REGULAR ARTICLES



Alterations in vaginal temperature during the estrous cycle in dairy cows detected by a new intravaginal device—a pilot study

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Abstract

Estrus identification is important in dairy cow production. At present, estrus identification is automated with a pedometer or accelerometer and the results remain unsatisfactory. It was previously reported that body temperature changes during estrus. In the present study, dairy cow vaginal temperature (VT) was monitored during various seasons, and an increase in VT of 0.3 °C was suggested for the onset of estrus, using an automated VT monitoring system developed in-house. Natural and synchronized estrus were measured simultaneously. The VT was determined to be in circadian rhythm and significantly higher in summer than in either autumn or winter (P < 0.05). VT difference (between estrus VT and average VT 7 days earlier) gradually increased, reached a peak of 0.56 °C ± 0.17 at 4 h before the end of estrus, and then decreased to the normal. The VT of cows in estrus and the duration of their estrus were significantly affected by seasons and estrus types (P < 0.05). VT gradually decreased in response to prostaglandin (PG) injection and was significantly lower (0.15–0.35 °C) from 9 to 33 h after the drug administration than the average VT at the same time 7 days earlier (P < 0.05). Changes in circadian and seasonal VT and in the estrous cycle can be monitored to assess the physiological status of cows and will help in developing an effective automated estrus identification technique. Results of this pilot study should be validated in further studies.

Keywords Dairy cattle · Estrus identification · Estrous cycle · Vaginal temperature

Introduction

Estrus identification is of great importance and is receiving increasing attention in the assessment of dairy cow reproductive performance (Holman et al. 2011; Talukder et al. 2015). Pedometers have been used in many farms to identify estrus and improve the cows' reproductive efficiency. However, only \sim 70% of cows in estrus were detected with pedometer (Fricke et al. 2014). Estrus intensity and duration vary significantly among individuals and are affected by many factors (Reith

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and Hoy 2018). An earlier study reported that milk yield gradually increases as breeding management improves but the duration and intensity of estrus decrease (Lopez et al. 2004). Consequently, pedometric estrus detection becomes less sensitive and accurate. In addition, with the intensification of farming and the use of enclosures, the space available for dairy cow movement decreases and pedometry does not effectively detect estrus (Palmer et al. 2010). Synchronized estrus and artificial insemination timing are gaining popularity. Nevertheless, synchronized treatment may induce inadequate ovulation with small, incompetent follicles, luteal insufficiency, short estrous cycles, and, consequently, reduced embryo survival (Santos et al. 2004). Therefore, estrus identification technology plays an important role in reproduction management of dairy herds (Miller et al. 2007; Fricke et al. 2014). It is necessary to explore physiological estrus indices that are relatively resistant to changes in external factors and to develop automated estrus detection systems.

It was reported that estrus (Suthar et al. 2011), pregnancy (Gil et al. 2001), parturition (Wright et al. 2014), and the postpartum period (Burfeind et al. 2014) in cows are all accompanied by changes in body temperature. Compared with

surface temperature measurement, vaginal temperature (VT) is strongly correlated with core body temperature (EI-Sheikh et al. 2013; Miura et al. 2017) and is not significantly affected by external factors. Therefore, VT measurement was a more reliable and realistic method to use for the purposes of regulating reproduction and managing production (Hossam et al. 2013). Studies also showed that the estrous detection based on temperature measurement was superior to using ultrasound and hormone detection (Sakatani et al. 2016; Miura et al. 2017). Therefore, it is useful to monitor VT continuously and accurately. Although some studies had explored the VT alterations on beef cows (Sakatani et al. 2012, 2016), few studies on Holstein cows have been reported (Suthar et al. 2011; Talukder et al. 2014). Owing to their greater milk production, dry matter intake, and larger body mass than that of beef cows, the body temperature in Holstein cows is significantly affected by summer heat stress (West et al. 2003; Collier et al. 2006). Therefore, in the present study, we investigated seasonal- and estrus-type VT characteristics during estrus using our real-time vaginal temperature detection device (patent number CN201620035605.9) designed for automated estrus prediction.

Materials and methods

Time and place

This study was conducted on a dairy farm in Shijiazhuang, Hebei Province, China, housing 1043 dairy cows with an average 305-day milk yield of 11,000 kg. Vaginal temperature was measured during summer (July 11, 2017–August 4, 2017; indoor temperature-humidity index (THI = 80.1 ± 3.2)), autumn (October 20, 2017–November 15, 2017; THI = 53.8 ± 5.2), and winter (December 24, 2017– January 18, 2018; THI = 39.5 ± 3.8). No testing was done in the springtime because the climatic conditions during spring closely resemble those of autumn.

Equipment

The automated VT- and activity-measuring system developed by our team consisted of 20 sets of real-time VT- and activitymeasuring devices (Fig. 1a), a data collector (Fig. 1b), and a computer system. VT was measured every 0.5 h, wirelessly transmitted to the data collector every 2 h, and then uploaded to the computer system.

Animal feeding and management

Animal care was approved and conducted according to the standard established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Twenty healthy young (19–24 months) Holstein dairy cows were randomly selected per trial per season. All cows were housed in a free stall barn and fed with total mixed rations (TMR) three times daily (07:00, 12:00, and 17:00). Drinking water was freely available.

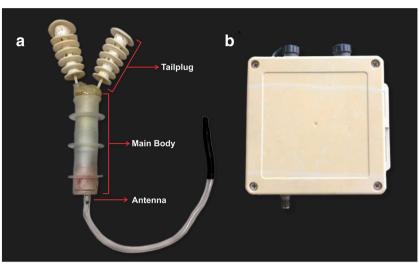
Experimental design

Referring to reference (Kou et al. 2017), we randomly selected seven detecting devices and a mercury thermometer kept them in the incubator. At the beginning, we set the temperature of the incubator at 31 °C and left it for 0.5 h, then we increased 1 °C and detected another 0.5 h; we repeated this detecting method till 39 °C. Meanwhile, the data was sent by the sensors to collectors and then automatically saved in the computer system. According to our previous study, before the automated detection system was used to monitor the estrus of dairy cows, a temperature increase of 0.3 °C between the current VT and the average VT recorded at the same time point 7 days earlier, and which lasted for 2.5 h, was set as the estrus prediction threshold. In addition, activity greater than 2.5 times the average one at the same time point 7 days earlier, which lasted at least 2.5 h, was defined as another estrus prediction threshold. Therefore, in this study, the end of estrus was defined when the difference in VT or activity between two adjacent time points was < 0.3 °C or 2.5-fold, respectively. The detecting devices were surface-sterilized with 0.01% potassium permanganate solution and then inserted into the vagina of the cows.

A total of 60 young Holstein cows (20 in summer, 20 in autumn, and 20 in winter), aged between 19 and 24 months and with normal estrus, were used in this study. In summer, autumn, and winter, 15, 14, and 13 cows were used for natural estrus. Meanwhile, five, six, and seven cows were used for synchronized estrus (treated with 4 mL PG) in summer, autumn, and winter, respectively. Activity and VT were used to detect estrus. Estrus was also observed four times daily between 08:00 and 20:00. Each observation required 15-20 min. When estrus behavior, such as standing and acceptance being mounted, was observed, or estrus was identified by the automated detection system, the follicles were measured using a type B ultrasound scanner. True estrus was characterized by the presence of a preovulatory follicle larger than 10 mm in diameter and absence of an active corpus luteum (Madureira et al. 2015).

Statistical analyses

Relationship between the devices and mercury thermometer was evaluated using both Pearson correlation and linear regression. A Bland-Altman test was used to test the consistency between the two measurements. Estrus was confirmed in 14 (in summer), 13 (in autumn), and Fig. 1 Real-time VT- and activity-measuring device and a data collector. a Real-time VTand activity-measuring device. The measuring device is equipped with temperature sensors (ADT7320) and activity sensors (ADXL362), its size is 210 mm length and 20 mm in diameter, and the width of the tail plug is 80 mm. b Data collector



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12 (in winter) natural estrus cows and in 12 synchronized estrus cows (four in summer, four in autumn, and four in winter). VT and activity data for each cow were recorded for the diestrus period, the estrus period, and 48 h after PG injection. Starting time points (at which VT began to increase) and estrus durations differed among individual cows. Therefore, when estrus was confirmed in a cow and VT met the estrus prediction threshold, the time point immediately before VT increased by 0.3 °C during estrus was designated 0. Circadian variations in VT during diestrus were analyzed with one-way ANOVA. Significant seasonal differences were also tested. Relative differences between the VT recorded within 48 h after PG injection and the average VT at the same time point 7 days earlier were calculated and their significance tested. Two-factor ANOVA was used to determine the influences of season and estrus type on estrus VT and the difference in VT. The latter was the difference between the estrus VT and the average VT at the same time point 7 days before estrus. The duration of estrus, the VT at estrus, and the differences in VT among seasons and estrus types were also tested. A P value < 0.05 denoted a statistically significant difference. Data were processed with SPSS v. 21.0 (IBM Corp., Armonk, NY, USA).

Results

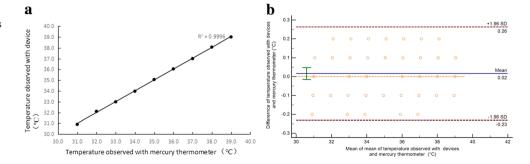
Accuracy experiment of the device

The pre-cattle laboratory test analysis demonstrated a close relationship between the detecting devices and the mercury thermometer, with temperature data sourced from incubator increasing from 31 to 39 °C. The strong relationship between the temperatures detected by the detecting devices and the mercury thermometer ($R^2 = 0.996$) was shown in Fig. 2 a, and the agreement between the temperature data measured by the devices and mercury thermometer was shown in Fig. 2 b (95% CI - 0.23 to 0.26 °C).

Circadian VT variations in diestrus

The measured VT was significantly higher in summer $(38.64 \text{ }^{\circ}\text{C} \pm 0.22)$ than in autumn $(38.26 \text{ }^{\circ}\text{C} \pm 0.08)$ and winter (38.26 °C \pm 0.07) (P < 0.05), and the differences in VT between autumn and winter were not significant (P > 0.05)(Fig. 3). Circadian variations indicate that VT increased in the daytime and decreased at night. In summer, VT decreased to a minimum of 38.27 °C \pm 0.36 at 07:00 and increased to a maximum of 38.97 °C \pm 0.70 at 16:00. Circadian variations

Fig. 2 Consistency of temperature measured by devices and mercury thermometer. a The linear curve of the measured temperature of devices and mercury thermometer. b Bland-Altman plot



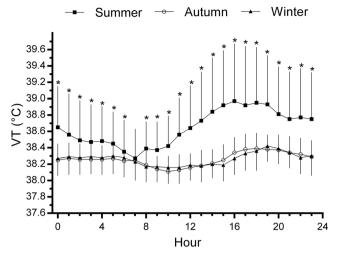


Fig. 3 Circadian VT variations in Holstein cows over three seasons. The data were the average VT of 20 cows in summer, autumn, and winter, respectively. Data are means \pm standard deviations (mean \pm SD). For clarity, only the upper SD for summer and autumn and only the lower SD for winter are shown. *Significant at P < 0.05

were delayed in autumn and winter, with a nadir at 10:00 (38.11 °C \pm 0.19 and 38.16 °C \pm 0.20 in autumn and winter, respectively). The VT increases to the highest levels at 18:00–19:00 (38.39 °C \pm 0.19 and 38.42 °C \pm 0.19 in autumn and winter, respectively). The maximum diurnal VT differences were 0.70 °C in summer, 0.28 °C in autumn, and 0.26 °C in winter. In summer, the diurnal VT values fluctuated substantially. Relative differences in VT among individuals were also greater in summer than in either autumn or winter.

Effect of PG injection on VT change

Estrus was detected in twelve cows (four per season) within 48–72 h after PG injection. The average VT values at 48 h after PG injection and at the same time point 7 days before PG

Fig. 4 Effects of PG injection on VT. The PG injection time point was designated 0. A total of 12 cows (four in summer, four in autumn, and four in winter) were used for this analysis. Data are means \pm standard deviations (mean \pm SD). For clarity, only the upper SD for average VT 7 days earlier and only the lower SD for VT after PG injection. *Significant at P < 0.05

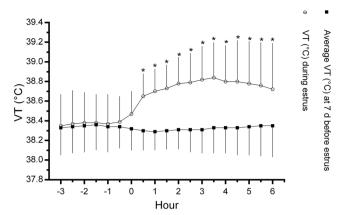
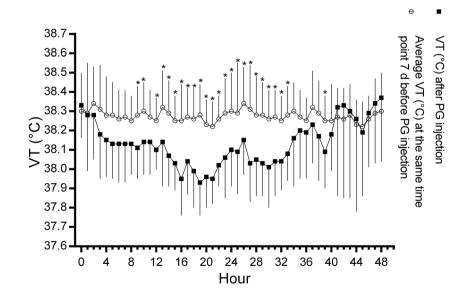


Fig. 5 Changes in VT before and after the onset of estrus. A total of 51 cows (18 in summer, 17 in autumn, and 16 in winter) were used for this analysis. Data are means \pm standard deviations (mean \pm SD). For clarity, only the upper SD for VT during estrus and only the lower SD for average VT at 7 days before estrus. *Significant at P < 0.05

injection were calculated (Fig. 4). VT values gradually decreased after PG injection and were 0.15–0.35 °C lower at 9–33 h post-injection than average VT at the same time point 7 days before PG injection (Fig. 4). The VT nadir (37.93 °C) was at 19 h after PG injection (P < 0.05), then gradually increased and recovered to its normal level at 41 h.

VT alteration during estrus

The relative differences between estrus VT and the average VT recorded at the same time point 7 days before estrus for 51 cows (18 in summer, 17 in autumn, and 16 in winter) are shown in Figs. 5 and 6. Prior to estrus, the differences in VT approached 0 (Fig. 5). The VT values then began to increase at 1 h before the onset of estrus and reached their maximum (38.84 °C \pm 0.36) at 3.5 h after estrus. The maximum VT difference was 0.56 °C \pm 0.17 at 4 h before the end of estrus



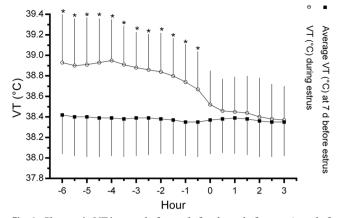


Fig. 6 Changes in VT in cows before and after the end of estrus. A total of 51 cows (18 in summer, 17 in autumn, and 16 in winter) were used for this analysis. Data are means \pm standard deviations (mean \pm SD). For clarity, only the upper SD for VT during estrus and only the lower SD for average VT at 7 days before estrus. *Significant at P < 0.05

(Fig. 6). Thereafter, estrus VT gradually decreased until it returned to its average value at 7 days before estrus started.

VT and activity characteristics of cows in estrus according to season

The effects of season and estrus type on estrus VT, differences in VT before and after estrus, and estrus duration are shown in Table 1. Estrus VT values (39.13 $^{\circ}C \pm 0.51$) and VT differences before and after estrus ($0.52 \text{ }^{\circ}\text{C} \pm 0.21$) were significantly higher in summer than in autumn and winter (P < 0.05). Autumn estrus duration (6.88 $h \pm 1.72$) was significantly shorter than those in summer and winter (P < 0.05). All estrus VT values (38.92 °C \pm 0.44), VT differences before and after estrus (0.50 °C \pm 0.20), and estrus duration (9.32 h \pm 3.53) were significantly higher in the cows with natural estrus than in those with synchronized estrus (P < 0.05). The average activity increase during estrus in summer (4.4 ± 1.1) was significantly lower than that in autumn (8.1 ± 3.9) and winter ($8.7 \pm$ 3.4) (P < 0.05). Duration of estrus in summer (3.0 h ± 0.5) was significantly lower than that in autumn (5.8 h \pm 2.6) and winter $(6.0 h \pm 3.4)$ (P < 0.05); however, average activity increase

during estrus and duration of estrus did not differ between autumn and winter (P > 0.05) (Table 2).

Discussion

In the present study, VT in Holstein cows was automated and continuously measured every 30 min using the real-time devices. Compared with previous studies (Burfeind et al. 2014; Sakatani et al. 2016), the changes in Holstein VT during estrus were more accurately explored in our study, which will be beneficial for the precise management in dairy reproduction.

Recently, a study showed that the diestrus VT in Japanese Black cows was significantly higher mostly during the daytime in summer than in the cool season (Sakatani et al. 2012). However, our study showed that summer circadian rhythm significantly differed from those in autumn and winter. The diestrus VT in Holstein cows was also significantly higher in summer than in autumn and winter, and the diurnal variation was 0.28-0.70 °C. Although a similar tendency was reported earlier (Piccione et al. 2003; Nabenishi et al. 2011; Sakatani et al. 2012), Piccione et al. (2003) did not find a significant seasonal difference during the diestrus period. Nabenishi et al. (2011) reported a higher temperature in summer (mostly higher than 39.5 °C), and study showed that environmental effects could not be avoided (Miura et al. 2017). Compared with other studies (Piccione et al. 2003; Nabenishi et al. 2011; Sakatani et al. 2012), our study systematically revealed the variation of diestrus VT in Holstein dairy cows in summer, autumn, and winter on the basis of the greater density VT data, a longer detection time, and larger sample size.

Prostaglandin was administered for the synchronized estrus because it dissolved the corpus luteum and promoted follicle development (Brito et al. 2002). Studies have reported that cattle body temperature falls after PG injection (Suthar et al. 2012; Sakatani et al. 2016). In our present study, the VT also decreased after PG injection and earlier than Sakatani's experiment (Sakatani et al. 2016). It was also determined that using the average VT at the same time point 7 days before PG injection was a more reliable control than using the VT data

Table 1	VT characteristics of
cows in	estrus according to
season a	nd estrus type

		N	Estrus VT (°C)	VT difference (°C)	Duration (h)
Season	Summer	18	$39.13\pm0.51^{\rm a}$	0.52 ± 0.21^{a}	9.78 ± 4.80^a
	Autumn	17	38.68 ± 0.19^b	0.45 ± 0.17^b	6.88 ± 1.72^{b}
	Winter	16	38.71 ± 0.25^b	0.49 ± 0.20^b	$8.25\pm3.08^{\rm a}$
Estrus type	Nature	39	38.92 ± 0.44^a	0.50 ± 0.20^a	9.32 ± 3.53^a
	Synchronization	12	38.63 ± 0.19^{b}	0.41 ± 0.14^b	5.13 ± 1.30^{b}

Data shown are means \pm standard deviations (mean \pm SD); mean values within the same column with different superscript letters^(a-b) differ significantly (P < 0.05). N, number of cows used in the study; VT difference, the difference between estrus VT and average VT 7 days earlier

Table 2Average activity increaseof cows in estrus according toseason

	Ν	Average activity during estrus	Average activity increase	Duration of estrus (h)
Summer	10	82.7 ± 22.9^{b}	$4.4\pm1.1^{\rm b}$	3.0 ± 0.5^b
Autumn	10	118.6 ± 42.6^{ab}	8.1 ± 3.9^{a}	5.8 ± 2.6^a
Winter	11	$160.0 \pm 89.7^{\mathrm{a}}$	8.7 ± 3.4^a	6.0 ± 3.4^a

Data shown are means \pm standard deviations (mean \pm SD); mean values within the same column with different superscript letters^(a-b) differ significantly (P < 0.05). N, number of cows used in the study

from only 1 day before or after PG injection (Suthar et al. 2012; Sakatani et al. 2016).

Several studies have reported that bovine body temperature increased by 0.43-1.27 °C during estrus (Wrenn et al. 1958; Dolecheck et al. 2015; Miura et al. 2017). In the present study, bovine VT was continuously monitored every 30 min for 1 month. Vaginal temperature progressively increased from the onset of estrus reaching a maximum at 3.5 h, then decreased gradually starting from 4 h before the termination of estrus and remained at a normal level thereafter. Vaginal temperature is affected by environmental temperature, physiological state, and locomotor activity (Kendall and Webster 2009). Sakatani et al. (2012) and Sakatani et al. (2016) found that estrus VT in Japanese Black cows in the hot season was similar to that in the cool season. However, our results were different from those reported by Sakatani et al. (2016), possibly because synchronized estrus induced follicle development and ovulation by breaking the normal cycle via exogenous hormones, thereby causing shorter estrus periods and smaller increase in VT. In addition, average activity increase and duration of estrus in the present study were different from those reports (Sakatani et al. 2012, 2016). The different experimental cattle breeds, climates, and estrus prediction thresholds used may be the main causes for this difference. Therefore, in practical applications, attention should be paid to the effects of these factors on estrus detection.

For each estrus cow, both increase of body temperature and preovulatory follicle larger than 10 mm in diameter were detected, and active corpus luteum was not detected in all estrus cows. However, no false negative and false positive individuals were found in this study. Another experiment on large population should be designed to obtain the information of false positive and false negative individual in the future.

In conclusions, the VT and activity of cows were monitored using a new real-time device in this study. This technology measured alterations in VT and activity in response to environmental variations via a high reading density and a long measurement interval. Our results can help to evaluate the physiological state of the cow accurately and promote efficient reproduction management by combining activity and temperature parameters. It is hoped that the results and methodology presented here will improve the accuracy of the prediction of estrus and increase the efficacy of artificial insemination. This pilot study suggested a threshold of 0.3 °C increase that should be validated in further studies with a large number of animals and under different housing conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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