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Original Article

Cervix–rectum temperature differential at the time of insemination is correlated with the potential for pregnancy in dairy cows

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Abstract. This study sought to establish whether temperature gradients between the cervix, vagina, and rectum at and 7 days post-artificial insemination (AI) were associated with the incidence of pregnancy in lactating dairy cows (Experiment I; n = 90 ovulating cows) and to evaluate temperature gradient dynamics from the time of insemination to 7 days post-AI under heat stress conditions (Experiment II; n = 16 ovulating and 4 non-ovulating cows). In Experiment I, 39 cows (43.3%) became pregnant. The odds ratio for pregnancy was 2.5 for each one-tenth of a degree drop in cervical temperature with reference to the control rectal temperature at the time of AI (P = 0.01), whereas the same decrease in the cervix–rectum temperature differential 7 days post-AI resulted in an odds ratio of 0.44 (P = 0.02). In Experiment II, 5 of the ovulating cows (31.3%) became pregnant. The mean values of the vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials at AI (day 0), 8 h, 24 h, and 7 days post-AI changed significantly from day 0 to day 7 (within-subject effect; P < 0.02) in ovulating cows but not in non-ovulating cows. Temperature differentials on days 0 and 7 were similar between ovulating cows and cows of Experiment I. Overall, our findings support the notion that a temperature differential between the caudal cervical canal and rectum at AI may be an indicator of the likelihood of pregnancy. Possible prospects of confirming estrus at the herd-level are also suggested.
Key words: Artificial insemination, Body temperature differentials, Delayed ovulation, Low cervical temperature, Ovulation failure

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The female genital organs experience a decrease in temperature before ovulation to facilitate male and female gamete maturation [1–3]. There is substantial evidence indicating that pre-ovulatory follicles are ≤ 1.0°C cooler than ovarian stroma in eutherian mammals, and both compartments have a lower temperature compared to neighboring tissues [1, 3, 4]. The oviduct is the closest to the ovary. In the caudal region of the oviduct isthmus, the pre-ovulatory functional sperm reservoir is 1–2ºC cooler than the cranial end of the oviduct ampulla [1, 4]. This pre-ovulatory cooling of Graafian follicles and the caudal oviduct is consistent with the results from other studies [1–4]. The continuous recording of ovarian parenchyma temperature for > 18 days in beef cows revealed that the ovaries require a lower temperature than other organs to maintain their functions [5]. In lactating dairy cows, a relationship was established between the extent of cooling of pre-ovulatory follicles and the occurrence of pregnancy, whereas pre-ovulatory follicles that did not undergo cooling failed to ovulate [6–8]. This evidence regarding the ovaries of cows indicates that intra-follicular temperature regulates ovulation in mammals [9].

As high environmental temperatures correlate with rectal and body temperatures [10, 11], heat stress (HS) will likely compromise the cooling of the reproductive system in cows [12, 13]. Temperature gradients across reproductive tissues are primarily regulated by changes in steroid hormones secreted by the ovaries; the pre-ovulatory follicle is particularly sensitive to thermal stress [13]. For example, cows showing estrous signs exposed for a brief period (< 45 min) to solar radiation just after sunrise (dawn) failed to ovulate, whereas their counterparts in the shade ovulated [6]. In addition, the quality of the oocyte is greatly affected by HS conditions [12, 14, 15]. It is likely that a damaged follicle-enclosed oocyte is responsible for follicular cooling failure and subsequent ovulation failure. The role of mammalian oocytes in promoting cooling in responsive Graafian follicles has been recently hypothesized [16].

While temperature changes within the ovary and oviduct have been a topic of intense research, to the best of our knowledge, temperature gradients across the caudal genital tract have been examined only in a single study [17]. In beef cows, temperature increases steadily from the vagina to the uterine horns and is higher during the luteal phase compared to the follicular phase [17]. As copious secretion and flow of the uterine mucous through the tubular genital organs may favor local cooling during estrus [13], we hypothesized that the temperature differential between the caudal cervical canal and rectum during artificial insemination (AI) could be an indicator of pregnancy. The present study was, therefore, designed to determine whether temperature differences between the cervix, vagina, and rectum at AI and at 7 days post-AI could be related to the incidence of pregnancy in lactating dairy cows (Experiment I) and to evaluate dynamics of temperature gradients between the cervix, vagina, and

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rectum from the time of insemination to 7 days post-AI under HS conditions (Experiment II).

Materials and Methods

Experimental animals

This study was performed from December 2018 to January 2020 on a commercial dairy herd of Holstein-Friesian lactating dairy cows in north-eastern Spain (41.13 latitude, 0.24 longitude). During the study period, the mean number of lactating cows in the herd was 225, and the mean annual milk production was 9,440 kg per cow. The cows were milked twice daily and fed with complete rations. Participants were healthy cows with a 2.5–3.5 body condition score on a scale of 1 to 5 [18] and producing > 30 kg of milk per day. During the study period (days –7–28 of insemination), they were free of detectable reproductive disorders and clinical diseases. Cows were selected from groups synchronized for fixed-time AI that were treated using a controlled intravaginal progesterone (P4)-releasing device (CIDR, containing 1.38 g of progesterone; Zoetis Spain SL, Alcobendas, Madrid, Spain). The CIDR was left in place for 5 days, and these animals were also administered cloprostenol (500 µg i.m.; PGF Veyx Forte, Ecuphar, Barcelona, Spain) for CIDR removal. Twenty-four hours later, the cows received a second dose of cloprostenol, followed by insemination and a gonadotropin releasing hormone (GnRH) agonist dose (dephereline: 100 µg gonadorelin acetate [6-D-Phe] i.m.; Gonavet Veyx, Ecuphar, Barcelona, Spain) administered 36 h after the second dose of cloprostenol [19]. A cow was confirmed to be in estrus and ready for insemination by both ultrasonography using a portable B-mode ultrasound scanner (E.I. Medical IBEX LITE; E.I. Medical Imaging, Loveland CO, USA) equipped with a linear 5–10 MHz transducer for transrectal use and manual rectal palpation. As the sequence until ovulation of the largest ovarian follicle goes through palpation during the spontaneous (non-synchronized) estrus and is a firm/soft follicle (young pre-ovulatory follicle), followed by a very soft follicle (mature pre-ovulatory follicle), and evacuated follicle [20], only cows with a mature (soft) pre-ovulatory follicle > 12 mm in diameter in the absence of a corpus luteum were included. Further prerequisites were a very tonic uterus contractile to touch and a vaginal discharge of copious clear fluid. The selected study population consisted of 120 cows artificially inseminated by the same technician using semen from two bulls of proven fertility. At the time of AI, mean milk production, number of lactations, and mean pre-ovulatory follicular diameter were 38.6 ± 7.4 kg (20–52 kg), 2.6 ± 1.6 lactations (1–7 lactations), and 18.4 ± 2.4 mm (range 12–29 mm), respectively (mean ± standard deviation [SD], ranges between parentheses). All gynecological examinations and pregnancy diagnoses were performed by the same surgeon. All procedures were approved by the Ethics Committee on Animal Experimentation of the University of Lleida (license number CEEA.06-01/12).

Temperature measurements

Ventral vaginal fornix, cervical canal, and rectal temperatures were measured using a Crison thermometer (Crison thermometer 638 Pt, Alella, Barcelona, Spain) with a 2 mm × 220 mm platinum probe (Fig. 1). The thermometer resolution was 0.1ºC from –99 to 200ºC, with a reproducibility of ± 0.1ºC, and adjusted by the manufacturer to the deviation factor of the probe. Immediately before the temperature measurements, the vulva and perineal region of the cows were washed with 0.05% iodine solution (Betadine, ASTA Médica, Coslada, Madrid, Spain). The thermometer probe was positioned and pre-warmed for 60 sec in the ventral vaginal fornix and maintained for 20 sec for reading.

Fig. 1. The thermometer with a platinum probe used for measuring temperatures in the ventral vaginal fornix, 2–3 cm within the cervical canal, and 20 cm within the rectum.

Immediately after reading, the thermometer probe was carefully removed from the fornix and introduced again to a depth 2–3 cm for 40 sec to take a reading in the cervical canal. Finally, the probe was introduced 20 cm into the rectum for 60 sec for rectal temperature reading. No signs of discomfort were observed during temperature measurements. Cows were artificially inseminated immediately after the temperature measurements were performed at 0800 h.

Experiment I: Significance of temperature gradients between the cervix, vagina, and rectum at insemination and seven days post-insemination

This experiment was designed to determine whether temperature gradients between the cervix, vagina, and rectum at AI and at 7 days post-AI correlated with the incidence of pregnancy in lactating dairy cows. The study population comprised 100 cows. By ultrasonography, ovulation was recorded as the presence of a corpus luteum assessed at 7 days post-AI, and pregnancy diagnosis was performed 28 days post-AI. In our geographical area in northeastern Spain, a clear negative effect of HS on the reproductive performance of lactating dairy cows has been extensively described [21, 22]. Ovulation failure was found to increase dramatically under HS conditions [6, 23]. The maximum temperature–humidity index (THI) on the day of AI was used to examine the effects of HS, defined as a THI > 72 [24], on the likelihood of subsequent pregnancy.

Data collection and statistical analyses

The following data were recorded for each animal: parturition and AI dates; lactation number (parity: primiparous *vs*. pluriparous); HS (THI > 72), milk production at AI (low producers < 37 kg *vs*. high producers ≥ 37 kg); follicular size at AI; vaginal, cervical, and rectal temperatures and their corresponding differentials at AI and 7 days post-AI; semen-providing bull; and pregnancy rate 28 days post-AI. The threshold for milk production was derived from the median value of the production of primiparous cows. Differences in temperature between the vagina–rectum, vagina–cervix, and cervix–rectum and their corresponding differentials between pregnant and non-pregnant cows were assessed by a one-way analysis of variance (ANOVA) in the SPSS software package (version 18.0; SPSS Inc., Chicago, IL, USA) (means ± SD). A binary logistic regression analysis was performed using pregnancy as the dependent variable. The factors entered in the model as independent dichotomous variables (where 1 denoted presence and 0 denoted absence) were HS, milk production, and parity. The semen-providing bull was considered as a class variable, and follicular size (mm) at AI, vaginal, cervical, and rectal temperatures (ºC) and their corresponding differentials at AI and 7 days post-AI (continuous variables) were considered as factors in the analyses. Estimates and Wald 95% limits were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). A regression analysis was conducted according to the method of Hosmer and Lemeshow [25] using the logistic procedure of PASW Statistics for Windows Version 18.0 (SPSS Inc., Chicago, IL, USA). Significance was set at P < 0.05. Values are expressed as mean ± SD.

Experiment II: Dynamics of temperature gradients between the cervix, vagina, and rectum from the time of insemination to seven days post-insemination under heat stress conditions

Temperature gradient dynamics in the cervix, vagina, and rectum from the time of insemination to 7 days post-AI under HS conditions were evaluated in this experiment. Data were recorded in August 2019 and were derived from 20 cows: 16 ovulating and 4 non-ovulating cows. Gynecological ultrasound examinations and vaginal, cervical, and rectal temperature measurements at the time of AI and 8 h, 24 h, and 7 days post-AI were performed for all cows. As noted above, because ovulation failure has been found to increase dramatically under HS conditions in our working area [6, 23], this experiment was performed with a THI > 72 at AI to increase the chances of ovulation failure in a pre-ovulatory follicle.

Data collection and statistical analyses

The data recorded for each animal were the same as those for Experiment I plus ovarian structures and temperature measurements at 8 h and 24 h post-AI. Differences between the vagina–rectum, vagina–cervix, and cervix–rectum temperatures and their corresponding differentials for each time point were assessed by a one-way ANOVA. Temperature differential changes over time were evaluated by generalized linear model (GLM) repeated measures ANOVA using the SPSS software package (version 17.0; SPSS Inc., Chicago, IL, USA).

Results

Experiment I: Significance of temperature gradients between the cervix, vagina, and rectum at insemination and seven days post-insemination

Of the 100 cows selected as the primary study population for this experiment, 3 had 2 corpora lutea 7 days post-AI, and 7 further cows failed to ovulate. These 10 cows were excluded from the experiment. The final study population consisted of 90 ovulating cows.

Of the 90 cows included in this experiment, 35 (38.9%) were inseminated under HS conditions, 56 (62.2%) were high producers, 28 (31.1%) were primiparous cows, and 39 (43.3%) became pregnant. In Table 1, vaginal, cervical, and rectal temperatures and vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials at AI and 7 days post-AI are presented for pregnant and non-pregnant cows. Vaginal temperatures at AI and 7 days post-AI were 0.52–0.77% lower (P = 0.0001) than cervical and rectal temperatures for all cows. Cervix–rectum temperature differentials at AI were larger in pregnant cows than in non-pregnant cows (P = 0.0004), whereas cervix–rectum differentials 7 days post-AI were larger in non-pregnant cows than in pregnant cows (P = 0.001). No significant differences were detected in vagina–rectum and vagina–cervix temperature differentials between pregnant and non-pregnant cows at AI or at 7 days post-AI.

Table 2 summarizes the pregnancy rate, OR, and 95% CIs for all cows. The OR for pregnancy was 2.5, for each one-tenth of a degree drop in cervical temperature with reference to control rectal temperature at the time of AI (P = 0.01), whereas the same decrease in the cervix–rectum temperature differential at 7 days post-AI resulted in an OR of 0.44 (P = 0.02). Parity, HS, milk production, semen-providing bull, follicular size, rectal, cervical, and vaginal temperatures, and vagina–cervix and vagina–rectum temperature differentials at AI and 7 days post-AI had no significant effect, as indicated by the binary logistic regression results.

Experiment II: Dynamics of temperature gradients between the cervix, vagina, and rectum from the time of insemination to seven days post-insemination under heat stress conditions

Of the 20 cows included in this experiment, 16 ovulated. Mean

Table 1. Summarized data for vaginal, cervical, and rectal temperatures (ºC), and vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials at artificial insemination (AI) (D0) and 7 days post-AI (D7) for pregnant and non-pregnant cows

Temperature	All cows (n = 90)	Temperature differential	Pregnant cows (n = 39)	Non-pregnant cows (n = 51)	P (pregnant *vs*. non-pregnant)
Vagina D0	38.16 * ± 0.42 (36.5–39.5)	Vagina–rectum D0	–0.36 ± 0.36	–0.26 ± 0.40	N.S.
Vagina D7	38.27 * ± 0.41 (37.3–39.9)	Vagina–rectum D7	–0.22 ± 0.33	–0.33 ± 0.35	N.S.
Cervix D0	38.45 ** ± 0.37 (36.1–39.9)	Vagina–cervix D0	–0.21 ± 0.34	–0.28 ± 0.34	N.S.
Cervix D7	38.46 ** ± 0.38 (37.3–40.1)	Vagina–cervix D7	–0.28 ± 0.33	–0.15 ± 0.42	N.S.
Rectum D0	38.46 ** ± 0.39 (37.2–40)	Cervix–rectum D0	–0.25 * ± 0.36	0.01 *** ± 0.30	0.0004
Rectum D7	38.55 ** ± 0.40 (37.7–39.9)	Cervix–rectum D7	0.04 ** ± 0.26	–0.17 **** ± 0.32	0.001

N.S.: non significant. Mean ± S.D., ranges between parentheses. Values with different superscripts differ significantly within columns when tested by one-way ANOVA (*-**: P = 0.0001; ***-****: P = 0.004).

Table 2. Odds ratios of the pregnancy rate variables included in the final logistic regression model (n = 90)

Factor	Class	n	% Pregnancy	Odds ratio	95% Confidence interval	P
CR-D0	Continuous	90	43.3	2.5	1.25–3.45	0.01
CR-D7	Continuous	90	43.3	0.44	0.32–0.61	0.02

Hosmer and Lemeshow goodness-of-fit test = 4.8; P = 0.91. R^2 Nagelkerke = 0.35. CR–D0: cervix–rectum temperature differential at AI. CR–D7: cervix–rectum temperature differential 7 days post-AI.

Table 3. The summarized findings concerning vagina, cervix, and rectum temperatures and vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials at the time of artificial insemination (AI) (D0), and 8 h, 24 h, and 7 days post-AI (D7) in ovulating cows

	D0	8 h	24 h	D7
Temperatures (ºC)				
Vagina	38.70 ± 0.35	38.69^{*} ± 0.22	38.65 ± 0.22	38.59^{***} ± 0.32
Cervix	38.85 ± 0.23	38.58^{*} ± 0.21	38.83 ± 0.23	38.90^{****} ± 0.21
Rectum	38.84 ± 0.29	39.02^{**} ± 0.29	38.78 ± 0.26	38.86^{****} ± 0.23
Differentials				
Vagina-rectum	–0.14 ± 0.32	-0.32^{*} ± 0.38	-0.17^{***} ± 0.27	-0.26^{***} ± 0.38
Vagina-cervix	–0.15 ± 0.28	0.13^{**} ± 0.40	-0.17^{***} ± 0.27	-0.31^{***} ± 0.35
Cervix-rectum	0.01 ± 0.17	-0.40^{*} ± 0.33	0.05^{****} ± 0.16	0.04^{****} ± 0.26

Mean values ± S.D., n = 16. Values with different superscripts differ significantly within columns when tested by one-way ANOVA (*–**: P < 0.001; ***–****: P < 0.01).

pre-ovulatory follicular diameter at AI was 18.4 ± 3.0 mm (12–24 mm) and 17.8 ± 2.1 mm (15–20 mm) for ovulating and non-ovulating cows, respectively. All cows ovulated between 8 and 24 h after AI. The summarized findings concerning vagina, cervix, and rectum temperatures and vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials at AI and 8 h, 24 h, and 7 days post-AI for the ovulating cows are presented in Table 3. Vaginal and cervical temperatures at 8 h post-AI were 0.77% and 1.03% (P = 0.001), respectively, lower than rectal temperatures, whereas vaginal temperatures at 7 days post-AI were 0.77% (P = 0.01) lower than cervical and rectal temperatures. Vagina–rectum and cervix–rectum temperature differentials at 8 h post-AI were larger than the vagina–cervix temperature differentials (P = 0.003 and P = 0.0001, respectively). Vagina–rectum and vagina–cervix temperature differentials at 24 h post-AI were larger than the cervix–rectum temperature differentials (P = 0.003). Vagina–rectum and vagina–cervix temperature differentials at 7 days post-AI were larger than the cervix–rectum temperature differentials (P = 0.01 and P = 0.003, respectively).

The mean vagina–rectum, vagina–cervix, and cervix–rectum temperature differential at AI and 8 h, 24 h, and 7 days post-AI for the ovulating cows changed significantly from day 0 to day 7 (within-subject effect; P < 0.02). Vagina–rectum, vagina–cervix, and cervix–rectum temperature differentials changed significantly from 0 h to 8 h post-AI (P = 0.02), and cervix–rectum temperature differentials changed significantly from 8 h to 24 h post-AI (P = 0.02). Vagina–cervix temperature differential 8 h post-AI (0.13°C ± 0.40ºC) was different from that of the cervix–rectum (–0.44 ± 0.33ºC; P = 0.0001) and vagina–rectum (–0.32 ± 0.38ºC; P = 0.003).

Of the 16 ovulating cows, 5 (31.3%) became pregnant. Comparing values between pregnant and non-pregnant cows, only significant difference in cervix–rectum temperature differential at AI (pregnant and non-pregnant cows, –0.14 ± 0.05ºC and 0.07 ± 0.16ºC, respectively; P = 0.01). Comparing values between ovulating (n = 16) and non-ovulating (n = 4) cows, only significant differences in cervix–rectum temperature differential were detected at 8 h post-AI (–0.44 ± 0.33ºC and –0.05 ± 0.10ºC, respectively; P = 0.03) and 24 h post-AI (0.05 ± 0.06°C and –0.10 ± 0.22ºC, respectively; P = 0.02).

Discussion

Low cervical temperature at AI was correlated with a greater likelihood of pregnancy, supporting our hypothesis that the temperature differential between the caudal cervical canal and rectum at AI may be a predictive factor for pregnancy. The data emerging from our study are consistent with those of other studies, which showed that Graafian follicles undergoing pre-ovulatory cooling before insemination are most likely to give rise to pregnancy [6–8]. Furthermore, the cervical temperature of non-pregnant cows was significantly lower than the rectal temperature on day 7 post-AI, whereas the cervix–rectum temperature differential at this time was close to zero for the pregnant cows. Thus, cervix–rectum temperature differentials at AI and 7 days post-AI were significantly different between pregnant and non-pregnant cows (P = 0.0004 and P = 0.001, respectively). Rectal, cervical, and vaginal temperatures and vagina–cervix and vagina–rectum temperature differentials at AI and 7 days post-AI could not be related to the likelihood of pregnancy, whereas cervix–rectum temperature differentials at 8 h and 24 h post-AI could be associated with the process of ovulation. As a simple and easily available measurement, cervix–rectum temperature differentials could be used in routine practice in dairy herds, at least as an additional tool to confirm estrus readiness for insemination.

By confirming that a cow was in estrus and ready for insemination, we achieved a pregnancy rate of 43.3%, which is more than acceptable. The state of the pre-ovulatory follicle at AI could overcome the effects of strong factors, such as HS and parity, influencing fertility. The presence of a very soft follicle means that the time of ovulation approaches in both spontaneous and synchronized estrus [26]. However, the remaining non-pregnant cows were probably inseminated too early. Low cervical temperature was not recorded at AI and, unlike the situation in pregnant cows, a significant cervix–rectum temperature differential was recorded at 7 days post-AI. Cervix–rectum temperature differential was also not recorded from the time of insemination to 7 days post-AI in cows with ovulation failure. In cows not showing low cervical temperature at AI, an elevated dose of GnRH at insemination may facilitate ovulation and increase fertility [27].

Vaginal temperature has been linked to rectal temperature [28, 29], and under HS conditions, it shows stronger relationships with THI values [11] and uterus temperature [17] than rectal temperatures. Effectively, vaginal temperature serves to predict the onset of estrus in cattle [30, 31]. However, in our study, vaginal temperatures and vagina–cervix and vagina–rectum temperature differentials at AI and 7 days post-AI could not be related to the likelihood of ovulation or pregnancy. It is likely that in cattle, the lowest vaginal temperature is recorded 3 days prior to estrus and that this temperature increases by at least 0.4ºC at > 6 h of estrus and declines again at the time of ovulation [30, 31], which implies a higher degree of individual variation than that of cervical temperatures at AI. Vaginal temperature was lower than cervical and rectal temperatures at AI and 7 days post-AI, whereas vagina–rectum and vagina–cervix temperature differentials were similar for pregnant and non-pregnant cows at both times (Table 1). These temperature patterns failed to reflect the relationship between low vaginal temperature and fertility. Although extensive studies are required to assess the effects of HS on the process of cervical/vaginal temperature decrease, the present results indicate that cervix–rectum temperature differentials are a better indicator than vagina–cervix and vagina–rectum temperature differentials at AI and 7 days post-AI of the likelihood of pregnancy at insemination.

Lastly and most importantly, uterine mucus secretion and flow throughout the cervical canal and vagina during the peri-ovulatory period will favor local cooling. The water content of uterine mucus represents approximately 95–99% of the total amount during the follicular phase, whereas the percentage may decrease to 85% during the luteal phase [32]. Copious production of bovine vaginal fluid is a sign of estrus readiness for service [20] and may influence the cervix–rectum temperature differential at AI in pregnant cows. In contrast, a significant cervix–rectum temperature differential was recorded at 7 days post-AI in non-pregnant cows, suggesting a delayed ovulation with a more aqueous cervical mucus. In an extended study, the full physical nature of the cervical/vaginal fluid of pregnant and non-pregnant cows needs to be addressed.

Overall, our findings support the notion that the temperature differential between the caudal cervical canal and rectum at AI may be an indicator of the likelihood of pregnancy. In Experiment I, a one-tenth drop in the degree in cervical temperature with reference to control rectal temperature at the time of AI gave rise to an odds ratio of 2.5 for pregnancy, whereas the same decrease in the cervix–rectum differential at 7 days post-AI resulted in an odds ratio of 0.44. In Experiment II, ovulating cows showed a similar cervix–rectum temperature differential dynamic to that of cows in Experiment I.

Conflict of interests: The author declares no conflict of interest.

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