JAST (Journal of Animal Science and Technology) TITLE PAGE

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Short Communication
Article Title (within 20 words without abbreviations)	Measuring the effects of estrus on rumen temperature and environment, behavior and physiological attributes in Korean Native breeding cattle
Running Title (within 10 words)	Interpretation of estrus-related changes using a rumen bolus sensor
Author	Jae-Young *Kim*[a], Jae-Sung *Lee*[a], Yong-Ho *Jo* and Hong Gu *Lee*[*]
Affiliation	Department of Animal Science and Technology, Sanghuh College of Life Sciences, Konkuk University, Seoul 05029, Korea [a] These authors contributed equally to this work.
ORCID (for more information, please visit https://orcid.org)	Jae-Young *Kim* (https://orcid.org/0000-0001-5656-5121) Jae-Sung *Lee* (https://orcid.org/0000-0001-8940-9862) Yong-Ho *Jo* (https://orcid.org/0000-0002-9842-5765) Hong-Gu *Lee (*https://orcid.org/0000-0002-0679-5663)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
Acknowledgements	This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) and the Korea Smart Farm R&D Foundation (KosFarm) through Smart Farm Innovation Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) and the Ministry of Science and ICT (MSIT), Rural Development Administration (RDA)(421014-04).
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization and hypothesis: Jae-Sung *Lee,* Hong-Gu *Lee*. Data curation: Jae-Young *Kim*, Yong-Ho *Jo.* Formal analysis: Jae-Young *Kim*, Yong-Ho *Jo.* Methodology: Jae-Young *Kim*, Yong-Ho *Jo*, Jae-Sung *Lee*. Writing first draft: Jae-Young *Kim*, Jae-Sung *Lee.* Writing, reviewing, and editing: Jae-Young *Kim*, Jae-Sung *Lee*, Yong-Ho *Jo,* Hong-Gu *Lee*. Preparation, experiments, and discussion: Jae-Young *Kim*, Jae-Sung *Lee*, Yong-Ho *Jo*, Hong-Gu *Lee*. Supervised the experiment, supporting the experiment financially: Hong-Gu *Lee*.
Ethics approval and consent to participate	This article does not contain any studies with human subjects performed by any of the authors. The experimental procedure and methods were approved by the Animal Welfare and Ethics Authority of Konkuk University, Seoul, Korea (KU22106).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Hong-Gu *Lee*
Email address – this is where your proofs will be sent	hglee66@konkuk.ac.kr
Secondary Email address	
Address	Department of Animal Science and Technology, Sanghuh College of Life Sciences, Konkuk University, Seoul 05029, Korea
Cell phone number	+82-10-3307-4387
Office phone number	+82-2-450-0523
Fax number	+82-2-455-1044

Abstract

In this study, rumen temperature and environment in estral and non-estral Korean Native breeding cattle were evaluated by using a bolus sensor. Behavioral and physiological changes in study animals were also assessed. To assess the rumen temperature and environment, we inserted bolus sensors into 12 Korean Native cattle with an average age of 35.5 months, then measured temperature and activity within the rumen using the wireless bolus sensor. Drinking, feeding and mounting behavior, and measured vaginal temperature and levels of intravaginal mucus resistance were recorded. We found that cattle in estrus exhibited more acts of mounting (37.4 vs. 0 times/day), increased vaginal temperature (39.0℃ vs. 38.4℃), and decreased vaginal mucus resistance (136.3Ω vs 197.4Ω), compared with non-estral animals. Furthermore, increased levels of rumen activity were most significant in estrus cattle at the highest activity levels ($p < 0.01$). Overall, the estrus group exhibited increased rumen temperature ($p = 0.01$), compared with the non-estrus group. In conclusion, the results of this study not only provide basic physiological data related to estrus in improved Korean Native breeding cattle, but also suggest that monitoring of rumen temperature and activity might be used as an effective smart device for estrus detection.

Keywords: Bolus wireless sensor, Breeding cattle, Estrus, Rumen temperature

Introduction

For farmers who raise Korean Native breeding cattle, the optimal time of insemination is a critically important matter because calf production directly affects farm income. The best way to predict the timing of fertilization is to observe the estrus behavior and condition of cattle. In South Korea, as elsewhere, visual observations reveal that cattle in estrus typically mount other animals, or allow themselves to be mounted. Korean studies found that that 98.3% of cattle in estrus allowed themselves to be mounted, and 74.6% mounted other animals [1,2]. Other indicators of estrus include mucus secretion from the vagina, changes in vulval color, anxiety, and excitement [3,4]. However, studies of behavioral, physiological, and endocrine changes during estrus have not yet considered fully the particular breeding environment associated with Korean beef cattle [5-7].

In small farm settings, estrus indicators are usually easy to identify visually. On larger farms, it can be difficult to determine optimum insemination times because there is less time available for visual observation of individual animals. However, new developments in information and communications technology (ICT) such as bolus sensors may help to overcome such human resource limitations. Recently, researchers have monitored the rumen environment in exotic species using a rumen-inserted bolus sensor in order to identify estrus [8-11]. The estrus state can also be confirmed by behavioral observations [3,4]. Several studies have reported behavioral changes due to estrus including changes in standing time, mounting behavior, activity levels, and rumen movement associated with action of the nervous system and endocrine system [12-14]. Researchers have also recorded increased rumen temperature during estrus in exotic breeding cattle [11,15]. However, the use of ICT for estrus verification in Korean Native cattle has not been attempted to date. Therefore, in this study, we sought to investigate changes in behavior, physiology, and endocrine indicators during estrus in recently genetically improved Korean Native animals, and to interpret estrus-related changes in rumen temperature and activity automatically recorded by rumen-inserted bolus sensors.

Materials and Methods

Animals and diet

The experimental procedure and methods were approved by the Animal Welfare and Ethics Authority of Konkuk University, Seoul, Republic of Korea (KU22106).

For our study, 12 healthy Korean Native breeding cattle aged 38.8 months with fertility of 2.67 ± 1.37 were used. This study was carried out at the Farm at Chungcheongnam-do in South Korea, from September to November, 2021. All animals in a roofed feedlot area with open sides were housed. Feed consisted of commercial cattle concentrate and rice straw, with free access to water (Table S1). Feed was given twice daily (0700 and 1700).

The wireless bolus sensor

Bolus wireless sensor probes (Dongbang S&D Co., Ltd, Seoul, Korea) which are disposable, cylinder-shaped devices suitable for oral administration to cattle were acquired. The probe detects and communicates rumen temperature and activity by means of a temperature sensor, a three-axis accelerometer (STMicroelectroincs, Geneva, Switzerland), a transmitter (Dongbang S&D Co., Ltd) and a battery (Sensirion AG, Staefa, Switzerland). The probe diameter is 3.2 cm, its length is 10.8 cm, and its weight is 180 g. The probe is administered orally via a bolus gun (Leedstone, Inc., MN, USA) into the rumen. Transmissions are in the 900 MHz band. Average current consumption is less than 100 uA. In our study, data on rumen temperature and activity were transmitted at ten-minute intervals from the temperature sensor and three-axis accelerometer, respectively. A receiver which stored all data on a cloud-connected server and could be remotely monitored via a website (http://218.144.67.74:8080/board/logout) was used. The distance from the probe to the receiver was about 30 m.

Behavior, physiology, endocrine indicators, rumen temperature and activity in estrus and non-estrus cattle

CCTV (IDIS, Ltd., Daejeon, Korea) was used to acquire data on animal behavior during estrus and non-estrus periods. We gathered data concerning mounting (other animals), drinking, and feeding behavior for a non-estrus period of 3 days before estrus from 10 to 12 days, and then determined average values for the 12 heads. Estrus animal data covered a 24-hour period consisting of 12 hours prior to the confirmation of estrus, and the 12 hours following. Then, an average value of 16 periods of estrus from 12 heads of cattle was determined. Mounting behavior as the number of attempts made to mount another animal was measured. We assessed water intake by the number of occasions on which water was consumed.

Specialist equipment to collect data concerning vaginal temperature (MT1681, Microlite, Germany) and electrical resistance of vaginal mucus (EDC2, Draminski, Poland) were used, On average, we carried out these measurements once for estrus cattle and three times for non-estrus animals. Vaginal temperatures by inserting the temperature-measuring part of the thermometer into the animal's vagina for 1 minute were measured. To measure the electrical resistance of vaginal mucus, we inserted the measuring device up to the entrance of the cervical canal. Then, rotated the device three times in the same direction, and recorded the value obtained.

To measure luteinizing hormone, blood from animals on two occasions was collected. Blood was collected from the jugular vein 10 to 12 days before the expected onset of estrus, then took blood again within 3 hours of estrus detection. The collected blood was immediately centrifuged at 2,700 x g, 4℃, transferred to a 1.5-mL tube, and stored at -80℃. Later, after thawing, concentrations of luteinizing hormone in serum were analyzed by measuring absorbance at 450 nm using a microplate reader (PMT49984, oTek Instruments, RI, USA) and ELISA kit (EK760147, AFG Scientific, Shizuoka, Japan).

Data on temperature and activity in the rumen were obtained by using the bolus sensor. We first collected data 10 to 12 days before the expected onset of estrus. As this time approached, the experimenter and a veterinarian visually checked for signs of estrus every 4 hours. After estrus was confirmed, data for the 12 hours before and after confirmation time were used. The onset of estrus by measuring vaginal temperature and electrical resistance of vaginal mucus was also confirmed. We expressed our rumen temperature data in terms of means, minimum values, maximum values, proportions of animals with below-average and above-average temperatures, as well as proportions of animals with temperatures exceeding 39.5℃. For rumen activity, we determined average, minimum and maximum activity levels, and classified activities recorded in terms of numbered levels rising from zero to eight or above.

Statistical analysis

Data of LH, behavioral activity and physiological changes were analyzed, as well as activity data obtained from the ruminal sensor, by means of repeated measures analysis using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Our model was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma(\alpha)_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} is the observation of breeding cattle k at sampling time j with or without estrus i, μ is the overall mean, α_i is the fixed effect of treatment i (non-estrus and estrus), β_j is the fixed effect of sampling time j, $\gamma(\alpha)_{ik}$ is the random effect of breeding cattle k nested in treatment i and ε_{ijk} is the residual effect. We included calf identity in the model as a random effect. Covariance structures (autoregressive order 1, compound symmetry, unstructured, and variance components) for the repeated measures model were tested and chose the structure which best fitted the model based on the lowest value of the Schwarz–Bayesian information criterion. Data were present as least square means and associated standard error of means. We compared least square means among treatments using Student's *t*-test comparison when the treatment effect was tendency or significant. We considered differences to be statistically significant if the *p*-value was lower than 0.05. Means with *p*-values between 0.05 and 0.10 reflected a tendency to differ.

Results and Discussion

Our data on the concentration of serum luteinizing hormone revealed that hormone concentration increased from 22.79 mIU/mL in non-estrus cattle to 38.38 mIU/mL in estrus animals, i.e., an increase of 15.59 mIU/mL ($p < 0.01$) (Table 1). Estrus involves cyclical changes in levels of various hormones; the most representative of these are progesterone and luteinizing hormone. In dairy cows, the authors of [16] found that progesterone, a hormone secreted from the corpus luteum, was maintained at a low level of less than 1 ng/mL, then rose to a peak level of 4.50 ng/mL on subsequent days 15 to 17 and then decreased rapidly before estrus. Contrarily, Snook and coworkers [17] found that luteinizing hormone maintained an average level of 5.1 ng/mL in non-estrus cattle but increased to 20.7 ng/mL on the day of estrus [17]. Studies by Henricks et al. [18] and Chenault et al. [19] found that increases in luteinizing hormone varied across studies. The data in our study revealed an increase in concentration of serum luteinizing hormone on the day of estrus.

Concerning mounting behavior, our CCTV observations showed that mounting was not attempted when cattle were not in estrus. However, when in estrus, animals performed mounting behaviors on an average of 37.4 occasions (Table 1). Cattle in estrus typically mount other animals or allow themselves to be mounted. Previous studies have found that, during estrus, 98.3% of cattle mount other animals, and 74.6% allow themselves to be mounted [1,2]. One study found that cattle performed an average of 7.6 mounting behaviors over a period of approximately 8 to 9 hours [20,21]. Factors affecting the number of mountings include external temperature, the physical properties of the breeding area, and the number of animals raised together [22]. The study of Sveberg et al. [21] involved twenty-two breeding animals in a space of 17 x 24 m. In our study, twelve animals were housed in an area of 12.5 x 25 m. When considering average numbers of mounting behaviors, the nature of the breeding environment should be considered when carrying out comparisons between studies, as well as the species involved.

We also found increased water intake during estrus, from 3.1 to 3.6 drinking occasions, on average ($p < 0.01$) (Table 1). One previous study [23] reported a lower frequency of water intake of cattle during estrus, due to a decrease in the intake of roughage without compensating changes in the intake of concentrate feed. The authors of [24] found that water intake fell from an average of four occasions in non-estrus cattle to an average of three in estrus animals. In addition, the duration of water visits also decreased during estrus. In our study, though we did not measure the volume of water consumed, we did find an increase in water-drinking occasions in estrus animals, as stated above, and this might be associated with increased cattle activity during estrus periods.

Average vaginal temperature in our cattle increased from 38.4℃ in the non-estrus state to 39.0℃ during estrus ($p < 0.01$) (Table 1). This is in line with studies of another cattle breed [25,26], which found that the average vaginal temperature of Holstein cows increased by between 0.4℃ and 0.6℃, and this increase in vaginal temperature was maintained for 3 to 6.8 hours.

Average level of electrical resistance in the vaginal mucus of cattle in our study was 44.8% lower in estrus than non-estrus animals (136.3Ω vs. 197.4Ω) ($p < 0.01$) (Table 1). Factors affecting the electrical resistance in vaginal mucus include higher amounts of mucus in the vagina due to an increase in estrogen secretion and an increase in ions such as NaCl which leads to lower resistance, as reported by the authors of [27]. Other researchers have measured electrical resistance of vaginal mucus during the estrus cycle and found that it gradually decreases before estrus, remains low during estrus, and returns to its original level after estrus [28,29]. In our study, we observed a similar pattern. The authors of [30] also measured electrical resistance in vaginal mucus of cattle. Although they obtained differing results for different breeds, they still found resistance decreased during estrus, by an average of 40.04%, from 49.7 Ω to 29.8 Ω.

The rumen-inserted bolus sensor used in this study indicated activity levels by means of an acceleration sensor which measured gravitational acceleration every 2.5 seconds for 10 min. We determined all activities recognized by the sensor in the rumen to be 100%. We then found that times of inactivity decreased from 46.25% in non-estrus cattle to 28.76% in estrus animals, i.e., a reduction of 17.49% (Table 2). We thus numerically confirmed that inactivity decreased significantly during estrus. Level '1' activity also significantly decreased during estrus, from 18.94% to 14.39% ($p < 0.01$). For activity levels '2' to '6', we found no statistically significant differences between the non-estrus and estrus periods ($p > 0.05$). Activity at the high levels of '7' or above was 10.79% in non-estrus cattle and 27.65% in estrus animals ($p < 0.01$). These findings contrast with the above-described activity levels 'zero' and '1', and confirmed that the activity level increased during estrus. This increase rate was matched by the decrease in the activity level of 'zero' (17.49%) and the activity level of '7' or more (16.86%). Previous studies have considered such levels of activity. The authors of [31] found that walking activity increased by 290% on the day of estrus confirmation, compared with 7 days previously, and the authors of [12] found increased standing time prior to ovulation. In line with previous studies, therefore, we found that activity during estrus increased significantly. Using the rumen-inserted bolus sensor, we found that estrus could be confirmed if activity levels ‘zero’ and ‘1’ decreased by 22.0%, while activity levels ‘7’ or higher increased by 16.8%.

As shown in Table 3, when we observed rumen temperature, average temperature in non-estrus animals was 38.76℃. During estrus, the corresponding figure was 38.8℃, i.e., a rise of 0.13℃ ($p = 0.01$). We found no significant difference in minimum rumen temperature between the two groups ($p > 0.05$), while maximum temperature was 39.91℃ in estrus cattle and 39.67℃ in non-estrus animals, i.e., an increase of 0.24℃ ($p = 0.03$). The rumen-inserted bolus sensor used in this study might have influenced these temperature changes by affecting drinking water intake, which did not show a clear change as did vaginal temperature. However, we did identify a significant difference between estrus and non-estrus animals. Because the average temperature in the rumen for differs between the two groups, we determined a base value for rumen temperature on the 10th to the 12th days before estrus. Subsequently, we checked for values higher or lower than the base temperature. First, we defined measurements of temperature lower than or equal to the base temperature as ‘below-average’ temperature. We defined temperature measurements higher than the base temperature as ‘above-average’ temperature. ‘Below-average’ measurements made up 34.29% of the non-estrus and 28.71% of the estrus data, while ‘above-average’ measurements made up 65.71% of the non-estrus and 71.29% of the estrus data ($p = 0.04$). Proportions for temperatures beyond 39.5℃ were 2.78% and 15.97% for non-estrus and estrus groups, respectively, i.e., a large difference of 13.19% ($p > 0.01$). In summary, when considering the variability between individuals, we judged that the use of rumen temperature data could contribute to a more accurate means of estrus detection than visual inspection alone.

Conclusion

In this study, we investigated changes in behavior, physiology, and endocrine indicators during estrus in Korean Native breeding cattle. We interpreted estrus-related changes in rumen temperature and activity automatically recorded by a wireless bolus sensor. Compared with non-estrus cattle, animals in estrus exhibited increases in luteinizing hormone levels, mounting behavior, drinking water intake, and vaginal temperature, as well as decreased electrical resistance of vaginal mucus. Using the rumen-inserted bolus sensor, we also found increased rumen temperature and activity. The results of our study not only provide basic physiological data related to estrus in improved Korean Native breeding cattle, but also suggest that monitoring of rumen temperature and activity can be used as an effective smart device for estrus detection in South Korea.

Acknowledgments

This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) and the Korea Smart Farm R&D Foundation (KosFarm) through the Smart Farm Innovation Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) and the Ministry of Science and ICT (MSIT), Rural Development Administration (RDA)(421014-04).

References

1. Yang JS, Heo YT, Uhm SJ, Ko DH. Effect of pregnancy rate following timing of artificial insemination after estrus of Hanwoo female. Reprod Dev Biol 2013;37:75-77. http://dx.doi.org/10.12749/RDB.2013.37.2.75.

2. Uhm HB, Jeong JK, Kang HG, Kim IH. Effect of timed artificial insemination protocols on the pregnancy rate per insemination and pregnancy loss in dairy cows and Korean native cattle under heat stress. J Vet Clin 2020;37:235-241. https://doi.org/10.17555/jvc.2020.10.37.5.235.

3. Foote RH. Estrus detection and estrus detection aids. J Dairy Sci 1975;58:248-56. https://doi.org/10.3168/jds.S0022-0302(75)84555-3.

4. Fricke PM, Carvalho PD, Giordano JO, Valenza A, Lopes G, Amundson MC. Expression and detection of estrus in dairy cows: the role of new technologies. Animal 2014;8:134-143. https://doi.org/10.1017/S1751731114000299.

5. Seong HH, Woo JS, Im SK, Ko YG, Baek KS, Park JK, Koo Y, Lee JH. Reproductive physiology and endocrinology: Change of serum progesterone and insulin-like growth factor-1 concentration during the estrous cycle in Hanwoo. Korean J Anim Sci 1997;39:237-242.

6. Chung HJ, Yoon HI, Lee SD, Ko JS, Choy YH, Choi SB, Jeon GJ, Ko YG, Lee HC, Im SK, Lee MS. Concentration differences in LH, FSH and progesterone secretion among seasonal changes in Hanwoo and Holstein heifers in Daegwallyeong. J Emb Trans 2008;23:257-261.

7. Son JK, Park SJ, Baek KS, Choi YL, Lee MS, Kim SB, Kim HS, Shin SO, Park CK, Park SB. Studies on the relationship between number of repeat and duration of estrus behavior in Hanwoo and Holstein cattle. J Emb Trans 2008;23:141-145.

8. Nogami H, Arai S, Okada H, Zhan L, Itoh T. Minimized bolus-type wireless sensor node with a built-in three-axis acceleration meter for monitoring a cow's rumen conditions. Sensors 2017;17: 687. https://doi.org/10.3390/s17040687.

9. Halachmi I, Guarino M, Bewley J, Pastell M. Smart animal agriculture: application of real-time sensors to improve animal well-being and production. Annu Rev Anim Biosci 2019;15:403-425. https://doi.org/10.1146/annurev-animal-020518-114851.

10. Tedeschi LO, Greenwood, PL, Halachmi I. Advancements in sensor technology and decision support intelligent tools to assist smart livestock farming. J Anim Sci 2021;99:1-11. https://doi.org/10.1093/jas/skab038.

11. Cooper-Prado M, Long N, Wright E, Goad C, Wettemann R. Relationship of ruminal temperature with parturition and estrus of beef cows. J Anim Sci 2011;89:1020-1027. https://doi.org/10.2527/jas.2010-3434.

12. Higaki S, Okada H, Suzuki C, Sakurai R, Suda T, Yoshioka K. 2021. Estrus detection in tie-stall housed cows through supervised machine learning using a multimodal tail-attached device. Comput Electron Agric 2021;191:106513. https://doi.org/10.1016/j.compag.2021.106513.

13. Sveberg G, Refsdal AO, Erhard H, Kommisrud E, Aldrin M, Tvete IF, Buckley F, Waldmann A, Ropstad E. Behavior of lactating Holstein-Friesian cows during spontaneous cycles of estrus. J Dairy Sci 2011;94:1289-1301. https://doi.org/10.3168/jds.2010-3570.

14. Cheon SN, Yoo GZ, Kim CH, Jung JY, Kim DH, Jeon JH. Study on behavioral change of estrus in Hanwoo (Korean native cattle). Journal of the Korea Academia-Industrial cooperation Society 2020;21:825-832. https://doi.org/10.5762/KAIS.2020.21.11.825.

15. Kim DH, Ha JJ, Yi JK, Kim BK, Kwon WS, Ye BH, Kim SH, Lee Y. Differences in ruminal temperature between pregnant and non-pregnant Korean cattle. J Anim Reprod Biotechnol 2021;36:45-50. https://doi.org/10.12750/JARB.36.1.45.

16. Adriaens I, Saeys W, Lamberigts C, Berth M, Geerinckx K, Leroy J, De Ketelaere B, Aernouts B. Sensitivity of estrus alerts and relationship with timing of the luteinizing hormone surge. J Dairy Sci 2019;102:1775-1779. https://doi.org/10.3168/jds.2018-15514.

17. Snook R, Saatman R, Hansel W. Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. Endocrinology 1971;88:678-686. https://doi.org/10.1210/endo-88-3-678.

18. Henricks D, Dickey J, Niswender G. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. Biol Reprod 1970;2:346-351. https://doi.org/10.1095/biolreprod2.3.346.

19. Chenault J, Thatcher W, Kalra P, Abrams R, Wilcox C. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J Dairy Sci 1975;58:709-717. https://doi.org/10.3168/jds.S0022-0302(75)84632-7.

20. Walker W, Nebel R, McGilliard M. Time of ovulation relative to mounting activity in dairy cattle. J Dairy Sci 1996;79:1555-1561. https://doi.org/10.3168/jds.S0022-0302(96)76517-7.

21. Sveberg G, Refsdal A, Erhard H, Kommisrud E, Aldrin M, Tvete I, Buckley F, Waldmann A, Ropstad E. Sexually active groups in cattle—a novel estrus sign. J Dairy Sci 2013;96:4375-4386. https://doi.org/10.3168/jds.2012-6407.

22. Gwazdauskas F, Lineweaver J, McGilliard M. Environmental and management factors affecting estrous activity in dairy cattle. J Dairy Sci 1983;66:1510-1514. https://doi.org/10.3168/jds.S0022-0302(83)81966-3.

23. Reith S, Brandt H, Hoy S. Simultaneous analysis of activity and rumination time, based on collar-mounted sensor technology, of dairy cows over the peri-estrus period. Livest Sci 2014;170:219-227. https://doi.org/10.1016/j.livsci.2014.10.013.

24. Cairo F, Pereira L, Campos M, Tomich T, Coelho S, Lage C, Fonseca A, Borges A, Alves B, Dórea JRR. Applying machine learning techniques on feeding behavior data for early estrus detection in dairy heifers. Comput Electron Agric 2020;179:105855. https://doi.org/10.1016/j.compag.2020.105855.

25. Redden K, Kennedy A, Ingalls J, Gilson T. Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. J Dairy Sci 1993;76:713-721. https://doi.org/10.3168/jds.S0022-0302(93)77394-4.

26. Kyle B, Kennedy A, Small J. Measurement of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. Theriogenology 1998;49:1437-1449. https://doi.org/10.1016/s0093-691x(98)00090-9.

27. Albrecht, B. H., R. S. Fernando, J. Regas, and G. Betz. 1985. A new method for predicting and confirming ovulation. Fertil Steril 44:200-205. https://doi.org/10.1016/s0015-0282(16)48736-4.

28. Gupta KA, Purohit GN. Use of vaginal electrical resistance (VER) to predict estrus and ovarian activity, its relationship with plasma progesterone and its use for insemination in buffaloes. Theriogenology 2001;56:235-245. https://doi.org/10.1016/s0093-691x(01)00559-3.

29. Gartland P, Schiavo J, Hall CE, Foote RH, Scott NR. Detection of estrus in dairy cows by electrical measurements of vaginal mucus and by milk progesterone. J Dairy Sci 1976;59:982-985. https://doi.org/10.3168/jds.S0022-0302(76)84307-X.

30. Schams D, Schallenberger E, Hoffmann B, Karg H. The oestrous cycle of the cow: hormonal parameters and time relationships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. Eur J Endocrinol1977;86:180-192. https://doi.org/10.1530/acta.0.0860180.

31. Spicer, L., and S. Echternkamp. 1986. Ovarian follicular growth, function and turnover in cattle: a review. J Anim Sci 62:428-451. https://doi.org/10.2527/jas1986.622428x.

Table 1. Comparison of LH levels, mounting behavior, drinking behavior, vaginal temperature and electrical resistance of vaginal mucus between non-estrus and estrus cattle.

Item	Non-estrus	Estrus	SEM[3]	*p*-value[4]
Luteinizing hormone, mIU/mL[1]	22.79	38.38	3.26	<0.01
Mounting, times[2]	0.0	37.4	2.30	<0.01
Drinking, times[2]	3.1	3.6	0.14	0.03
Vaginal temperature, ℃[2]	38.4	39.0	0.04	<0.01
Electrical resistance of vaginal mucus, Ω[2]	197.4	136.3	5.57	<0.01

[1] Luteinizing hormone level of non-estrus shows the average value of 12 heads. Estrus data is an averaged value of 16 estrus periods of 12 heads.

[2] Non-estrus animal data collected for 3 days before estrus from 10 to 12 days is an averaged value of 12 heads. Estrus data obtained for 12 hours prior to estrus confirmation and for 12 hours following is an averaged value of 16 periods of estrus of 12 heads.

[3] SEM means standard error of the mean.

[4] Statistical analysis carried out used Student’s t-test.

Table 2. Comparison of activity levels between non-estrus and estrus cattle based on data obtained using the bolus sensor.

Item	Non-estrus	Estrus	SEM[3]	*p*-value[4]
Average activity[1]	2.9	5.8	0.38	<0.01
Minimum activity[1]	0.0	0.0	0.00	1.00
Maximum activity[1]	57.8	49.6	8.12	0.49
Activity, %[2]				
0	45.8	26.7	2.63	<0.01
1	17.6	13.9	0.83	<0.01
2	10.0	9.2	0.84	0.36
3	6.7	6.5	0.75	0.84
4	4.5	5.7	0.62	0.05
5	3.8	4.0	0.57	0.67
6	2.4	3.4	0.43	0.02
7	1.9	3.6	0.34	<0.01
≥ 8	10.1	25.9	2.00	<0.01

[1] Non-estrus animal data collected for 3 days before estrus from 10 to 12 days is an averaged value of 12 heads. Estrus data obtained for 12 hours prior to estrus confirmation and for 12 hours following is an averaged value of 16 periods of estrus of 12 heads.

[2] Activity levels 0, 1, 2, 3, 4, 5, 6, 7, and ≥ 8 refer to the percentage of total daily activity measurements, as follows: (0-≥8 number of activity measurements / total daily activity measurements) × 100

[3] SEM means standard error of the mean.

[4] Statistical analysis carried out using Student's t-test.

Table 3. Comparison of ruminal temperatures between non-estrus and estrus of cattle based on data obtained using the bolus sensor.

Item	Non-estrus	Estrus	SEM[4]	*p*-value[5]
Average temperature, ℃[1]	38.8	38.9	0.04	0.01
Minimum temperature, ℃[1]	34.6	34.7	0.29	0.73
Maximum temperature, ℃[1]	39.7	39.9	0.08	0.03
Below average, %[2]	34.3	28.7	2.24	0.04
Excess average, %[2]	65.7	71.3	2.24	0.04
Over 39.5 ℃, %[3]	2.8	16.0	2.19	<0.01

[1] Non-estrus animal data collected for 3 days before estrus from 10 to 12 days is an averaged value of 12 heads. Estrus data obtained for 12 hours prior to estrus confirmation and for 12 hours following is an averaged value of 16 periods of estrus of 12 heads.

[2] Based on the average ruminal temperature of non-estrus animals, the data refers to the probability of a number of times below or excess the average temperature.

[3] (Number of times of over 39.5℃ / total daily ruminal temperature measurements) × 100

[4] SEM means standard error of the mean.

[5] Statistical analysis carried out using Student's t-test.