

The efficiency of vaginal temperature measurement for detection of estrus in Japanese Black cows

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Abstract. Recently, weak estrous behavior was assumed to be the cause of a decline in breeding efficiency in cattle. The present study investigated the effect of measuring the vaginal temperature on the detection of estrus in Japanese Black cows. First, the effect of hormone administration to cows with a functional corpus luteum on the vaginal temperature was evaluated by continuous measurement using a temperature data logger. After 24 h of cloprostenol (PG) treatment, the vaginal temperature was significantly lower than on day 7 after estrus, and the low values were maintained until the beginning of estrus ($P < 0.05$). The cows that received PG and exogenous progesterone (CIDR) did not show a temperature decrease until the CIDR was removed. This finding suggested that the vaginal temperature change reflected the progesterone concentration. The rate of detection of natural estrus was lower for a pedometer than for the vaginal temperature ($P < 0.05$); synchronization of estrus resulted in a high estrus detection rate regardless of the detection method. In a subsequent experiment, the effect of vaginal temperature measurement and the use of a pedometer on estrus detection was evaluated in the cool and hot seasons. The average activities during non-estrus and the activity increase ratio (estrus/non-estrus) changed according to season ($P < 0.01$, $P < 0.05$). However, the average vaginal temperatures during estrus and non-estrus were not affected by season. The estrus detection rate of the pedometer was lower in summer and lower than that obtained using the vaginal temperature. These results indicated that vaginal temperature measurement might be effective for detecting estrus regardless of estrous behavior.

Key words: Beef cattle, Estrus detection, Season, Vaginal temperature

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The low pregnancy rate of cattle in the hot season has been a major issue in many countries. A decrease in breeding efficiency in both dairy and beef cattle might be associated with a low pregnancy rate. The low breeding efficiency in the hot season is thought to be partly due to a low estrus detection rate.

Generally, detection of estrus has been based on the detection of standing heat. However, standing heat and the conception rate have been decreasing due to the increase in milk yield in dairy cows [1, 2]. The common occurrence of silent heat is a serious problem in the dairy industry. According to Roelofs *et al.* [3], only 42% of cases of standing heat were detected by visual observation (performed twice a day for 30 min each time), and some groups have shown a standing heat detection rate of only 19% in dairy cows. Environmental factors such as high ambient temperature and high temperature humidity index (THI) also affect the expression of estrus [4, 5]. Besides, the duration of standing heat has been shorter than the duration of increased activity during estrus in Japanese Black cows [6]. Therefore, the detection of estrus is essential for successful breeding of cattle.

To reduce labor and cost, the use of automated sensors is increasing

in the cattle industry [7]. Estrus detection methods such as detecting standing heat using sensors or detectors or determining the increase in activity using a pedometer have been commonly utilized [7–9]. However, these methods are not effective for detecting estrus in cows that undergo silent heat because they are based on estrous behavior. Additionally, even beef cattle considered to be of thermotolerant breeds have shown a lower increase in activity during estrus in summer than in winter [10]. It has been suggested that the expression of standing heat is affected by the hierarchy of the herd or by the feeding system [11]. Cows kept in tie-stalls sometimes do not show an increase in activity during estrus. Thus, estrus detection based on estrous behavior might have some limitations depending on the situation. Therefore, a combination of several detection methods is effective for precisely detecting estrus [5].

The measurement of body temperature has recently been the focus of new estrus detection methods. Vaginal temperature can be continuously monitored without restraint [12]. Therefore, studies that have measured vaginal temperature have been reported. Elevation of body temperature was observed on the day of estrus or during estrus [10, 13–17]. Furthermore, previous studies indicated that elevation of body temperature is not related to an increase in activity but instead is related to hormonal secretion [15, 18]. Fisher *et al.* [15] reported that transient temperature elevation during estrus was associated with the expression of a luteinizing hormone (LH) surge. Besides, it has been suggested that the temperature change during the estrous cycle or a decrease in body temperature before estrus might be associated with corpus luteum function in the ovaries [18]. Moreover, these

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body temperature changes during estrus were observed not only in free-stalled dairy cows but also in tie-stalled dairy cows [17].

Considering this evidence, it is possible that these body temperature changes may still occur in animals whose estrous behavior is decreased due to heat stress if ovarian function is normal. Estrus synchronization methods have been reported to be effective for the expression of estrus because the corpus luteum is forced to regress by administration of hormones [19–21]. Therefore, in the present study we performed three experiments using non-lactating Japanese Black cows. The first experiment evaluated the effect of hormone administration on the change in vaginal temperature. Then, the effect of hormone administration or the absence of it on estrus duration and the estrus detection rate was evaluated. Finally, the effect of heat stress on estrus expression and the estrus detection rate was evaluated by comparing use of a pedometer with vaginal temperature measurement to elucidate the efficiency of using body temperature for detection of estrus and improvement of summer breeding efficiency.

Materials and Methods

Animals

All animal treatments and procedures were approved by the Animal Care and Use Committee at Kyushu Okinawa Agricultural Research Center. Experiments were conducted from July to September in 2012 and 2013 (hot season), from November to June in 2012 and 2013 and from November in 2013 to January in 2014 (cool season). A total of 10 non-lactating Japanese Black cows were used for the experiments (35 to 84 months old, 400 to 550 kg). All cows exhibited regular signs of estrus (average 21 days), and no reproductive problems were observed prior to the experiments. The cows were kept in groups of five or six animals in a concrete-floored paddock without a roof. Cows were fed Bahía grass hay freely with water *ad libitum*.

Meteorological data

The meteorological data (maximum, minimum, average ambient temperatures and humidity) were obtained from our institutional weather data collection system. The THI was calculated using the following formula [22]:

$$\text{THI} = (0.8 \times \text{AT} + (\text{RH}/100) \times (\text{AT} - 14.4)) + 46.4,$$

where is the ambient temperature (°C) and RH is the relative humidity (%).

Measurement of vaginal temperature and walking activity

Measurement of vaginal temperature and walking activity was performed as described in a previous report [10]. Briefly, a temperature data logger (Thermoclone type SL, NS Laboratories, Osaka, Japan) was utilized for measuring vaginal temperature. The temperature data logger was attached to a CIDR containing no progesterone (blank-CIDR, Zoetis Japan, Tokyo, Japan) and inserted into the vagina with an applicator. The vaginal temperature was measured every hour without restraint. After the experiment, the data logger was removed from the vagina, and the temperature data were collected with the Rh-Manager software (NS Laboratories).

Walking activity was measured with a pedometer (Gyuhosystem, Comtec, Miyazaki, Japan). The pedometer was worn on the foreleg and attached with a belt. The walking activity was sent wirelessly

to a computer every hour and analyzed with the Gyuhosystem software (Comtec). The software calculated the two-week average for the pedometer data. If the number of steps was 1.5-fold higher than the average and the increased level continued for at least 3 h, the cow was automatically considered to be in estrus.

Estrus synchronization

Two different estrus synchronization methods were utilized. The presence of a functional corpus luteum was confirmed by rectal palpation and ultrasound diagnostics (HS-2000, Honda Denshi, Aichi, Japan) on Days 7 to 14 after estrus (Day 0 = the day of estrus). Then, 0.5 mg of cloprostenol (PG, Estrumate, MSD, Tokyo, Japan) was injected (intramuscularly, i.m.) into the first group, which was labeled the PG group. In a second group, 0.5 mg of cloprostenol was injected (i.m.), and an Eazi-Breed CIDR (including progesterone [P4], Zoetis Japan, Tokyo, Japan) was inserted. The CIDR was removed 6 days (144 h) after the PG treatment. In both treatments, a temperature data logger attached to a blank-CIDR was inserted into the vagina at the time of PG treatment. Vaginal temperature data were recorded every hour until the second or third estrus.

Estrus detection

Estrus expression was determined by the observation of behavior, the presence of standing heat and the change in color of a Heatmount Detector (Kamar, Plain City, OH, USA). On Day 7, the presence of a corpus luteum was confirmed by rectal palpation and ultrasonic diagnostics.

To calculate the estrus duration, the beginning of estrus was determined using the pedometer as the time at which the number of steps (/h) became 1.5-fold higher than the monthly average (steps/h) and remained at this higher value for more than 3 h [6, 10]. The end of estrus was defined as the time when the steps/h returned to less than 1.5 times the monthly average level. To determine the estrus detection rate, the estrus detection algorithm in the Gyuhosystem pedometer's software was utilized. Cases in which estrus was automatically detected by the system were classified as successful estrus detection.

The vaginal temperature was analyzed as described in a previous report [10]. The beginning of estrus was judged to be when the vaginal temperature was 0.3°C higher than it had been at the same time the previous day and the higher temperature continued for more than 3 h. The end of estrus was judged to be when the vaginal temperature was less than 0.3°C above the temperature before estrus.

Experimental design

Experiments 1, 2 and 3 included some of the same data for analysis.

Experiment 1. The effect of hormone administration on vaginal temperature: Fourteen estrous cycles in 8 cows were treated with PG only (PG) in the morning (10 estruses) or the afternoon (4 estruses). Also, 6 estrous cycles in 3 cows were treated with PG and a CIDR (PG + CIDR). The vaginal temperature measurement device was inserted at the same time as the hormonal treatment, and data were collected every hour. The temperature data from 1 to 44 h after PG treatment and at the same clock time on Day 7 were compared to elucidate the temperature change induced by PG, which regressed the corpus luteum. The temperature data from every hour after PG + CIDR treatment were analyzed to determine the effect of

forced regression of the corpus luteum and exogenous progesterone administration on the vaginal temperature.

Experiment 2. The effect of estrus synchronization methods on estrus expression and detection rate: Twenty-eight natural estrous cycles in 6 cows, 14 PG-synchronized estrous cycles in 6 cows and 6 PG + CIDR-synchronized estrous cycles in 3 cows were analyzed. The vaginal temperature differences during estrus (compared Day -1 or Day 1) were calculated in each treatment. The estrus duration time and estrus detection rates were calculated using two different estrus detection systems, namely a pedometer and vaginal temperature.

Experiment 3. The effect of heat stress on estrus expression and detection rate as determined by different methods: Twenty-three estrous cycles in the cool season and 24 estrous cycles in the hot season in 6 cows under various treatments (natural, PG and PG + CIDR) were used for the analysis. The data included both vaginal temperature and walking activity, and the estrus duration and estrus detection rate were calculated. In addition, the average walking activity (steps/h) during estrus or non-estrus, activity increase ratio (estrus/non-estrus) and walking activity during daytime (0800 h to 1900 h) or night-time (2000 h to 0700 h) were calculated in each season. The average vaginal temperature during estrus or non-estrus and the temperature difference between estrus and non-estrus were calculated in each season.

Statistical analysis

In experiment 1, the vaginal temperature changes caused by the PG treatment were analyzed by Student's *t*-test (StatView program; Abacus Concepts, Berkeley, CA, USA). The vaginal temperature under the PG + CIDR treatment was compared between days using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test (StatView program).

In experiments 2 and 3, the estrus detection rate was analyzed by the chi-square test and Fisher's exact test (StatView). The estrus duration was compared between seasons, detection methods and synchronization methods by Student's *t*-test (StatView). Average walking activity, activity increase ratio, average vaginal temperature and temperature differences between estrus and non-estrus were compared between seasons by Student's *t*-test (StatView). The temperature differences during estrus in experiment 2 were analyzed by treatment, time, season and each interactions with the SAS GLM procedure (SAS 9.4, SAS Institute Japan, Tokyo, Japan).

Results

Experiment 1

The vaginal temperature changes induced by PG treatment and the temperatures at the same clock time on Day 7 are shown in Fig. 1. One of the cows exhibited estrus 46 h after PG treatment, and the vaginal temperature transiently increased due to estrus. Therefore, data recorded up to 44 h after the PG treatment are shown. From 24 to 44 h after the PG treatment, the vaginal temperature was significantly lower than that on Day 7. From 21 to 35 h after treatment, the vaginal temperature was 0.15 to 0.30°C lower than that on Day 7. Furthermore, the vaginal temperature at 27 to 37 h after PG treatment was significantly lower compared with the temperature recorded at the same time on the previous day (at 3 to 13 h after PG

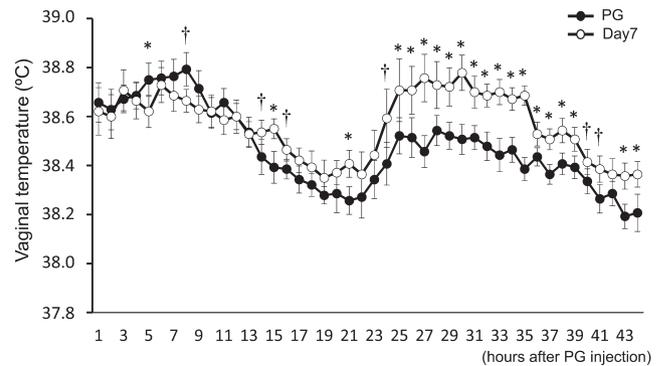


Fig. 1. Changes in the vaginal temperatures following PG treatment. Vaginal temperature was measured every hour after PG administration (black marker) or at the same time on 7 days after estrus (open marker). Fourteen replicates (7 in summer, 7 in spring or autumn) of 8 cows are shown as averages \pm SEM. PG administrations were conducted in the AM (10 replicates) or PM (4 replicates). * Significant difference between PG and day 7 ($P < 0.05$), † $P < 0.1$ between PG and Day 7.

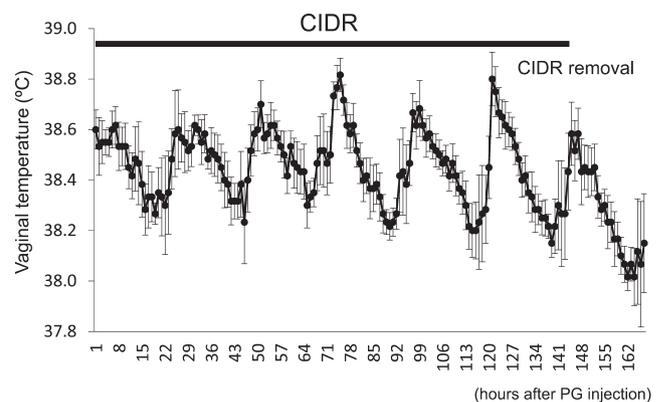


Fig. 2. Changes in vaginal temperatures following PG and exogenous P4 treatment. Vaginal temperature was measured every hour after PG and CIDR administration. The CIDR was placed in the vagina for 6 days (black bar). At 6 days after CIDR administration, the CIDR was removed from the vagina (grey bar: CIDR removal). Six replicates (2 in spring, 4 in summer) of 3 cows are shown as averages \pm SEM. All hormone treatments were performed in the morning (0900 to 1000 h).

treatment) ($P < 0.05$). All cows started estrus from 46 to 94 h after PG treatment, and their vaginal temperatures increased during estrus.

The vaginal temperature changes induced by PG + CIDR administration are shown in Fig. 2. The vaginal temperature decreased after CIDR removal ($P < 0.05$), and no differences were observed while the CIDR was inserted. Also, in contrast with treatment with PG alone, no significant differences were observed in vaginal temperature at 1 and 24 h after the PG + CIDR treatment was initiated. No cows started estrus until CIDR removal. All cows started estrus from 38 to 118 h after CIDR removal, and their vaginal temperatures increased during estrus.

Experiment 2

The temperature differences between Day 0 and Day -1 (from -4 to 12 h after estrus beginning) and between Day 0 and Day 1 (from -6 to 12 h after estrus ending) are shown in Figs. 3A and B. The vaginal temperatures during estrus were consistently higher than those on Day -1 and 1 ($P < 0.0001$). Also, there were significant differences by treatment, time and treatment-season interaction (Fig. 3A, $P < 0.0001$). PG- and PG + CIDR-treated cows showed higher vaginal temperatures than natural estrus cows during estrus (Fig. 3A). However, the temperature differences in the vicinity of the end of estrus were affected by time (Fig. 3B).

The effect of estrus synchronization on estrus duration and estrus detection rate is shown in Table 1. Neither the synchronization method nor the estrus detection method affected the estrus duration. The estrus detection rate in natural estrus was lower when determined by the pedometer than by the vaginal temperature ($P < 0.05$). The estrus detection rates using the pedometer were increased by estrus synchronizations. However, the estrus detection rates using the vaginal temperature were stably high and not affected by synchronization.

Experiment 3

The ambient temperature and THI in the cool and hot seasons were 11.3°C and 27.2°C and 53.4 and 77.9, respectively. The estrus duration in the cool and hot seasons were 17.3 ± 0.9 h and 15.2 ± 0.5 h as measured by the pedometer and 16.5 ± 0.6 h and 15.6 ± 1.4 h as measured by the vaginal temperature, respectively. The estrus duration detected by the pedometer tended to be shorter in the hot season compared with that in the cool season ($P < 0.08$). However, there was no difference in the estrus duration detected using the vaginal temperature. No difference in the estrus duration was observed between the detection methods.

Table 2 shows the average walking activities (steps/h), activity increase ratios, average vaginal temperatures during estrus and during non-estrus and temperature differences between estrus and non-estrus in each season. The average walking activities (steps/h)

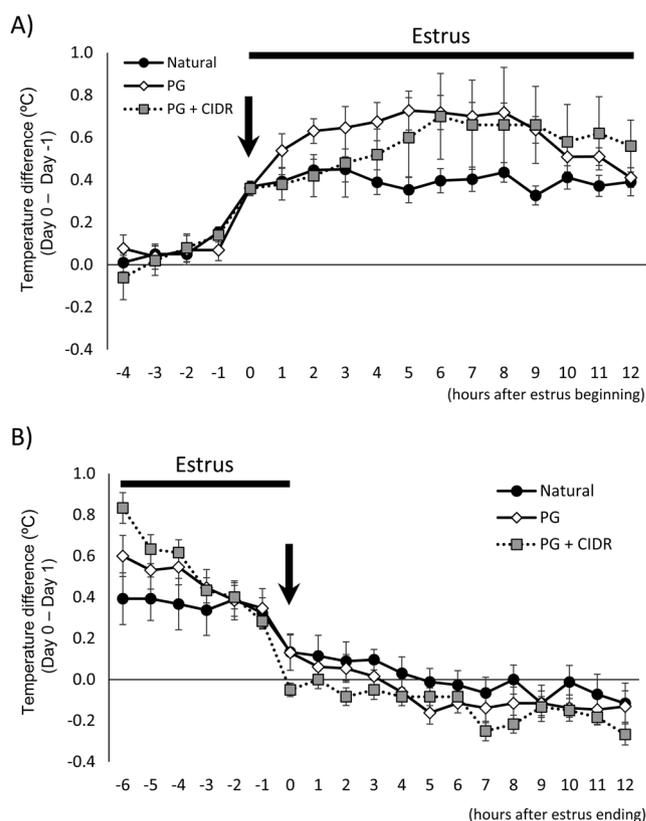


Fig. 3. Temperature differences in the vicinity of the beginning or end of estrus. The temperature differences between Day 0 (day of estrus) and Day -1 from -4 to 12 h after the estrus beginning (A) and between Day 0 and Day 1 from -6 to 12 h after the estrus ending (B) are shown averages \pm SEM (circle, natural; open marker, PG; square, PG + CIDR). An arrow indicates the beginning or end of estrus, and the black bar above the graph indicates estrus. There are significant differences by treatment, time and treatment-season interaction in A ($P < 0.0001$) and by time in B ($P < 0.0001$).

Table 1. Estrus duration and estrus detection rate by the different methods

	Estrus duration (h)		Estrus detection rate (%)		N (cows)
	Pedometer	Vaginal temperature	Pedometer	Vaginal temperature	
Natural	16.8 \pm 0.9	16.3 \pm 0.9	75.0 ^a	96.4 ^b	28 (6)
PG only	14.1 \pm 1.2	14.1 \pm 1.5	85.7	92.9	14 (6)
PG + CIDR	17.0 \pm 2.6	19.2 \pm 2.9	100.0	100.0	6 (3)

^{a-b} $P < 0.05$ between the pedometer and vaginal temperature methods by chi-square analysis. Data for two estruses induced by the PG + CIDR method and detected by the pedometer were removed because the pedometer came off during estrus.

Table 2. Vaginal temperature and steps during estrus or non-estrus in the cool and hot seasons

	Pedometer			Vaginal temperature (C)			N (cows)
	Estrus (steps/h)	Non-estrus (steps/h)	Estrus/non-estrus	Estrus	Non-estrus	Temp differences	
Cool	334.1	101.2 ^a	3.55 ^a	38.80 \pm 0.07	38.47 \pm 0.04	0.33 \pm 0.03	23 (6)
Hot	363.3	157.8 ^b	2.33 ^c	38.73 \pm 0.09	38.43 \pm 0.05	0.30 \pm 0.06	24 (6)

^{a-b} $P < 0.01$ between seasons by Student's *t*-test. ^{a-c} $P < 0.05$ between seasons by Student's *t*-test.

Table 3. Effect of season on estrus detection rates of different estrus detection methods

	Cool		Hot		Total	
	Pedometer	Vaginal temp	Pedometer	Vaginal temp	Pedometer	Vaginal temp
Detected	18	23	15	22	33	45
Not detected	4	0	8	2	12	2
Detection rate (%)	81.8 ^a	100 ^b	65.2 ^a	91.7 ^b	73.3 ^a	95.7 ^c

^{a-b} P < 0.05 between detection methods by chi-square test. ^{a-c} P < 0.01 between detection methods by chi-square test. There was no significant difference between the cool and hot seasons using the same detection method. Data for one estrus each in the cool and hot seasons detected by the pedometer were removed because the pedometer came off during estrus.

Table 4. Activities during daytime and nighttime in the cool and hot seasons

	Daytime ¹⁾ (steps/h)	Nighttime ²⁾ (steps/h)	Number of cows
Cool	147.7 ± 7.8 ^a	57.1 ± 5.6	6
Hot	260.5 ± 27.3 ^b	62.0 ± 7.8	6

¹⁾ Average steps (/h) during 0800 h to 1900 h. ²⁾ Average steps (/h) during 2000 h to 0700 h. ^{a-b} P < 0.05 between seasons by Student *t*-test.

during estrus were not different between seasons. However, the levels of activity during non-estrus in the hot season were higher than those in the cool season ($P < 0.01$), and the activity increasing ratio (estrus/non-estrus) was significantly lower in the hot season ($P < 0.05$). By contrast, none of the average vaginal temperatures during estrus and non-estrus or the temperature differences were different between seasons. Also, the vaginal temperature differences between Day 0 and Day -1 or 1 were not affected by season. The number of times estrus started during the daytime and the number of times it started during nighttime were not different between seasons (12 daytime vs. 11 nighttime estruses in the cool season, 13 daytime vs. 11 nighttime estruses in the hot season).

Estrus detection rates determined using the pedometer were lower compared with those determined using the vaginal temperature ($P < 0.05$ in the cool or hot season, $P < 0.01$ in total, Table 3). However, the estrus detection rates determined using the same detection method were not different between the seasons (Table 3).

The average walking activities (steps/h) during the daytime and nighttime in all cows are shown in Table 4. Basal walking activities (activities on non-estrus days) and daytime activities were high and varied enormously in the hot season compared with those in the cool season ($P < 0.05$). However, the average activities during the nighttime were not different between the seasons.

Discussion

A decrease in standing heat is a serious issue because it decreases the success of artificial breeding (artificial insemination [AI] or embryo transfer [ET]) and results in a low conception rate in the dairy industry [2]. Factors such as individual differences between cows [23], housing [24–27], herd size [11] and seasons [7, 10, 21, 28] also affect the expression of estrous behavior.

It has been suggested that there is a correlation between the

progesterone concentration secreted from the corpus luteum and body temperature in humans, rodents and cattle [13, 18, 29]. Recently, continuous measurement of body temperature revealed that body temperature is associated with changes in PG or progesterone concentration and prepartum body temperature in cattle [30, 31]. Pregnant cows showed higher vaginal temperatures due to the secretion of progesterone by the corpus luteum than nonpregnant cows [18].

A previous study showed that the vaginal temperature changed with the induction of estrus by PG administration in dairy cows [18]. In the present study, PG treatment in the presence of a functional corpus luteum on Days 7 to 14 revealed that vaginal temperatures were significantly lower from 24 h after PG treatment to the beginning of estrus than on Day 7 when a functional corpus luteum was present. Vaginal temperatures after 24 h of PG treatment were significantly lower than those on the day of PG treatment. According to Ginther and Beg [32], the length of luteolysis is 24 h in heifers, and the progesterone concentration decreases steadily during luteolysis. Besides, Levy *et al.* [33] revealed that PG administration in the presence of a functional corpus luteum decreases the progesterone concentration promptly and that the progesterone concentration reaches its minimum from 14 to 24 h after PG administration. We did not measure the blood progesterone concentration. However, the existence of a functional corpus luteum was confirmed by rectal palpation and by ultrasonic diagnosis before PG administration, and all cows treated with PG showed estrus. Thus, our results indicated that the vaginal temperature decrease after the PG treatment would be linked to functional regression of the corpus luteum as the progesterone concentration decreases.

On the other hand, vaginal temperatures remained high from the day of hormone administration to CIDR removal in PG + CIDR-treated cows. The vaginal temperatures were significantly lower at 24 h after CIDR removal (168 h) compared with those measured on the day of PG + CIDR treatment. Besides, no cows expressed estrus until CIDR removal. It was reported that PG + CIDR-treated cows showed a decreased blood progesterone concentration, but the concentration was still higher than those of cows not treated with a CIDR [31]. Administration of exogenous progesterone (CIDR) was reported to be effective for preventing the LH surge and ovulation [19, 31]. Vaginal temperatures did not drop as in the cows treated with only PG; they were maintained by the exogenous progesterone until CIDR removal in the present study. This finding suggested that the slight decrease in vaginal temperature the day after PG administration could be the result of a reduction in the progesterone concentration due to corpus luteum regression. To examine this hypothesis, a

further experiment evaluating the relationship between the plasma progesterone concentration and the vaginal temperature changes during hormone administration is needed.

In experiment 2, a comparison of natural estrus and estrus synchronization methods was performed. No difference in estrus duration was present between natural estrus and estrus synchronization. Synchronized cows showed high estrus detection rates, more than 80%, using either the pedometer or vaginal temperature methods. However, the estrus detection rate obtained using the pedometer was significantly lower than that obtained using the vaginal temperature in natural estrus. In addition, as shown in Fig. 3, the temperature differences during estrus were higher in PG and PG + CIDR cows than natural estrus cows. It has been reported that silent-heat cows showed slower corpus luteum regression than normal-estrus cows [28]. Silent-estrus dairy cows treated with PG showed clear estrus [34, 35], but sometimes treatment with PG is not sufficient to induce estrus and ovulation [36]. In addition, treatment with exogenous progesterone leads to a greater release of LH pulses and results in enhanced secretion of estradiol-17 β ; cows receiving this treatment would express definitive estrus behavior [19–21, 37, 38]. Thus, some natural-estrus cows showed prolonged corpus luteum regression and lower levels of estradiol-17 β ; therefore, estrus expression would become weak, and detection using a pedometer might be difficult. On the other hand, the corpus luteum of the synchronized cows regressed promptly, and the PG + CIDR treatment induced estradiol-17 β secretion; therefore, cows receiving this treatment showed clear estrus behavior as previously reported [19–21]. Our temperature difference results in Fig. 3 agreed with these reports. Despite this, the vaginal temperature showed a high detection rate for natural estrus. Thus, it is thought that the change in vaginal temperature was less affected by the corpus luteum regression process than the changes in activity, and the estrus detection rate using the vaginal temperature was high.

Although beef cattle are considered to be a thermotolerant breed, they show lower standing heat expression and activities during estrus in summer [7, 10, 28, 39]. The pedometer is a useful aid for estimating estrus, ovulation and the time of artificial insemination, because walking activity is increased at the start of estrus [8]. Also, body temperature is considered to be an efficient index for detecting estrus because it is less affected by the animals' housing system (tie-stall, free-stall and grazing) and herd size [17]. It was reported that approximately 80% of incidences of estrus were detected using a pedometer [40] and body temperature [41]. In the present study, the average ambient temperature and THI in the hot season were more than 27°C and 77, respectively. This indicated that the cows suffered medium heat stress during the hot season. The calculated estrus duration did not differ between the detection methods. This indicated that both the pedometer and vaginal temperature are effective for estimating the start and end of estrus. However, interestingly, the estrus detection rate differs between the detection methods. Vaginal temperature measurement showed a high detection rate in all seasons (> 90%), whereas the pedometer method showed a lower rate. The estrus detection rate obtained using the pedometer was only 65.2% in the hot season. It has been reported that estrous activities in dairy cows were decreased by a high ambient temperature and high relative humidity [7, 21, 42]. As shown in Table 2, the activities during estrus

were not different between seasons, but the activities of non-estrus were significantly higher in the hot season ($P < 0.05$). Furthermore, the activity increase rates (estrus/non-estrus) were lower in the hot season. A previous study reported that the basal activity was high in the summer and that the increase in activity on estrus days was difficult to detect [10]. In the present study, the daytime activity in the hot season was significantly higher than that in the cool season, but no difference was observed in the nighttime activities. Furthermore, the number of times estrus started during the daytime and the number of times it started during the nighttime did not differ by season. This result indicated that the increased activity during the daytime and non-estrus time in the hot season would increase in the presence of ectozoon-like flies. Thus, it is likely that the accuracy of estrus detection using the pedometer depended on the threshold number of steps used to signify a meaningful increase in activity. Also, this result suggested that a different threshold is needed for the pedometer in the hot season in order to accurately detect estrus. In contrast, measurement of the vaginal temperature showed high estrus detection rates in both seasons, and the average temperature did not differ between seasons. In addition, the temperature differences during estrus were not affected by season. Thus, it is likely that the vaginal temperature changes during estrus cycles might not be affected by walking activity. The present study suggests that measurement of the vaginal temperature is the superior method for detection of estrus in Japanese beef cows in the hot season.

There are very few reports about the conception rate associated with cows that exhibit silent heat. However, it was reported that ovulation, corpus luteum growth and the progesterone concentration in silent-heat cows were not different compared with normal-estrus cows among Japanese Black cows [28]. This suggests that artificial insemination could be performed at optimum timing and result in pregnancy even in silent-heat cows. Thus, the cows with estrus detected by vaginal temperature only might show normal ovulation and corpus luteum growth because it is likely that the vaginal temperature changes during the estrous cycle reflect the progesterone concentration and LH surge.

In conclusion, our results indicated that the vaginal temperature changes observed during estrus cycles, especially the temperature decrease before estrus, reflect changes in progesterone concentration. Estrus detection using body temperature has the potential to be superior to methods based on the activity of cows (i.e., the use of a pedometer) in terms of reflecting ovarian function. It seems that pedometer detection would not be effective in the hot season. However, detection might be improved if an appropriate threshold is set. Our results suggest that estrus detection using body temperature, especially measurement of the vaginal temperature, could be effective throughout the year.

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