Theriogenology 157 (2020) 33–41

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Comparison of behavioral patterns of dairy cows with natural estrus and induced ovulation detected by an ear-tag based accelerometer

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A R T I C L E I N F O

Article history:
Received 17 January 2020
Received in revised form
15 May 2020
Accepted 31 May 2020
Available online 12 July 2020

Keywords:
Accelerometer
Ovsynch
Behavioral pattern
Activity
Rumination

A B S T R A C T

Dairy farms face many challenges and changes. With increasing herd sizes and fewer farmers or employees per cow, new strategies to maintain or improve reproductive management are required. One of the major challenges is to detect cows in estrus and to estimate the perfect time for artificial insemination (AI). Several estrus and ovulation synchronization programs with timed AI as well as estrus detection aids, e.g., tail-paint, pedometer, accelerometer, and others are available. A combination of ovulation synchronization programs and technical solutions, however, has rarely been tested. This study was designed to gain insights into behavioral patterns of cows subjected to an Ovsynch program and to test if behavioral data could be used to optimize the timing of insemination within an Ovsynch program. In this study, we used an ear-tag based 3D-accelerometer system (SMARTBOW, Smartbow GmbH, Weibern, Austria) to generate data of behavioral patterns, i.e., rumination and activity. In Part 1 of this study, behavioral patterns during the peri-estrus period were compared between cows with physiological estrus and cows subjected to an Ovsynch protocol. On the day before estrus and on the day of estrus/AI, cows with natural estrus showed a clear drop in rumination and “inactivity” and an increase in “high activity”, based on an algorithm of the accelerometer system, whereas, cows in the Ovsynch protocol showed only minor changes in behavioral patterns. In Part 2, we analyzed behavioral patterns between synchronized cows that became pregnant after AI and synchronized cows that remained open. As a result, no differences were detected between these two Ovsynch groups before AI. Thus, in this study we found no evidence that behavioral patterns can be used to improve conception rates within an Ovsynch protocol.

1. Introduction

Estrus detection (ED) is one of the major challenges on dairy farms worldwide. Besides the correct identification of animals in estrus, it is important to estimate the best time for artificial insemination (AI) [1]. Undetected estruses result in great economic losses due to prolonged calving intervals and reduced milk production [2,3].

An efficacious ED is based on the identification of specific signs of estrus, e.g., mounting, standing to be mounted, licking, sniffing, and restlessness [4,5]. Visual observation is the traditional method of ED [6], but in recent decades several changes have affected its efficiency on a herd level, e.g., increased herd size [7,8], reduced time available per cow [9], and changing personal structure on farms [7]. Unskilled workers often do not have a sufficient understanding of a cows' reproductive physiology [10], and frequently they are not familiar with signs of estrus [7]. Furthermore, high-producing dairy cows show shorter periods of estrus related behaviors [11,12] and spend less time showing standing to be mounted, the primary sign of estrus [12,13] compared with the past.

To achieve satisfying ED rates, it is recommended to observe cows for signs of estrus for at least 20–30 min up to three times per day [14,15]. This method is associated with a high investment in time and labor [8]. Thus, to make ED more efficient, synchronization protocols, e.g., Ovsynch [16] or ED tools, e.g., tail painting or

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https://doi.org/10.1016/j.theriogenology.2020.05.050

sensor-based technologies, such as pedometers, activity-meters, accelerometers, are used on many dairy farms [17–19]. Usually, dairy farms opt for synchronization protocols or for ED tools, but not for both at the same time. Nowadays, timed AI programs are widely used on dairy farms worldwide, but the use of hormones is subject to criticism by general public [7]. For this reason, it would be of interest to increase efficiency of synchronization program (i.e., Ovsynch) by combination with ED systems to reach pregnancy rates after Ovsynch of 35%–60% [17].

In the present study, we combined ED by use of the previously validated [20] ear-tag based accelerometer system SMARTBOW with synchronized ovulation, with the aim of analyzing whether accelerometer-based behavioral patterns of synchronized cows can be used to optimize the time for AI within an Ovsynch protocol. For this, we compared accelerometer-based behavioral patterns, i.e., rumination and activity, between cows with physiological estrus and synchronized ovulation. Furthermore, we analyzed differences between synchronized cows that became pregnant after timed AI and cows that remained open after synchronization.

2. Materials and methods

This study was approved by the institutional ethics committee of the University of Veterinary Medicine Vienna, Austria, in accordance with the national authority according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012 (BMWFW-68.205/0004-WF/V/3b/2016), as well as by the Slovakian Regional Veterinary Food Administration.

2.1. Herd description

This study was conducted on a commercial dairy farm located in western Slovakia from March 2016 to December 2017. The farm was housing approximately 2700 Holstein-Friesian dairy cows, with an average energy-corrected milk yield of 9260 kg per cow in 2017 (based on 4.0% butterfat and 3.4% protein). Heifers and primiparous cows in early lactation were housed on another farm site, therefore, they were not included in the study. Milking cows were kept in pens of freestall barns with full concrete floors and high bed cubicles. Each pen offered space for approximately 250 animals. Cows were fed with a total mixed ration (TMR) delivered *ad libitum* twice daily. The components of the TMR were corn silage, alfalfa silage, beet pulp silage, wet distiller's grains with solubles, corn-cob-mix, rapeseed extraction meal, and minerals. Cows were milked twice daily in rotary parlors. Animal related records and procedures (e.g., clinical diseases, treatments, estrus, AI, pregnancy outcome) were entered into the herd management software DairyComp 305 (DC305, Valley Agricultural Software, Tulare, USA) by responsible farm staff or AI technicians.

All technical equipment needed for this study was installed in two pens for multiparous cows. The cows were kept in these pens up to approximately 120 days in milk (DIM). Therefore, acceleration data of the ear-tags were only obtained if study cows were allocated to these pens during the experimental period.

2.2. Reproductive management

The voluntary waiting period (VWP) was set at 50 DIM. Estrus was identified by visual observation and by an automated monitoring system (CowManager Sensoor, Agis, Harmelen, Netherlands). Visual observation was done twice daily by farm staff during milking preparation and by two AI technicians in the pens. Animals detected in estrus by farm staff or by the automated monitoring system were examined by one of the AI technicians by transrectal palpation. If estrus was confirmed, cows were inseminated based on the a.m.-p.m. rule [21]. Cows not detected in estrus or not inseminated were subjected to an Ovsynch protocol. The Ovsynch protocol [16] was scheduled to start at 73 ± 3 DIM at one fixed day every week. A resynch protocol was initiated one week before pregnancy check and proceeded for non-pregnant cows. The farm veterinarian conducted pregnancy checks by ultrasound 42 ± 3 days after AI. Pregnancies were confirmed by transrectal palpation by an AI technician approximately 90 days after AI. General and reproduction management routines were identical in all pens and not affected by the study protocol.

Mean herd reproductive performance parameters of multiparous cows, during the study period, were 74 ± 13 days to first AI, 103 ± 39 days to conception, calving interval of 370 ± 38 days, 55.0% first AI submission rate (FSSR; percentage of cows receiving at least one AI in the first three weeks after VWP), 41.0% first AI conception risk (FSCR; number of first AI resulting in pregnancies by total number of first AI x 100), and 22.0% pregnancy rate (FSSR x FSCR/100).

2.3. Accelerometer-based monitoring system

Study animals were equipped with an ear-tag integrated accelerometer (SMARTBOW ear-tag, Smartbow GmbH, Weibern, Austria; size and weight of 52 × 36 × 17 mm and 34 g) before calving. The ear-tag was fixed in the middle of the right ear. Acceleration was measured during head and ear movements of the animals in three dimensions (x-, y-, and z-axes). The obtained values ranged from −2 to +2 gravity and were recorded with a frequency of 1 Hz. The measured values were transmitted in real-time to receivers (SMARTBOW WallPoint). Receivers were installed with a distance of 20 m each throughout the study pens and transferred data to a local server (SMARTBOW FarmServer). There, the incoming acceleration data were processed and analyzed by SMARTBOW software and estrus alerts were sent, e.g., to mobile devices.

Accumulated acceleration data were recorded hourly and included derived measurements of the following variables: (a) active, (b) inactive, (c) high active, and (d) rumination. Variables a) to d) were based on algorithms developed by the company Smartbow. The measured minutes of "active", "inactive", and "high active" added up to 60 min per h. The variable rumination showed the minutes of ruminating behavior per hour [22,23].

2.4. Study design and definition of terms

Cow data and reproductive performance data were retrieved retrospectively from the herd management software, DC305. For each cow, the following data were recorded: parity, AI date, DIM at AI, non-induced estrus (NIE) or ovulation induced by Ovsynch, pregnancy check outcome 42 ± 3 days after AI. The day of AI was set as identical with the day of estrus. Acceleration data "inactive", "high active", and rumination time were analyzed for the peri-estrus period three days before to two days after AI to detect deviations at estrus (AI) compared to the following and previous days. Values of "high active", "inactive" and "active" cumulate to 60 min/h. The variable "active" is not presented, because it is primarily not important for the estrus decision function of the SMARTBOW system.

According to the objectives of this study, two analyses were conducted, the comparison between NIE and synchronized estruses (OvS; Part 1) and the comparison between cows with synchronized estrus that became pregnant (OvPreg) and cows that remained open (OvOpen; Part 2). In Part 1, cows that were not accurately detected in estrus or that did not respond to the Ovsynch protocol were excluded. Thus, in Part 1, only cows that became pregnant after AI were included in the analyses. This was decided to ensure that only cows that were truly in estrus were included to detect differences between NIE and OvS groups.

Cows in Parts 1 and 2 were matched as best as possible as pairs based on the following criteria: (1) AI date, (2) parity, and (3) DIM at AI.

As a primary outcome, the data of rumination and activity were analyzed in the groups in detail. Furthermore, all estrus events were matched with generated estrus alerts by the SMARTBOW system and the percentage of “true positive” (TP, a SMARTBOW estrus alert occurred before AI in NIE, OvS, OvPreg, or OvOpen) and “false negative” (FN, no SMARTBOW estrus alert occurred before AI in NIE, OvS, OvPreg, or OvOpen) was calculated. The performance of the SMARTBOW system in ED in NIE, OvS, OvPreg, and OvOpen was based on sensitivity and calculated as TP/(TP + FN).

2.5. Statistical analyses

Cow data and reproductive performance data based on DC305 entries were extracted and exported to Microsoft Excel spreadsheets (MS Excel, version 14.0.7194.5000, Microsoft Corporation, Redmond, USA). Additionally, acceleration data were converted into Excel spreadsheets.

For the analyses, the peri-estrus period was categorized. Acceleration data were every 6 h or 24 h constricted and the mean min per h and cow were calculated for the variables “inactivity”, “high activity”, and rumination. Thereafter, the results were summarized and presented as boxplots.

For statistical analyses, the program package SPSS (version 24, IBM Corporation, Armonk, NY) was used. The mean acceleration data of all four variables for Parts 1 and 2 were tested for normal distribution with the Kolmogorov–Smirnov test. They were not normally distributed and hence, analyzed using a Mann–Whitney *U* test. Averages were reported as mean ± SD. The level of significance was defined as $P < 0.05$.

3. Results

In total, 170 NIE were eligible to be matched with 123 OvS events, and 169 OvOpen events with 123 OvPreg events. After assignment, 102 and 108 pairs remained in the data set of Parts 1 and 2. The average parity and DIM (mean ± SD) were 2.8 ± 1.1 and 88.6 ± 32.2 for NIE and 2.7 ± 1.1 and 95.9 ± 26.3 for OvS cows in Part 1, and 2.6 ± 1.1 and 92.4 ± 19.9 for OvPreg and 2.6 ± 1.0 and 91.9 ± 19.1 for OvOpen cows in Part 2, respectively.

The SMARTBOW system generated estrus alerts on the day of AI of 99 NIE, 53 OvS, 55 OvPreg, and 40 OvOpen events. Sensitivity was calculated as 97.0% for NIE, 52.0% for OvS, 50.9% for OvPreg, and 37.0% for OvOpen events.

3.1. Part 1: Natural estrus vs induced ovulation

3.1.1. Rumination time during the peri-estrus period

The rumination time for every day during the peri-estrus period is presented as a boxplot for NIE and OvS cows (Fig. 1). Rumination time of NIE cows started to decrease one day before estrus (22.1 ± 4.6 min/h) and reached a nadir on the day of estrus (18.8 ± 4.6 min/h; $P < 0.001$). The OvS group showed no decline in rumination. Rumination time in the OvS group was 24.2 ± 3.7 min/h on the day of AI and ranged from 24.6 ± 4.0 to 25.1 ± 3.6 min/h during the peri-estrus period. Rumination times between the NIE and OvS groups differed one day before ($P < 0.001$), on the day of estrus/AI ($P < 0.001$), and one day after ($P = 0.036$).

Fig. 2 shows the rumination time during the peri-estrus period constricted for every 6 h. Cows of both groups showed a circadian

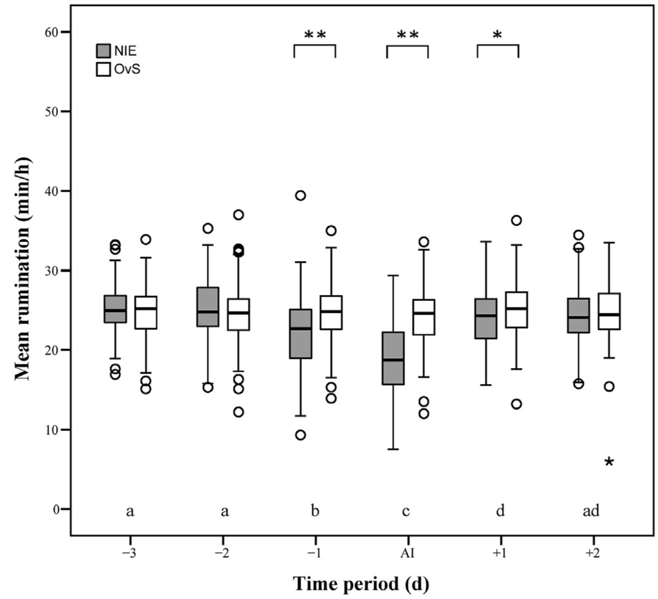

Fig. 1. Boxplots of mean rumination time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ⋆. Significant differences between both groups at one time point are shown by symbols (*$P < 0.05$; **$P < 0.001$). Significant differences within the NIE group between different time points are shown by different letters ($P < 0.05$).

rhythm of rumination on non-estrus days. Maximum rumination time from 28.9 ± 5.8 to 30.7 ± 5.7 min/h (NIE) and from 29.3 ± 5.4 to 30.3 ± 4.6 min/h (OvS) occurred during night (00:00 to 06:00) followed by a decrease in rumination ranging from 19.7 ± 5.8 to 21.0 ± 5.8 min/h (NIE) and from 20.4 ± 6.3 to 21.3 ± 5.4 min/h (OvS) between 06:00 and 12:00 on non-estrus days. A difference in rumination time between non-estrus days and the day of estrus was found in the group of NIE. The circadian rhythm dispersed on the day of estrus, and rumination time declined to 19.9 ± 5.2 min/h between 06:00 and 12:00. Rumination time between the NIE and OvS groups differed on the day before estrus/AI between 06:00 and 12:00 ($P = 0.029$), 12:00 and 18:00 ($P = 0.015$), and 18:00 and 24:00 ($P < 0.001$) and on the day of estrus/AI between 00:00 and 06:00 ($P < 0.001$), 06:00 and 12:00 ($P < 0.001$), and 12:00 and 18:00 ($P < 0.001$).

3.1.2. High activity during the peri-estrus period

“High activity” values are presented as boxplots for every day during the peri-estrus period for NIE and OvS groups (Fig. 3). The mean value of “high activity” of cows in the NIE group started to rise on the day before estrus (9.1 ± 6.2 min/h) and increased on the day of estrus to 14.5 ± 8.0 min/h ($P < 0.001$). The OvS group did not show a significant increase on the day of AI (7.7 ± 5.4 min/h) and values of “high activity” were between 7.1 ± 4.9 and 7.6 ± 5.2 min/h on non-estrus days. The greatest difference of the variable “high activity” was measured on the day of estrus/AI ($P = 0.001$) between the NIE and OvS groups.

The “high activity” values constricted for every 6 h are shown in Fig. 4. Values of both groups showed a circadian rhythm of “high activity” with the lowest values of “high activity” between 00:00 and 06:00 during the peri-estrus period (4.3 ± 5.4 to 5.0 ± 5.5 min/h in NIE, and 4.1 ± 4.7 to 4.9 ± 5.2 min/h in OvS). Cows in both groups showed maximum recordings of “high activity” between 06:00 and 12:00 (8.2 ± 5.1 to 9.5 ± 6.4 min/h in NIE, and 9.1 ± 6.4 to

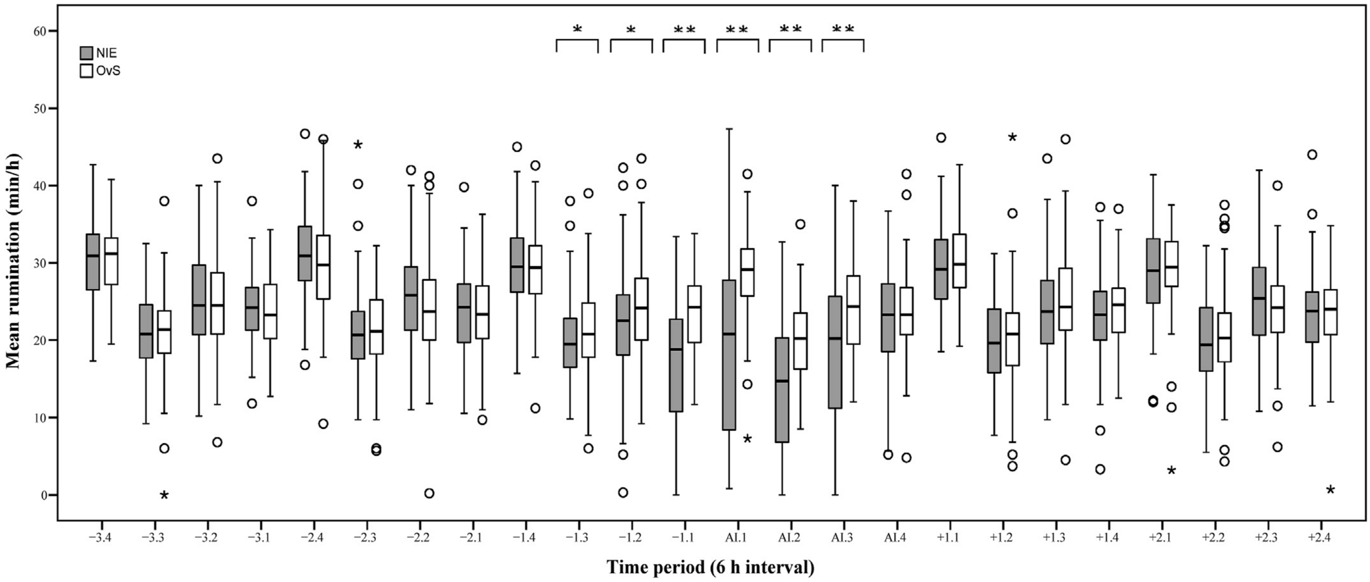

Fig. 2. Boxplots of mean rumination time (min/h) during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. Rumination activity was constricted to 6 h time intervals where −3.4 indicates mean rumination time between midnight and 06:00, −3.3 indicates mean rumination time between 06:00 and 12:00, −3.2 indicates mean rumination time between 12:00 and 18:00, and −3.1 indicates mean rumination time between 18:00 and midnight. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ★. Significant differences between both groups at one time point are shown by symbols (*$P < 0.05$; **$P < 0.001$).

9.7 ± 6.5 min/h in OvS). The circadian rhythm of the NIE group started to disperse one day before estrus and “high activity” recordings increased up to 18.5 ± 13.1 min/h between 06:00 and 12:00 on the day of estrus. “High activity” values between the NIE and OvS groups differed on the day before estrus/AI between 18:00 and 24:00 ($P < 0.001$) and on the day of estrus/AI between 00:00 and 06:00 ($P = 0.000$), 06:00 and 12:00 ($P < 0.001$), 12:00 and 18:00 ($P < 0.001$), and 18:00 and 24:00 ($P = 0.024$).

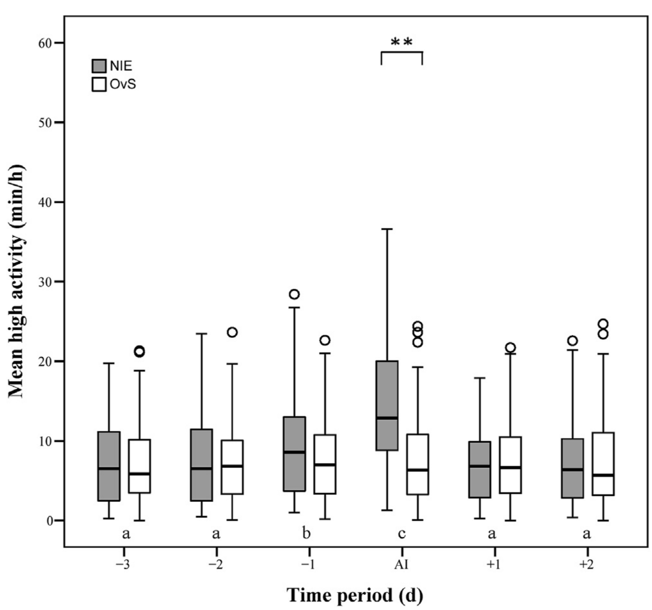

Fig. 3. Boxplots of mean high activity time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○. Significant differences between both groups at one time point are shown by ** ($P < 0.001$). Significant differences within the NIE group between different time points are shown by different letters ($P < 0.05$).

3.1.3. Inactivity during the peri-estrus period

“Inactivity” values for every 24 h during the peri-estrus period are presented as boxplots for the NIE and OvS groups (Fig. 5). “Inactivity” behavior of NIE cows started to decline one day before estrus (12.2 ± 3.6 min/h; $P < 0.001$) and reached its nadir on the day of estrus (8.8 ± 3.9 min/h). After estrus, NIE cows reached higher “inactivity” values than cows of the OvS group. In general, the OvS group showed no significant changes in “inactivity” during the peri-estrus period; “inactivity” values ranged from 14.1 ± 3.5 to 14.7 ± 3.7 min/h during peri-estrus days and were 14.0 ± 3.4 min/h on the day of AI. Differences between the NIE and OvS groups were found two days ($P = 0.007$) and one day before estrus/AI ($P < 0.001$), on the day of estrus/AI ($P < 0.001$), and one ($P < 0.001$) and two days ($P = 0.035$) after estrus/AI.

Fig. 6 shows the “inactivity” time during the peri-estrus period constricted for every 6 h. A circadian rhythm of “inactivity” was found for cows in the NIE and OvS groups. Maximum times of “inactivity” were measured between 00:00 and 06:00 with values ranging from 16.6 ± 5.2 to 19.7 ± 6.4 min/h (NIE) and from 17.4 ± 5.7 to 17.7 ± 6.0 min/h (OvS). Minimum times of “inactivity” occurred between 18:00 and 24:00 and ranged from 10.9 ± 5.2 to 12.8 ± 4.9 min/h (NIE) and from 11.2 ± 4.9 to 12.3 ± 4.9 min/h (OvS). “Inactivity” of the NIE group reached the lowest level (6.2 ± 5.6 min/h) one day before estrus between 18:00 and 24:000 and remained low until the end of the day of estrus. The circadian rhythm within the OvS group around AI remained similar to the peri-estrus days. Significant differences between the NIE and OvS groups were found on the day before estrus/AI between 12:00 and 18:00 ($P = 0.001$) and 18:00 and 24:00 ($P < 0.001$) and on the day of estrus/AI between 00:00 and 06:00 ($P < 0.001$), 06:00 and 12:00 ($P < 0.001$), and 12:00 and 18:00 ($P = 0.002$). Further significant differences between both groups were measured one day after estrus/AI.

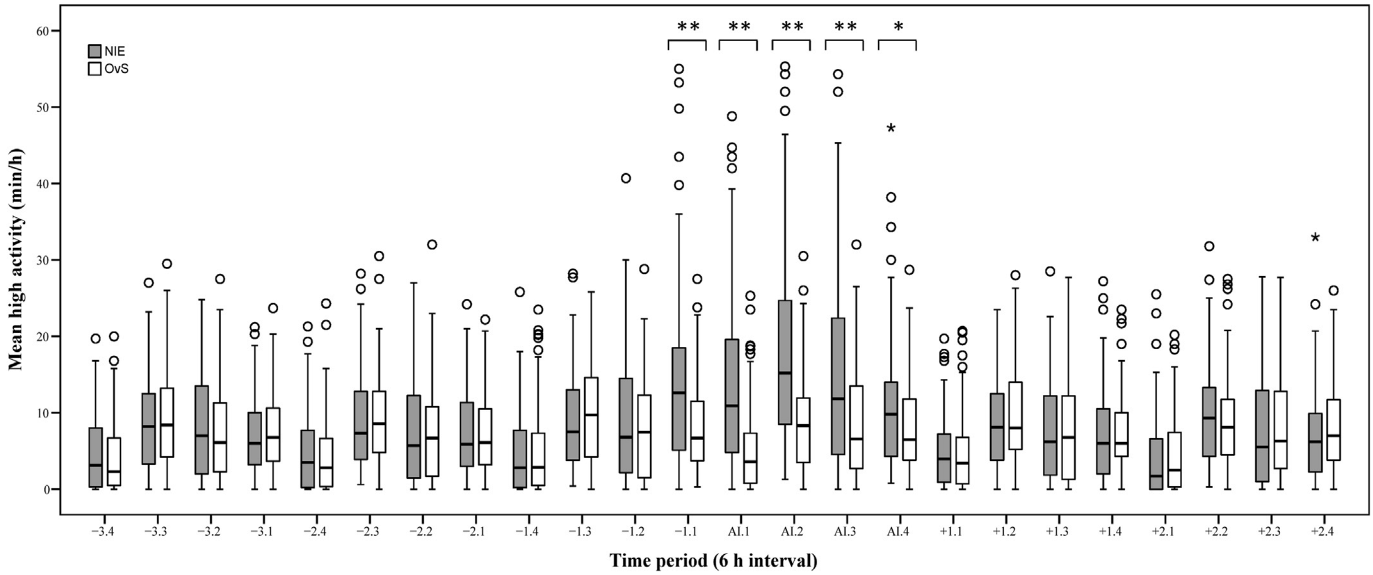

Fig. 4. Boxplots of mean high activity time (min/h) during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. Rumination activity was constricted to 6 h time intervals where −3.4 indicates mean rumination time between midnight and 06:00, −3.3 indicates mean rumination time between 06:00 and 12:00, −3.2 indicates mean rumination time between 12:00 and 18:00, and −3.1 indicates mean rumination time between 18:00 and midnight. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ⋆. Significant differences between both groups at one time point are shown by symbols (*$P < 0.05$; **$P < 0.001$).

3.2. Part 2: Pregnant vs non-pregnant Ovsynch cows

3.2.1. Rumination time during the peri-estrus period

Fig. 7 depicts the rumination time for every day during the peri-estrus period for cows of the OvPreg and OvOpen groups. In both groups, no differences in rumination time between the non-estrus days and AI were evident. Rumination times during the peri-estrus period were between 24.9 ± 3.4 and 25.5 ± 3.6 min/h (OvPreg) and 24.3 ± 3.4 and 25.0 ± 3.0 min/h (OvOpen) and were different one day after AI ($P = 0.017$). On the days of AI, no significant differences were found between the groups (24.7 ± 3.6 min/h in OvPreg, 24.5 ± 3.1 min/h in OvOpen).

Rumination time was constricted for every 6 h for OvPreg and OvOpen cows. Both groups showed a circadian rhythm of rumination on non-estrus days and on the day of AI. Maximum times of rumination occurred between 00:00 and 06:00 with values from 29.2 ± 5.3 to 30.7 ± 4.9 min/h for the OvPreg group and from 29.3 ± 4.8 to 29.9 ± 4.0 min/h for the OvOpen group ($P > 0.05$). Minimum times of rumination occurred between 06:00 and 12:00 ranging from 20.0 ± 6.0 to 21.8 ± 5.5 min/h for the OvPreg and from 20.0 ± 5.5 to 21.8 ± 6.3 min/h ($P > 0.05$) for the OvOpen group. On the day before AI, a difference in rumination time between the groups was found ($P = 0.049$) between 18:00 and 24:00.

3.2.2. High activity during the peri-estrus period

Fig. 8 provides data of “high activity” for every day during the peri-estrus period for cows in the OvPreg and OvOpen groups. Minor variations during the peri-estrus period were detected. “High activity” values were between 6.7 ± 4.9 and 7.3 ± 5.3 min/h in the OvPreg group and between 7.4 ± 4.8 and 7.9 ± 5.1 min/h in the OvOpen group. Furthermore, no differences were evident between non-estrus days and the day of AI. “High activity” on the day of AI was determined for 7.3 ± 5.4 min/h and 7.5 ± 4.7 min/h in the OvPreg and OvOpen groups ($P > 0.05$), respectively.

Values of “high activity” were constricted for every 6 h and a circadian rhythm was present on non-estrus days and on the day of AI in both groups. Minimum values of “high activity” were measured between 00:00 and 06:00 and maximum values between 06:00 and 12:00. During the peri-estrus period, minimum values ranged between 3.9 ± 4.7 and 4.9 ± 5.2 min/h (OvPreg) and 4.8 ± 5.0 and 5.6 ± 5.7 min/h (OvOpen) and maximum values between 8.9 ± 6.5 and 9.6 ± 6.5 min/h (OvPreg) and 9.0 ± 6.1 and 10.0 ± 6.0 min/h (OvOpen). No significant differences were found between both groups on non-estrus days and on the day of AI.

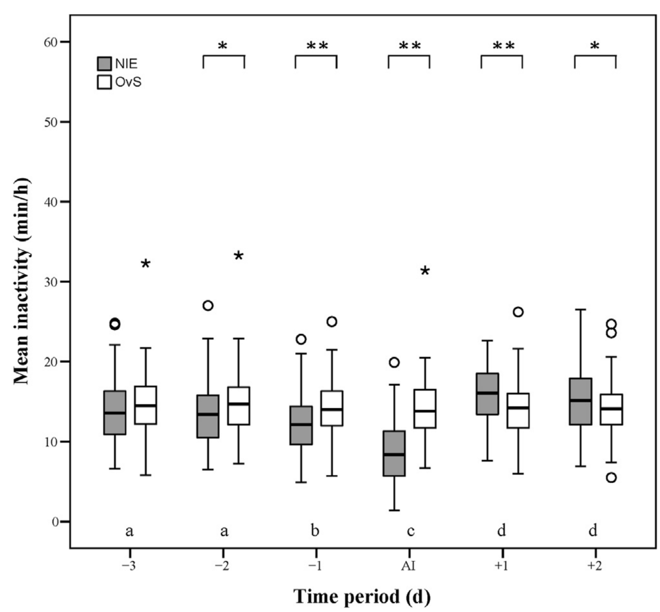

Fig. 5. Boxplots of mean inactivity time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ⋆. Significant differences between both groups at one time point are shown by symbols (*$P < 0.05$; **$P < 0.001$). Significant differences within the NIE group between different time points are shown by different letters ($P < 0.05$).

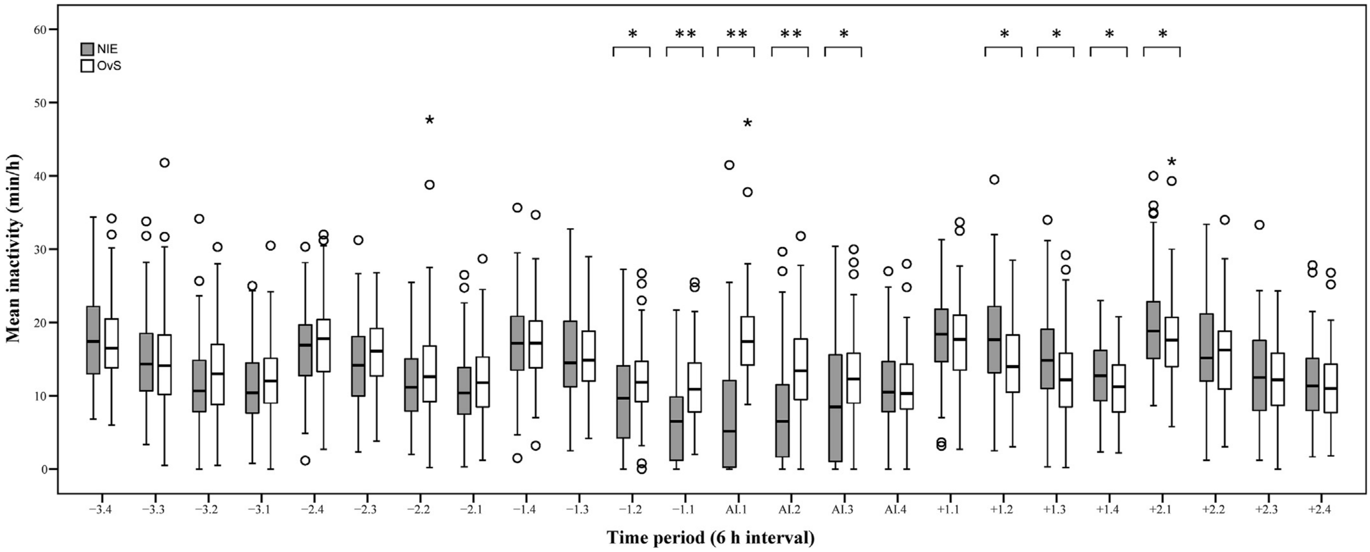

Fig. 6. Boxplots of mean inactivity time (min/h) during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the non-induced estrus (NIE) and induced ovulation (OvS) groups. Rumination activity was constricted to 6 h time intervals where −3.4 indicates mean rumination time between midnight and 06:00, −3.3 indicates mean rumination time between 06:00 and 12:00, −3.2 indicates mean rumination time between 12:00 and 18:00, and −3.1 indicates mean rumination time between 18:00 and midnight. The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ★. Significant differences between both groups at one time point are shown by symbols (*$P < 0.05$; **$P < 0.001$).

3.2.3. *Inactivity during the peri-estrus period*

Fig. 9 shows “inactivity” values for every day during the peri-estrus period for the OvPreg and OvOpen groups. No differences between the groups were found on the days of the peri-estrus period and on the day of AI. “Inactivity” times during the peri-estrus days ranged from 13.6 ± 3.3 to 14.4 ± 3.7 min/h (OvPreg) and from 13.5 ± 3.4 to 14.5 ± 3.6 min/h (OvOpen). On the day of AI, “inactivity” values were 13.6 ± 3.0 min/h (OvPreg) and 13.6 ± 3.3 min/h (OvOpen).

“Inactivity” values were constricted for every 6 h for OvPreg and OvOpen cows. Both groups demonstrated a circadian rhythm during the peri-estrus period and on the day of AI. Cows started with a maximum level of “inactivity” from 00:00 to 06:00, which constantly decreased during the day to minimum values between 18:00 and 24:00. Maximum “inactivity” values ranged from 16.7 ± 5.1 to 17.6 ± 5.3 min/h (OvPreg) and from 17.0 ± 5.4 to 17.9 ± 5.8 min/h (OvOpen) and minimum values from 10.8 ± 4.7 to 12.0 ± 4.9 min/h (OvPreg) and from 11.0 ± 5.5 to 12.1 ± 5.1 min/h

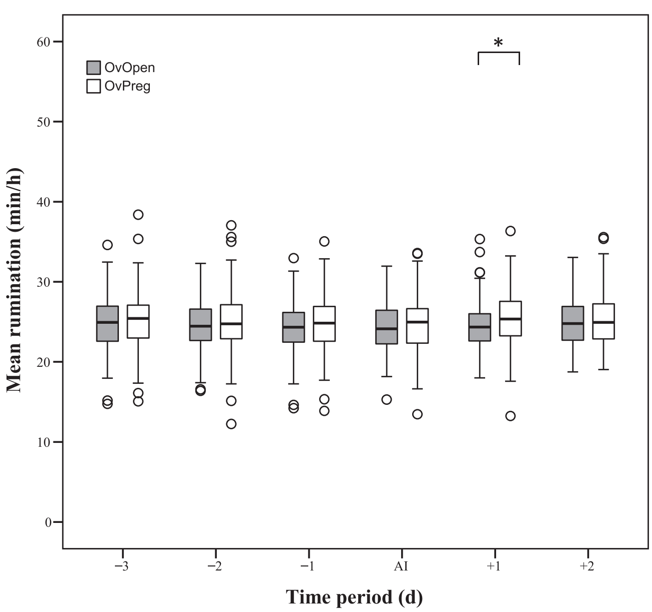

Fig. 7. Boxplots of mean rumination time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the induced ovulation group remaining open (OvOpen) and for the induced ovulation group resulting in pregnancy (OvPreg). The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ★. Significant differences between both groups at one time point are shown by * ($P < 0.05$).

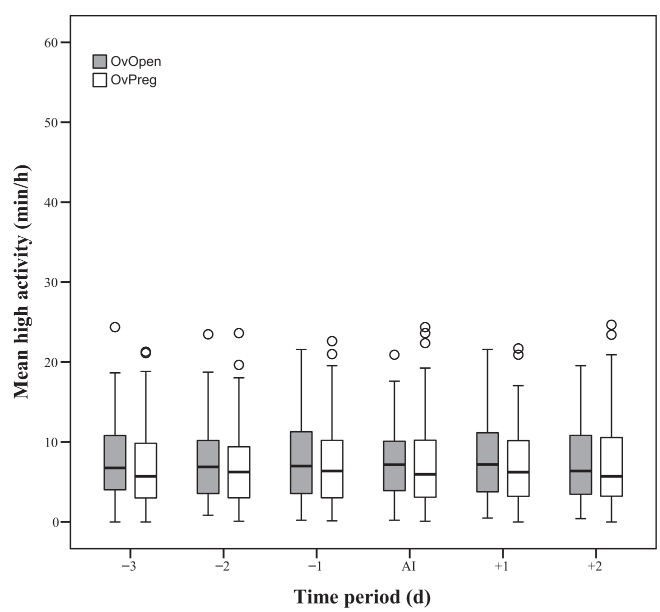

Fig. 8. Boxplots of mean high activity time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the induced ovulation group remaining open (OvOpen) and for the induced ovulation group resulting in pregnancy (OvPreg). The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○.

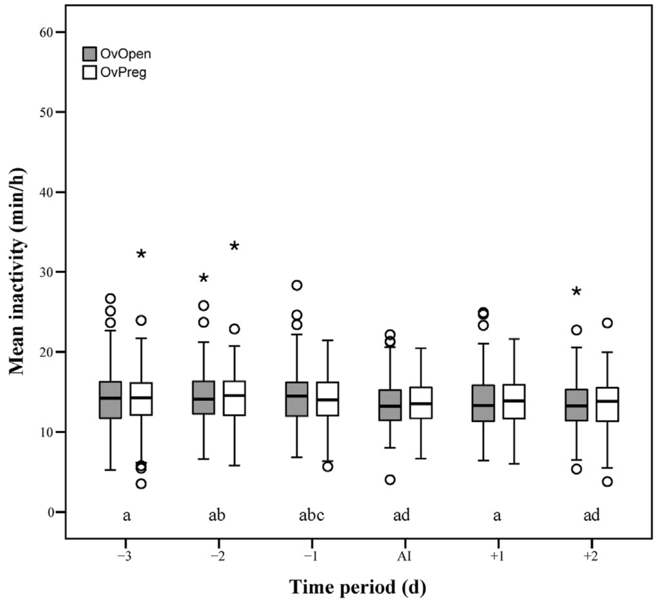

Fig. 9. Boxplots of mean inactivity time (min/h) per day during the peri-estrus period (−3, −2, −1, +1, +2) and estrus (AI) presented for the induced ovulation group remaining open (OvOpen) and for the induced ovulation group resulting in pregnancy (OvPreg). The black line inside the box marks the median (50th percentile); the box marks the 25th and 75th percentiles; the whiskers mark the minimum and maximum values; outliers are designated by ○ and ★ Significant differences within the OvOpen group between different time points are shown by different letters ($P < 0.05$).

(OvOpen). No significant differences were found between both groups on non-estrus days and on the day of AI.

4. Discussion

Various tools and sensors have been developed to identify cows in estrus and, therefore, to support or replace employees in visual ED [24]. Many dairy farms have adopted and integrated these technologies in their reproductive management, such as pedometers, activity-meters, accelerometer or synchronization protocols [19,24]. In the current study, we combined an accelerometer-based ED system with an Ovsynch protocol to compare different behavioral patterns during the peri-estrus period, i.e., rumination and activity measures.

The study was designed as a retrospective analysis of matched pair data. This approach was chosen to ensure that cows in estrus were not false positive (i.e. pregnant after AI) and to avoid effects of age, season, stage of lactation and others. In a prospective approach, hormonal analyses or repeated ultrasonography examination would be required to determine cows in estrus. This, however, was not possible on the commercial study farm. Thus, the described study design has some limitations, including, e.g., that effects of false negative ED cannot be evaluated.

An important component of estrus behavior is an increase in physical activity [4]. Restlessness is triggered by the release of estrogens [4,11] and can already be observed one day before estrus. In Part 1, "high activity" times of the NIE group, defined by the accelerometer system, started to increase one day before estrus and reached a maximum on the day of estrus. One day after estrus, "high activity" decreased back to baseline values. This change in activity is in accordance with results of other studies conducted with accelerometers during natural estruses [25–27]. A complementary change in behavioral pattern was found for "inactivity" and rumination in the NIE group. Mean "inactive" times per day started to decline one day before estrus, achieved its nadir on the day of estrus and reached higher values at the end of the observation period compared with the days before estrus.

A recent publication described similar patterns of activity and rumination times around estrus [25]. Activity measured by a neck-collar mounted accelerometer increased significantly during natural estrus by 38.7%, but with great variability among all cows. On the day of estrus, 24.0% showed not an increased but a reduced activity [25]. Rumination time was reduced by 19.6% on the day of estrus, but only 86.0% of the cows spent less time ruminating on the day of estrus. In total, only 69.0% of the cows showed "high activity" and reduced rumination behavior during estrus [25]. Finally, not all cows showed changes in behavior during natural estrus and, hence, will remain undetected by ED systems [25].

Using a modified Ovsynch protocol for estrus synchronization, Mayo et al. [29] found that activity, measured by different accelerometer and pedometer systems, increased on the day of AI between 69.0% and 170.0% compared to non-estrus days. This, however, was not detected in cows with synchronized ovulation in our study. A minor reduction of rumination time (4.2%) on the day of synchronized ovulation was measured with another ear-based accelerometer system [29], but this system showed a weak correlation ($r = 0.69$) with visually recorded rumination time [22]. Rumination time detected by the SMARTBOW system was highly associated with visual observations ($r = 0.97$) and video recordings ($r > 0.99$) [22,23]. Therefore, it can be assumed that generated rumination data of the here presented study are reliable and a deceased rumination time was only present in the NIE group but not in synchronized groups.

Constriction of data in 6-h time intervals allowed depiction of a circadian rhythm of "inactivity", "high activity", and rumination during non-estrus days. A peak in daily rumination time was detected between 00:00 and 06:00. This is in agreement with other studies which measured high rumination levels between 00:00 and 06:00 and a diurnal maximum between 02:00 and 04:00 [25,28]. A second diurnal maximum, as described in the literature, occurred around noon from 12:00 to 14:00 [25] and from 12:00 to 16:00 [28]. Lowest rumination times were recorded between 06:00 and 12:00, with phases of low rumination from 06:00 to 10:00 and from 16:00 to 20:00 [25,28]. It can be assumed that these peaks were related to, e.g., feeding and milking times. Accordingly, data demonstrated that night hours were the preferred time for cows to ruminate [28,30,31]. Around 63.0% of the total rumination time was recorded during nighttime [32].

After a minimum from 02:00 to 06:00 [25], activity increased, which was related to milking and feeding times [25,33,34]. During the day, two [25,34] or three [33] peaks of "high activity" occurred between 08:00 and 12:00 h, 16:00 to 18:00 [25,34], and around 21:00 [33]. After midnight, "high activity" decreased to a baseline level. These findings from the literature are in agreement with the results during non-estrus days in our study. On the day of estrus, this diurnal rhythm dispersed and "high activity" increased during night hours one day before estrus. This confirms that cows start showing estrus behavior during night hours, which may remain undetected by farmers if ED is based on visual observation [25,35]. This further reinforces the implementation of ED tools or synchronization protocols on farms to achieve higher ED rates.

In the present study, an ED tool was matched with an ovulation synchronization protocol. Data showed greater changes in behavioral patterns in natural estruses than in induced ovulation. Thus, natural estruses were more easily detected and alerted by the SMARTBOW system than in the Ovsynch group. In the second part of this study, we tested if differences in behavioral patterns between cows that became pregnant or remained open after an Ovsynch protocol can provide information that could be used for an optimized timed AI protocol. The results of this retrospective study, however, did not support this hypothesis, although cows were more likely to become pregnant if an estrus alert was generated by the SMARTBOW system. Some studies have shown that cows with a high increase in physical activity during estrus had higher odds of pregnancy [26,27,36,37]. Thus, further research, e.g. with a prospective study design are required to gain additional information about this research question. Furthermore, our finding that rumination was higher one day after AI in OvPreg compared with OvOpen could be a hypothesis for future research and tested in a prospective study about predicting pregnancy.

5. Conclusion

Sensor technologies and synchronization protocols are widely adopted on dairy farms to improve ED and fertility outcomes. The aim of this study was to analyze behavioral patterns, i.e., rumination and activity, for cows with natural estrus and induced ovulation. In our study, cows with natural estruses had a significant increase in activity and decrease in rumination around estrus compared to non-estrus days. Cows with synchronized ovulation showed only minor changes in behavioral patterns around timed AI. Cows that became pregnant after timed AI were detected by the SMARTBOW system. Hence, expressed estrus behavior can be used to predict fertility outcomes.

CRediT authorship contribution statement

V. Schweinzer: Investigation, Writing - original draft. **E. Gusterer:** Investigation, Writing - review & editing. **P. Kanz:** Investigation, Writing - review & editing, Validation. **S. Krieger:** Investigation, Validation. **D. Süss:** Investigation, Validation. **L. Lidauer:** Conceptualization, Project administration. **A. Berger:** Conceptualization, Writing - review & editing. **F. Kickinger:** Methodology, Software, Data curation. **M. Öhlschuster:** Methodology, Software, Data curation. **W. Auer:** Conceptualization, Supervision. **M. Drillich:** Conceptualization, Methodology, Supervision. **M. Iwersen:** Conceptualization, Methodology, Formal analysis, Project administration.

Declaration of competing interest

The authors declare no conflict of interest. Any data, results, or interpretation of this publication were not influenced by the company Smartbow. Authors LL, AB, WA, FK, MO are employees of Smartbow and were integral in the study design, technical support, and algorithm development. Authors VS, EG, PK, SK, DS, MI, and MD were responsible for the study design, data collection, statistical analysis, and preparation of the manuscript.

Acknowledgments

This work was supported by the Austrian Research Promotion Agency (FFG) of the Austrian Government (project number: 859331). Special thanks for their assistance in data collection are extended to Karen Wagener, Harald Pothmann, Paula Flick, Ulrike Reinländer, Andreas Palluch, and Patrick Schmidseder and the staff of the dairy farm in Slovakia for their support with the logistics of the project.

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