.16 to 0.24).

For heifers inseminated with conventional semen, there was a negative linear relationship between estrus intervals to AI and the predicted probabilities of pregnancy are shown in Fig. 3. The relationship between onset of high activity to AI was not significant ($P = 0.18$; Fig. 3a). However, for every 1-h increase from peak activity and end of high activity to AI, there was a reduction of 4.7% ($P = 0.05$; Fig. 3b) and 3.9% ($P = 0.09$; Fig. 3c) in predicted probability of pregnancy, respectively. For heifers inseminated with sex-sorted semen, albeit not significant, a quadratic relationship was observed between estrus intervals to AI and pregnancy (Fig. 4). Inseminations done −1 to 8 h or −1.5 to 4 h related to onset or peak of activity, respectively, resulted in the numerically highest predicted probability of pregnancy.

## 4. Discussion

The objectives of the current study were to evaluate performance of an ear tag AAM in heifers and determine any methods to optimize performance. Although AAM have been extensively studied in lactating cows [2,7,8], there is a lack of studies evaluating performance in heifers, particularly of a new ear tag AAM from SCR. There is evidence of differences in performance of AAM when looking at primiparous and multiparous cows separately. In general, primiparous cows have increased peak activity and duration of high activity compared to multiparous cows [10], in addition to higher conception rates and odds of pregnancy compared with multiparous cows [8,11]. Therefore, it stands to reason that performance would also differ from cows to heifers, especially as heifers have a longer duration of estrus activity compared with cows [13]. In addition, heifers are more often housed in bedded-pack or pasture compared with cows and AAM is more accurate in cattle on pasture [19].

In the present study, the pregnancy per AI to first AI in heifers was 67.6%, using almost entirely sex-sorted semen, and the overall pregnancy per AI was 65.9%. This is in-line with previously reported heifer pregnancy per AI using TAI (62.7% [20]) or visual estrus detection (69.6% [14]) with a combination of sex-sorted and conventional semen. Pregnancy per AI for inseminations based solely on AAM had only a small reduction when using sex-sorted compared with conventional semen, which is surprising, as the sperm sorting process is expected to reduce fertility [21]. However, this may be due to the circumstance that the most fertile heifers became pregnant to sex-sorted semen and subsequently less fertile heifers were inseminated with conventional semen. Based on previous studies using the SCR HeatTime collar AAM, the positive predictive value, or the ability to accurately detect true estrus events was between 87.8 and 89.6% for lactating cows [10,22] and was 84.7% for heifers [12]. In the current study the positive predictive value was 83.5%, and the sensitivity was 91.0%, with a false positive and false negative rate of 8.0%. This is similar to the 9.0% of cows flagged in estrus with an AAM that had serum progesterone concentrations > 1 ng/mL, as reported by LeRoy et al. [7]. While the positive predictive value was lower in the current study compared with previous studies in cows, the system still performed well in heifers for predicting estrus resulting in high overall odds of pregnancy.

Optimizing the performance of AAM in heifer reproduction programs may help to increase perceived value and uptake of this monitoring technology. One way to do this would be to assist producers in identifying potential false positive and false negative estrus events. The average heat index and duration of high activity for all recorded estrus in the current study were 79.2 and 11.3 h, respectively. Silper et al. [12] evaluated the SCR HeatTime collar AAM in Holstein heifers and reported a similar heat index of 77.3 and although the duration of high activity was longer at 14.3 h, there was a smaller range of 18 h, compared to 30 h in the current study. When looking at false positive estrus, the heat index, high activity duration, activity change and rumination change were all reduced compared with true positive estrus in the current study. Burnett et al. [10] reported a similar reduction in heat index and duration of high activity with false positive alerts in a study conducted in dairy cows using the SCR HeatTime collar AAM. Based on current results, an estrus event with a heat index below 45 and a minimum rumination change less than −20, indicates a false positive estrus 85% of the time. For false negative estrus, the current data indicates that although the activity change may not increase above the threshold for an estrus alert, the rumination change drops below −20 in 82% of the heifers with a false negative estrus event. Future work in AAM could improve performance of devices by considering estrus characteristics, drops in rumination time and/or health alerts and interval from last estrus.

In previous studies, cows with a higher activity peak had increased concentrations of estradiol at estrus and increase progesterone concentrations 7–10 d after AI, resulting in increased pregnancy per AI [22,23]. In addition, estrus events with a longer duration of high activity have resulted in increased pregnancy per AI in dairy cows [10,11]. In the current study, there was no

**Table 1**
The mean, standard deviation (SD) and range for number of AI and intervals between heat measurements (heat start, peak and end) and AI[a], ovulation[b] (OV) and estrus patch activation[c] (PA).

| Variable | Mean | ± SD | Range |
|---|---|---|---|
| Number of AI | 1.4 | 0.7 | 1.0–4.0 |
| Heat Start to AI (h) | 15.1 | 8.5 | −11.5 - 31.0 |
| Heat Peak to AI (h) | 9.6 | 8.5 | −18.5 - 27.0 |
| Heat End to AI (h) | 4.7 | 8.8 | −20.5 - 26.5 |
| Heat Start to OV (h) | 28.3 | 5.9 | 16.0–46.0 |
| Heat Peak to OV (h) | 21.5 | 5.5 | 10.0–40.0 |
| Heat End to OV (h) | 15.6 | 6.0 | 0.00–36.0 |
| AI to OV | 13.3 | 8.1 | 0.00–30.0 |
| Heat Start to PA (h) | 6.7 | 7.9 | −18.0 - 30.0 |
| Heat Peak to PA (h) | 0.8 | 7.2 | −25.0 - 22.0 |

[a] Heifers underwent AI with either conventional (n = 160) or sex-sorted semen (n = 256) at randomly attributed times after high activity alert from the SCR eSense ear tag activity monitor.
[b] In a subset of heifers (n = 149), ovaries were scanned every 12 h using transrectal ultrasonography to determine OV, which was defined as the disappearance of the dominant follicle ≥ 10 mm in diameter between subsequent scannings. OV was determined to occur 6 h before the scan the confirmed dominant follicle disappearance.
[c] All heifers were given an Estrotect estrus detection patch immediately after estrus synchronization for their first AI.

**Table 2**
The difference in estrus characteristics[a] (heat index, maximum activity change, maximum rumination change and heat length) between heifers pregnant or non-pregnant to AI[b] based on inseminations with conventional semen, sex-sorted semen or all inseminations combined.

| | Conventional Semen | | | | Sex-sorted Semen | | | | All Inseminations | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Non-Preg | Preg | SEM | P Value | Non-Preg | Preg | SEM | P Value | Non-Preg | Preg | SEM | P Value |
| Heat Index | 84.6 | 83.1 | 3.8 | 0.71 | 84.1 | 84.4 | 30 | 0.94 | 84.3 | 83.9 | 2.4 | 0.87 |
| Activity Change | 67.6 | 66.1 | 4.1 | 0.72 | 69.8 | 68.9 | 3.3 | 0.78 | 69.0 | 67.8 | 2.6 | 0.64 |
| Rumination Change | −49.6 | −51.9 | 4.0 | 0.57 | −50.3 | −54 | 3.0 | 0.22 | −50.0 | −55.2 | 2.4 | 0.19 |
| Duration of high activity (h) | 11.4 | 11.7 | 1.0 | 0.73 | 12.6 | 12.7 | 0.8 | 0.89 | 12.2 | 12.3 | 0.6 | 0.80 |

[a] All 275 heifers included in the final analysis were fitted with the SCR eSense ear tag activity monitor, which records a variety of heat characteristics. Heat index is a measure of heat strength, while maximum activity and rumination change is based on the greatest change from baseline. The duration of high activity is recorded from time of activity alert, when activity change surpasses a threshold of 25 units from baseline.
[b] Heifers underwent AI with either conventional (143 breedings) or sex-sorted semen (251 breedings) at random times based on the heat alert from the SCR ear tag activity monitor.

association between any estrus characteristics and pregnancy per AI. This could be due to a difference in the functioning of different AAM or the differences in estrus characteristics between heifers and cows. However, a couple of studies in cows have also reported a lack of association between heat index or duration of high activity with pregnancy [8,15]. In future studies using heifers, analysis of circulating concentrations of estradiol and progesterone may provide more insight into associations, or lack thereof, between measured estrus characteristics and pregnancy.

Although there was no association between estrus characteristics and pregnancy, all characteristics were significantly positively correlated with pre-ovulatory follicle diameter. However, only weak correlations were observed. While a positive relationship between pre-ovulatory follicle diameter and pregnancy was observed using TAI, there was no relationship between pre-ovulatory follicle diameter and pregnancy when OV was naturally occurring [24]. This is supported by the lack of association between estrus characteristics and pregnancy in the current study, indicating that pre-ovulatory follicle diameter was not related with pregnancy while using an AAM. Previous studies have observed a moderate correlation between pre-ovulatory follicle diameter and estrus intensity in heifers [12], as well as weak correlations between pre-ovulatory follicle diameter and estrus intensity and duration of high activity in cows [22]. However, studies that

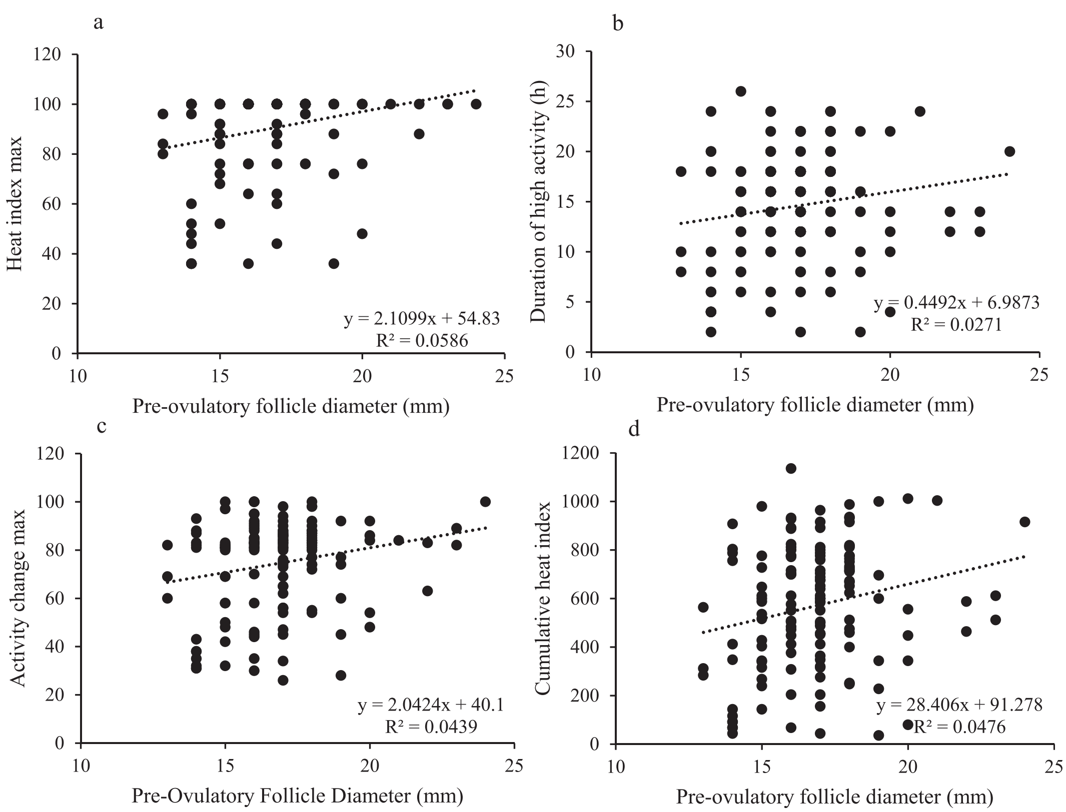


**Fig. 2.** The correlation between diameter of the pre-ovulatory follicle (mm) and estrus characteristics (heat index, duration of high activity, cumulative heat index and activity change) measured by the SCR eSense ear tag activity monitor. Time of OV and diameter of the pre-ovulatory follicle was measured in a subset of heifers (n = 149) by transrectal ultrasonography done every 12 h from AI until OV, or the disappearance of the dominant follicle between consecutive scannings. The SCR system measures heat index (heat strength), a maximum change in activity from baseline during a heat event and the duration of high activity starting with activity change surpassing a threshold of 25 units. Cumulative heat index was calculated by combining the heat index for each 2-h period during a heat event. The Pearson correlation of coefficient (r) was 0.24 ($P < 0.01$), 0.16 ($P = 0.05$), 0.21 ($P = 0.01$) and 0.22 ($P < 0.01$) between pre-ovulatory follicle diameter and heat index (a), duration of high activity (b), cumulative heat index (c) and activity change (d), respectively.

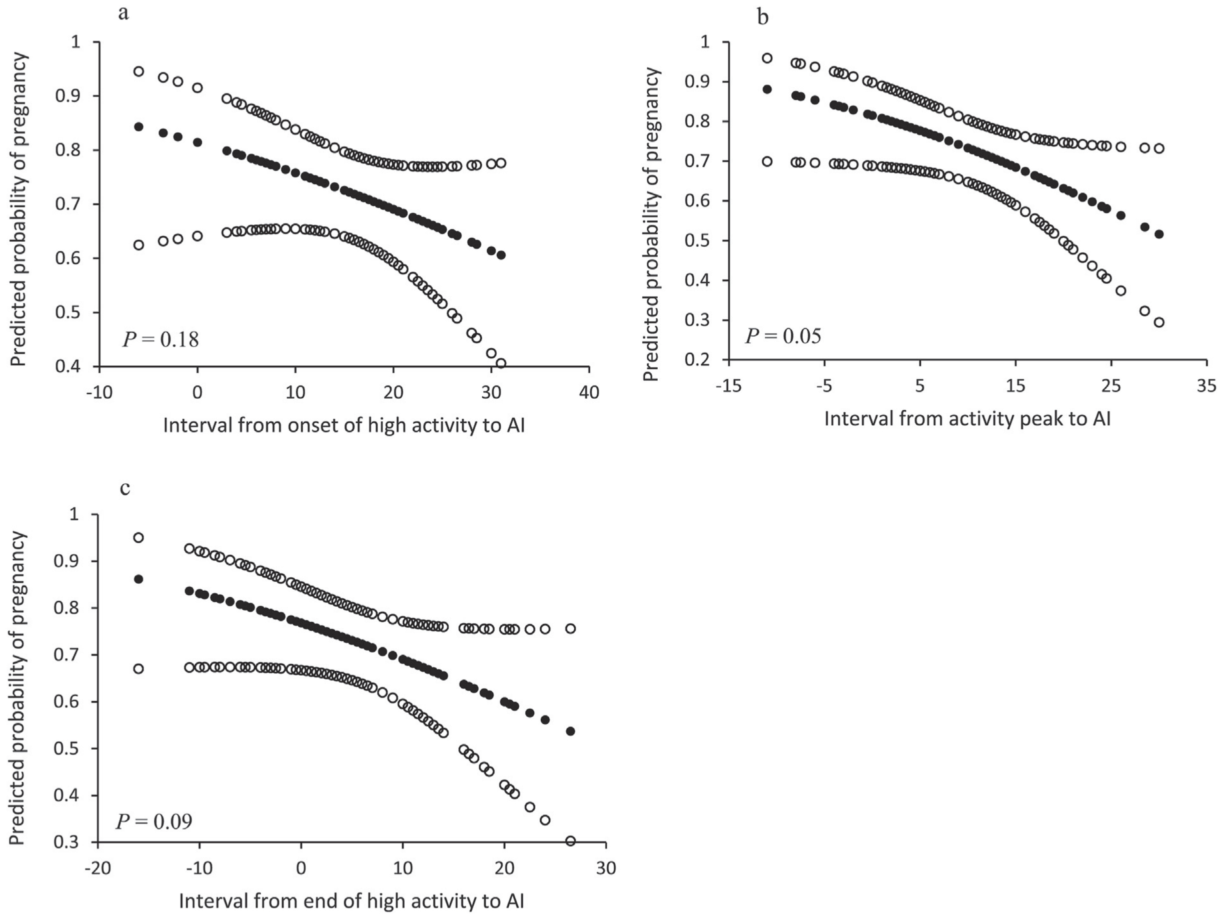


**Fig. 3.** The predicted probability of pregnancy (with 95% credible interval, CI) for heifers AI using conventional semen (143 breedings) based on estrus intervals (onset of activity, activity peak and end of high activity to AI) determined by the SCR eSense ear tag activity monitor. The SCR system recorded onset of high activity (a) as the time activity change increased past the threshold of 25 units from baseline, activity peak (b) as the time of the greatest activity change during a heat event and end of high activity (c) as the time that activity change dropped consistently below the threshold. The relationship between onset of high activity to AI, peak activity to AI or end of high activity to AI (predictor continuous variable) and pregnancy outcome (binomial outcome) was evaluated by logistic regression analysis. Although both linear and quadratic models were initially tested, the final model for conventional semen was linear.

established a relationship between peak activity change and pregnancy in cows, there was no association between pre-ovulatory follicle diameter and peak activity change [23,25]. Overall, results from the current study on the relationships between estrus characteristics and pre-ovulatory follicle diameter are weak and do not appear to affect fertility. It should also be noted that the sample size may not have been large enough to detect significant differences.

In previous studies, the interval from onset of visually detected estrus to OV in heifers has ranged from 25 to 32 h [26–28]. Saumande et al. [29] reported that the interval from estrus to OV was more variable than the interval from LH peak to ovulation, with the interval from estrus to LH peak explaining 80.6% of the variation in the interval from estrus to OV. In the current study, the interval from onset of activity to OV was 28.3 h, with a range between 16 and 46 h. This falls within the previously mentioned range, despite evidence that secondary signs of estrus, including high activity, occur 1–3 h before standing estrus in cows [30]. Although the average interval from high activity onset to patch activation was positive in the present study, the wide range indicates that many heifers would have passed the activity threshold both before and after coming into standing estrus. It should also be noted that estrus patches were only checked 3 times per day, as a means to confirm to an estrus event and not the timing. However, other studies have reported a similar average interval from onset of high activity to OV, of 25–28 h [8,9,15] and range, of 6–42 h [9], in lactating cows. This is despite evidence that interval from estrus to LH surge seems to be shorter and more variable in cows compared with heifers [29]. Indeed, the results from the present study do not appear to support conclusions from Bombardelli et al. [15] or Stevenson et al. [8], that primiparous cows had either a longer (25.7 vs. 19.6 h) or shorter (25.4 vs. 27.0 h) interval from onset of high activity to OV compared with multiparous, respectively. In addition, results from the present study do not corroborate previous reports that heifers have a longer duration of high activity compared with cows [13]. However, there also appears to be a wide range in average high activity duration among cows, from 12 to 17 h [8,9,15]. Overall, it is difficult to determine an accurate interval from onset of high activity to OV in heifers, due to the large amount of variation among animals indicated in the current and previous studies.

When using conventional semen, insemination within 36 h before OV resulted in acceptable pregnancy per AI (> 60%) in dairy heifers [14]. Based on the average interval from onset of high activity to OV in the current study, this would likely translate into insemination any time after estrus detection. The relationship between AAM estrus intervals (onset, peak and end to AI) and predicted probability of pregnancy was linear in the present study, although no significant relationship was observed between the

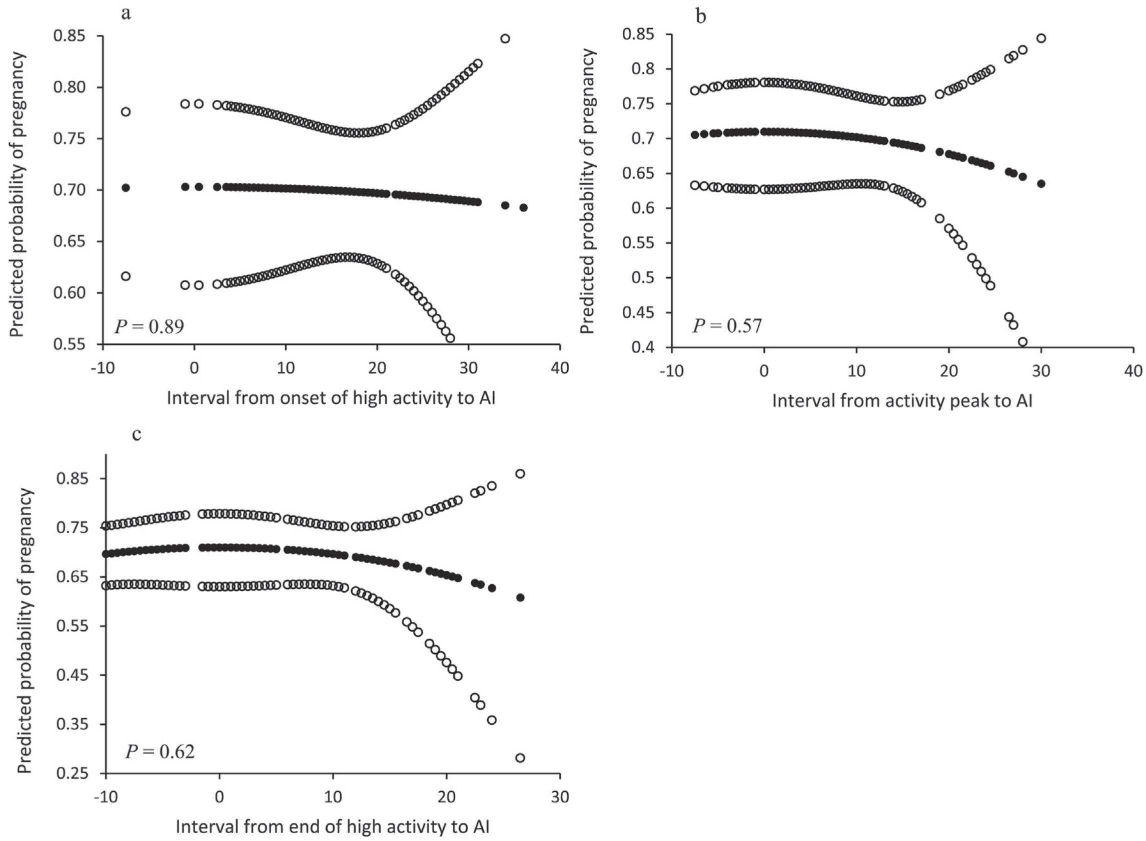


**Fig. 4.** The predicted probability of pregnancy (with 95% credible interval, CI) for heifers AI using sex-sorted semen (251 breedings) based on estrus intervals (onset of activity, activity peak and end of high activity to AI) determined by the SCR eSense ear tag activity monitor. The system recorded onset of high activity (a) as the time activity change increased past the threshold of 25 units from baseline, activity peak (b) as the time of the greatest activity change during a heat event and end of high activity (c) as the time that activity change dropped consistently below the threshold. The relationship between onset of high activity to AI, peak activity to AI or end of high activity to AI (predictor continuous variable) and pregnancy outcome (binomial outcome) was evaluated by logistic regression analysis. Although both linear and quadratic models were initially tested, the final model for sex-sorted semen was quadratic.

interval from onset of high activity to AI and pregnancy. However, for every hour increase in the interval from activity peak to AI there was a significant reduction in pregnancy. Based on the current results, the recommendation would be to breed heifers with conventional semen soon after activity peak, which occurred an average of 5.5 h after the onset of high activity. In dairy cows, LeRoy et al. [7] reported increased odds of pregnancy when primiparous cows were inseminated within 8 h of onset of high activity, whereas odds of pregnancy in multiparous cows were not affected based on interval from onset of high activity to AI between 0 and 24 h. However, Stevenson et al. [8] observed an increase in conception rate when primiparous cows were inseminated 13–16 h after the onset of high activity and when multiparous cows were inseminated less than 12 h after the onset of high activity. Based on the results from Colazo and Mapletoft [14], there is a large window of fertility for inseminating dairy heifers using conventional semen, and the current results suggest inseminating close to the activity peak will improve odds of pregnancy.

Conversely, sex-sorted semen does not maintain fertility for an extended time in the female reproductive tract, and therefore delaying insemination closer to OV is recommended [21]. There is evidence that delaying insemination in heifers 16–24 h after the onset of estrus results in the greatest pregnancy per AI [31]. However, there was no significant relationship between estrus intervals to AI and predicted probability of pregnancy when using sex-sorted semen in the current study. Bombardelli et al. [15] reported increased pregnancy per AI at 13–41 h after onset of high activity and 20 h after peak activity in Jersey cows inseminated with sex-sorted semen. In addition, delaying insemination with sex-sorted semen to within 12 h of OV was beneficial in Holstein heifers [14], which on average would occur 16 h after the onset of high activity and 10.5 h after peak activity. In the current study, the average interval from AI to OV was 13 h, which likely contributed to the high pregnancy per AI obtained with sex-sorted semen. Although, a quadratic relationship was observed, no recommendations can be made on a particular timing of insemination with sex-sorted semen based on AAM estrus alert from the current study.

## 5. Conclusion

Overall, the SCR eSense ear tag automated activity monitor performed well in heifers with a positive predictive value of 83.5%, a sensitivity of 91.0% and an overall pregnancy after four inseminations of 97.9%. There was no association between heat index (estrus intensity), activity change, rumination change or duration of high activity with pregnancy per AI. However, weak correlations were observed between all estrus characteristics and pre-ovulatory follicle diameter. One way to optimize performance of AAM is to better identify false positive heats, which had an average activity change $< 45$ and a rumination change $< -20$, and false negative heats, which had an average rumination change $\geq -20$ combined with a positive activity change. Another way to optimize performance is timing of AI relative to activity change. When using conventional semen, inseminating soon after peak activity increased the odds of pregnancy. There was no significant relationship for any estrus behavior intervals to AI for sex-sorted semen. Our results suggest that the automated activity monitoring technology utilized in the present study represents an alternative approach for reproductive management in dairy heifers.

## CRediT authorship contribution statement

**K. Macmillan:** Methodology, Investigation, Writing - original draft, Visualization. **M. Gobikrushanth:** Formal analysis, Investigation, Writing - review & editing, Visualization. **G. Plastow:** Writing - review & editing, Funding acquisition. **M.G. Colazo:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Funding acquisition.

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