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Research Report
Theriogenology

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Increased ruminoreticular temperature after simultaneous lumpy skin disease and foot-and-mouth disease vaccination in Hanwoo (*Bos taurus coreanae*) cows

Jisu Kim [1,2,†], Jae-Jung Ha [3,†], Mirae Kim [1,2], Woo-Sung Kwon [4], Junkoo Yi [5,*], Daehyun Kim [1,2,*]

[1]Department of Animal Science, Chonnam National University, Gwangju 61186, Korea
[2]Laboratory of Animal Reproduction, ARIF Inc., Naju 58307, Korea
[3]Gyeongbuk Livestock Research Institute, Yeongju 36052, Korea
[4]Department of Animal Science and Biotechnology, Kyungpook National University, Sangju 37224, Korea
[5]School of Animal Life Convergence Science, Hankyong National University, Anseong 17579, Korea

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***Corresponding authors:**

Junkoo Yi
School of Animal Life Convergence Science, Hankyong National University, 327 Jungang-ro, Anseong 17579, Korea.
Email: junkoo@hknu.ac.kr
https://orcid.org/0000-0003-2593-6529

Daehyun Kim
Department of Animal Science, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 61186; Laboratory of Animal Reproduction, ARIF Inc., 34 Hwangyong-gil, Bonghwang-myeon, Naju 58307, Korea.
Email: kimdhbio@jnu.ac.kr
https://orcid.org/0000-0002-4820-4438

†Jisu Kim and Jae-Jung Ha equally contributed to this work.

ABSTRACT

Importance: Foot-and-mouth disease (FMD) and lumpy skin disease (LSD) cause major economic losses in Hanwoo cattle. To reduce cost and improve convenience, simultaneous FMD–LSD vaccination is being promoted, yet its effects on ruminoreticular temperature and activity are unclear.
Objective: To evaluate ruminoreticular temperature and body activity after vaccination, stratified by pregnancy status.
Methods: This prospective observational cohort study investigated ruminoreticular temperature and body activity in Hanwoo cows after experimental administration of FMD and LSD vaccines. Sixty-three Hanwoo cows (pregnant n = 23 [100–175 days], nonpregnant n = 40) were monitored using a rumen-insertable biosensor that records ruminoreticular temperature and activity every 10 min. Groups received FMD, LSD, simultaneous FMD/LSD, or saline (control). Outcomes were analyzed using analysis of covariance with age and parity as covariates.
Results: In pregnant cows, simultaneous FMD/LSD vaccination increased ruminoreticular temperature at day 1 and returned to baseline by day 3; single-vaccine groups showed more prolonged elevations. In nonpregnant cows, simultaneous vaccination increased temperature at day 1, normalizing by day 3–4. Body activity did not differ among pregnant groups; in nonpregnant cows, the simultaneous group showed lower activity than single-vaccine groups on some days.
Conclusions and Relevance: In pregnant Hanwoo cows, post-vaccination temperature increase may enhance the risk of abortion, stillbirth, and premature birth, but this cannot be concluded to be the effect of vaccination. Since vaccination is the sole prevention for infectious diseases, it is unavoidable. Therefore, further research on reproduction related side effects after simultaneous vaccination are needed, and the findings of this study may provide valuable data to support such investigations.

Keywords: Cattle; rumen; biosensing techniques; monitoring, physiologic; telemetry

ORCID iDs
Jisu Kim
https://orcid.org/0009-0001-3459-5271
Jae-Jung Ha
https://orcid.org/0000-0001-6785-6346
Mirae Kim
https://orcid.org/0009-0004-9620-5832
Woo-Sung Kwon
https://orcid.org/0000-0002-0848-7189
Junkoo Yi
https://orcid.org/0000-0003-2593-6529
Daehyun Kim
https://orcid.org/0000-0002-4820-4438

Author Contributions
Conceptualization: Kim J, Ha JJ, Kim M, Kwon WS, Yi J, Kim D; Data curation: Kim J, Ha JJ, Kim M, Kwon WS, Yi J, Kim D; Formal analysis: Kim J, Ha JJ, Kim M, Kwon WS, Yi J, Kim D; Funding acquisition: Kim D; Investigation: Kim J, Ha JJ, Kim M, Kwon WS; Methodology: Kim J, Ha JJ, Yi J, Kim D; Project administration: Kim J, Ha JJ, Yi J, Kim D; Resources: Kim M, Kwon WS; Software: Kim J, Ha JJ, Kim M, Kwon WS; Supervision: Yi J, Kim D; Validation: Yi J, Kim D; Visualization: Kim J, Ha JJ; Writing - original draft: Kim J, Ha JJ; Writing - review & editing: Kim J, Ha JJ, Kim M, Kwon WS, Yi J, Kim D.

Conflict of Interest
The authors declare no conflicts of interest.

Data Availability Statement
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious disease in cloven-hooved animals caused by the FMD virus, which belongs to the Apthovirus genus of the *Picornaviridae* family [1,2]. The disease is mainly transmitted through contact with infected animals, exposure to contaminated vectors, and inhalation of the virus [3,4]. FMD is characterized by clinical symptoms such as fever, lameness, and blisters on the mouth and feet [5]. Vaccination is the most effective prevention strategy [6]. However, it may cause side effects such as abortion, delayed ovulation, acute immune response, elevated body temperature, reduced milk production, and skin lesions [7-9].

Lumpy skin virus, a member of the Capripoxvirus genus in the *Poxviridae* family, causes lumpy skin disease (LSD) [10]. LSD is mainly transmitted through insects and arthropods, including Aedes aegypti mosquitoes, stable flies, and ticks [11]. LSD primarily causes skin nodules and major economic losses for farmers by increasing abortion and stillbirth rates, reducing milk production, restricting animal movement, and lowering leather value [12,13]. Similar to FMD, vaccination is the most effective way to prevent LSD and its associated damages [14]. Reported side effects of the LSD vaccine include reduced feed intake, fever, reduced milk production, skin nodules, Neethling reactions, and others [15-17].

Changes in body temperature are the most easily detectable clinical symptoms and vaccine side effects [18,19]. Increased body temperature can reduce conception rates, cause early embryonic death, and disrupt hormone secretion, decreasing reproductive function [20,21]. Cow body temperatures can be measured in various ways: vaginal temperature using a vaginal temperature sensor, body temperature using infrared thermal imaging and auxiliary sensors, and ruminoreticular temperature using a rumen-insertable biosensor [22,23]. Typically, the rumen-insertable biosensor provide averaged body activity and ruminoreticular temperature every 5 to 10 min [24,25]. Based on the collected data, various information can be analyzed, including cow estrus, parturition, and ruminoreticular temperature changes before and after vaccination [8,9,26-28].

LSD emerged in South Korea in October 2023, so LSD and FMD vaccines have only been required for 1 year [29,30]. This highlights the need for a simultaneous vaccination approach to improve convenience for farmers, reduce costs, and increase efficiency. A study reports acute immune responses after simultaneous FMD and LSD vaccination in Hanwoo cows. The study reports that Acute-phase proteins, haptoglobin, and serum amyloid A levels peaked on day 3 post-FMD vaccination and then returned to normal. The LSD vaccine group showed gradual protein increases up to 10 days post-vaccination, while the simultaneous vaccination group maintained elevated levels from days 3–10 [31]. Furthermore, a study reports that simultaneous FMD and sheeppox-goatpox vaccination induced antibody titers [32].

In this study, rumen-insertable biosensors were utilized to analyze ruminoreticular temperature and body activity before and after simultaneous LSD and FMD vaccination. In cows, increased body temperature can have negative effects such as abortion and stillbirth [7,21]. cows. Previous research has predominantly focused on immune responses, such as acute phase proteins and antibody titers. However, temperature and body activity represent more practical indicators that can be readily monitored under field conditions. Therefore, the present study concentrated on evaluating these two parameters to assess physiological responses following vaccination. Also, no studies have analyzed ruminoreticular temperature

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and body activity changes after simultaneous FMD and LSD vaccination based on pregnancy status in Hanwoo. Accordingly, this study aims to analyze the changes in ruminoreticular temperature and body activity following vaccination status (control, FMD vaccination, LSD vaccination, simultaneous FMD and LSD vaccination).

METHODS

Experimental animal

This study is a prospective observational cohort study of 63 Hanwoo cows—23 pregnant (100–175 days) and 40 nonpregnant—obtained from the Gyeongsangbuk-do Livestock Technology Research Institute in 2024. As for the experimental axis, a ruminoreticular sensor was inserted among Hanwoo cows in the research institute, and in the case of pregnant cows were selected. Cows that have received other vaccines within the last 30 days and those with diseases were excluded. **Tables 1** and **2** show the status of the experimental groups based on the number of cows per group, pregnancy duration, and months of age. All studies were approved by the Ethics Committee of the Gyeongsangbuk-do Livestock Research Institute (approval No. 140). In addition, the experiment was conducted according to the checklist of Reporting Guideline, STROBE-Vet (observer).

The cows were raised following the Korean feeding standards program and housed in a facility equipped with stanchions. During the experiment, cows had free access to rice straw, mineral blocks, and water, with sufficient space.

Ruminoreticular temperature and body activity

Ruminoreticular temperature and body activity were measured using a Classic Volus, rumen insertable biosensor (smaXtec Co., Austria). This device has been tested and certified for gastric fluid resistance by the German Agricultural Society (DLG). This biosensor measures 132 mm in length and 35 mm in diameter, and has a battery life of approximately 5 years. This Volus is inserted directly into the rumen through the oral cavity of the cattle using a dedicated insertion tool. It measures ruminoreticular temperature, water intake volume cycle, and body activity every 10 min, transmitting data to the base station. Collected data can be analyzed

Table 1. Information for pregnancy groups (n = 23)

Groups	No. of cows	Age (mon)	Parity	Pregnancy period (day)
Pregnant control	1	26.10 ± 0.00	0.00 ± 0.00	166.00 ± 0.00
Pregnant FMD	4	43.70 ± 4.24	1.75 ± 0.25	164.40 ± 5.00
Pregnant LSD	10	45.53 ± 4.98	1.50 ± 0.34	159.00 ± 0.00
Pregnant FMD/LSD	8	35.73 ± 4.03	1.13 ± 0.23	133.38 ± 6.07
Total	23	40.96 ± 2.82	1.35 ± 0.18	152.74 ± 4.22

The number of cows per group, days of pregnancy, month of age, and parity are listed (mean ± standard error of the mean).
FMD, foot-and-mouth disease; LSD, lumpy skin disease.

Table 2. Information for nonpregnancy groups (n = 40)

Groups	No. of cows	Age (mon)	Parity
Nonpregnant control	5	100.51 ± 8.34	3.00 ± 0.84
Nonpregnant FMD	5	48.86 ± 5.85	1.55 ± 0.37
Nonpregnant LSD	11	62.74 ±10.99	2.60 ± 0.81
Nonpregnant FMD/LSD	19	46.01 ± 3.83	1.37 ± 0.27
Total	40	55.70 ± 4.02	1.78 ± 0.23

The number of cows per group, month of age, and parity are listed (mean ± standard error of the mean).
FMD, foot-and-mouth disease; LSD, lumpy skin disease.

using the smaXtec-Cloud TruD algorithm and accessed via a smartphone or computer application. When disconnected from the base station, data is stored in internal memory for up to 6 days. The device measures ruminoreticular temperature with ± 0.01°C accuracy. Body activity was measured using an intraruminal smaXtec bolus equipped with an accelerometer and expressed in V. This value represents a device-specific output provided by the system and was interpreted as a relative indicator of movement.

Ruminoreticular temperature and body activity were recorded every 10 min from days 1 (before vaccination) to day 10 (post-vaccination). The collected data were compared and analyzed based on pregnancy status and vaccination. For trend analysis, ruminoreticular temperatures were averaged at 6 and 12 h, respectively.

Vaccination

A 38 mm, 18 G syringe was used to inject 2 mL of FMD vaccine intramuscularly into the neck muscles, and 2 mL of LSD vaccine subcutaneously under the neck skin.

The unvaccinated group, labeled as "Control," was sham intramuscular (IM) injection (saline IM) to stimulate injection-related stress. The groups vaccinated against FMD, LSD, as well as FMD/LSD only, were labeled "FMD," "LSD," and "FMD/LSD," respectively (**Figs. 1-4**, **Tables 1** and **2**).

The FMD vaccine used in this experiment was BIOAFTOGEN (Biogénésis Bagó FMD vaccine, O1 Campos, A24 Cruzeiro, A2001 Argentina; Buenos Aires, Argentina). The FMD-only and FMD/LSD groups were administered a single 2 mL IM injection of the FMD vaccine.

The LSD vaccine used in this experiment was LUMPYVAC (VETAL Animal Health Products Inc., Türkiye). The LSD-only and FMD/LSD groups were administered a single 2 mL subcutaneous dose of the LSD vaccine.

Statistical analysis

Statistical analyses were performed using SPSS ver 29.0.2.0 (IBM, USA). Mean ruminoreticular temperature and body activity before and after vaccination were compared and analyzed across the four groups using analysis of covariance. The analyses included parity and age as covariates affecting each outcome.

RESULTS

Changes in ruminoreticular temperature (°C) after vaccination

Changes in ruminoreticular temperature (°C) after vaccination in pregnant cows

To confirm the overall ruminoreticular temperature trend, the measured temperatures were averaged every 6 h and analyzed comparatively (**Fig. 1**). All experimental groups of pregnant cows showed increased ruminoreticular temperature after vaccination. Ruminoreticular temperature in the FMD group remained elevated for up to 5 days post-vaccination. The temperature in the LSD group remained elevated for up to 6 days post-vaccination. In the FMD/LSD group, the temperature returned to normal levels on day 3 post-vaccination. Compared to the single vaccinated group, the FMD/LSD group showed a greater post-vaccination increase and a faster return to normal levels (**Fig. 1A**).

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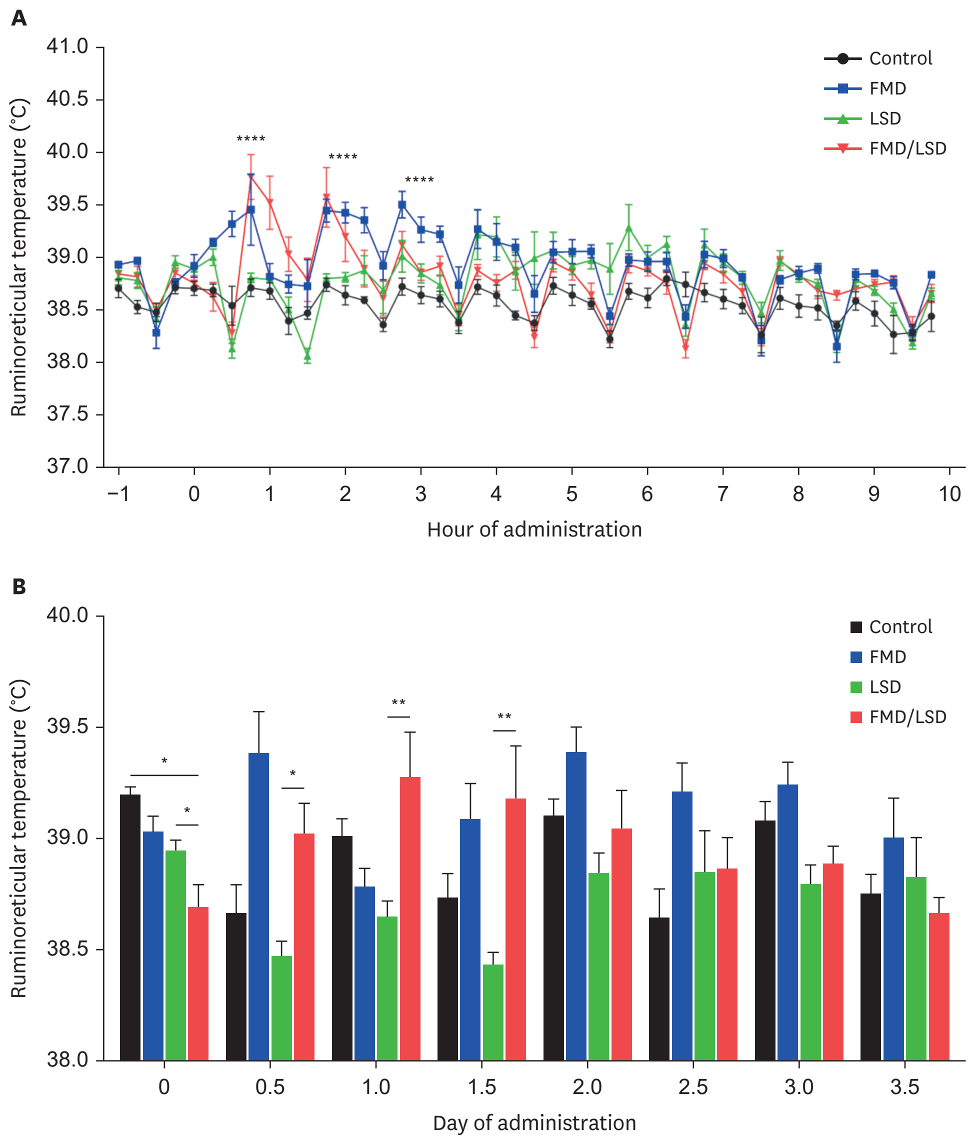

Fig. 1. Changes in the ruminoreticular temperature (°C) in pregnant cows. (A) Change in the 6-h average ruminoreticular temperature in pregnant cows (n = 23). Analysis was performed from day 1 (before vaccination) to day 10 post-vaccination. Ruminoreticular temperature was recorded every 10 min and averaged every 6 h. The black line connected by "●" represents the mean temperature of the Control group (sham injection). The blue line connected by "■" indicates the mean temperature of the FMD vaccination group. The green line connected by "▲" denotes the mean temperature of the LSD vaccination group. The red line connected by "▼" represents the mean temperature of the FMD/LSD vaccination group. (B) Change in the 12-h average ruminoreticular temperature in pregnant cows (n = 23). Analysis was performed from day 1 (before vaccination) to day 4 post-vaccination. Ruminoreticular temperature was recorded every 10 min and averaged every 12 h. The bars represent the average ruminoreticular temperatures of the Control, FMD, LSD, and FMD/LSD groups. The ruminoreticular temperature trends were observed across all groups of pregnant cows. Day 0 indicates the day of vaccination. Error bars represent the standard error of the mean.
FMD, foot-and-mouth disease; LSD, lumpy skin disease.
Statistics: analysis of covariance with age and parity as covariates; *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.

As the pregnant control group included only one animal, results related to this group are presented for descriptive purposes only and should be interpreted with caution. Analysis of covariance revealed significant group effects on day 1, day 2, day 3, and day 4 (day 1: $F[2,16] = 3.92$, $p = 0.039$), and more detailed statistical results for group effects are shown in

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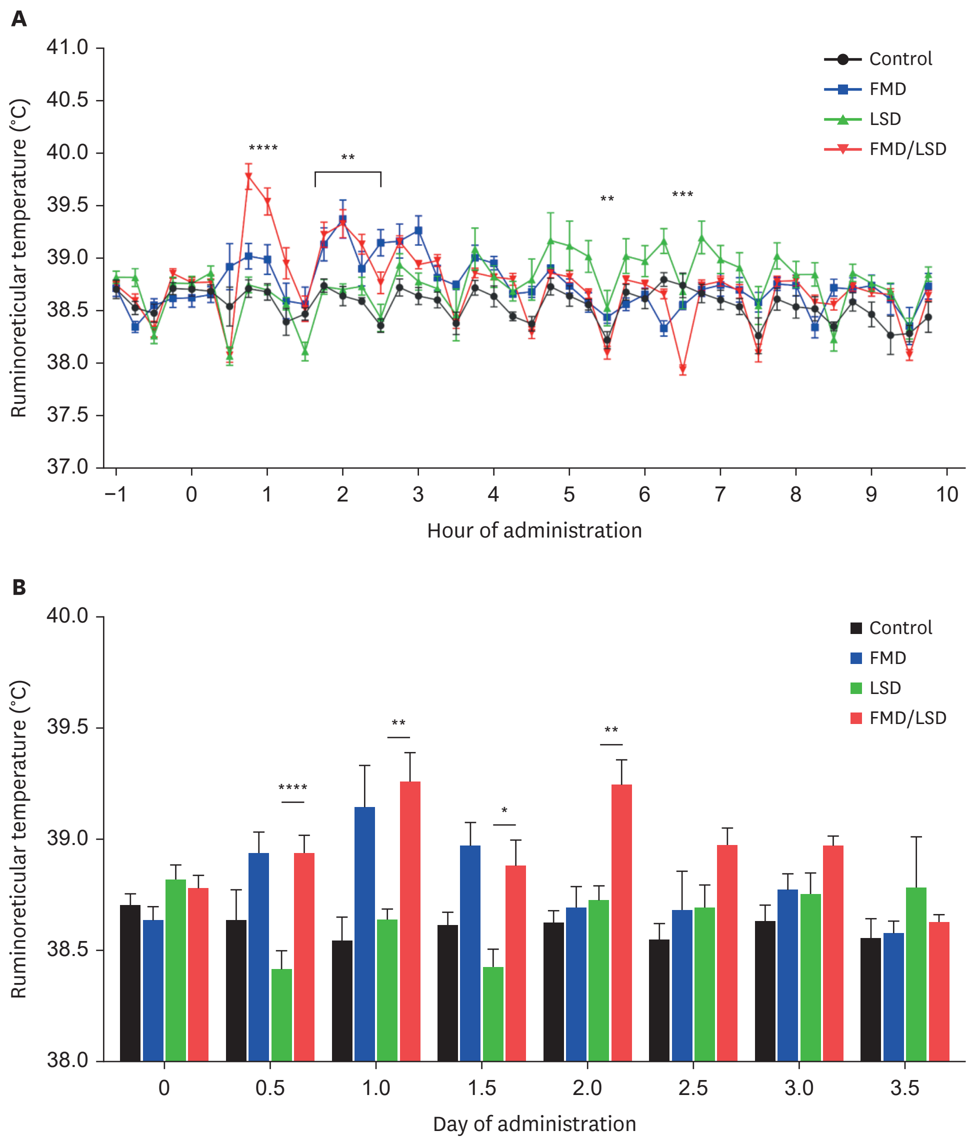

Fig. 2. Changes in the ruminoreticular temperature (°C) in nonpregnant cows. (A) Change in the 6-h average ruminoreticular temperature in nonpregnant cows (n = 40). (B) Change in the 12-h average ruminoreticular temperature in nonpregnant cows (n = 40). Analytical methods, time point definitions, and graphical formatting are identical to **Fig. 1** with the only difference being the nonpregnancy status of the cows. Error bars represent the standard error of the mean.
FMD, foot-and-mouth disease; LSD, lumpy skin disease.
Statistics: analysis of covariance with age and parity as covariates; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$.

supplementary **Table 1**. The FMD/LSD group was compared to other groups at 12-h intervals (day 0.5 = 12 h) from the day of vaccination to day 4 (**Figs. 2** and **4**). The FMD/LSD group had ruminoreticular temperatures of up to 0.75°C, and at least 0.55°C higher than those in the LSD group from day 0.5 (12 h) post-vaccination to day 1 ($p < 0.05$ and $p < 0.01$; **Fig. 1B**).

Changes in ruminoreticular temperature (°C) after vaccination in nonpregnant cows

In nonpregnant cows, ruminoreticular temperatures increased post-vaccination in the FMD/LSD and FMD groups. The LSD group showed no immediate increase in ruminoreticular temperature but exhibited a gradual increase from days 3–9. The FMD group reached peak ruminoreticular temperature on day 2 post-vaccination, returning to normal levels after

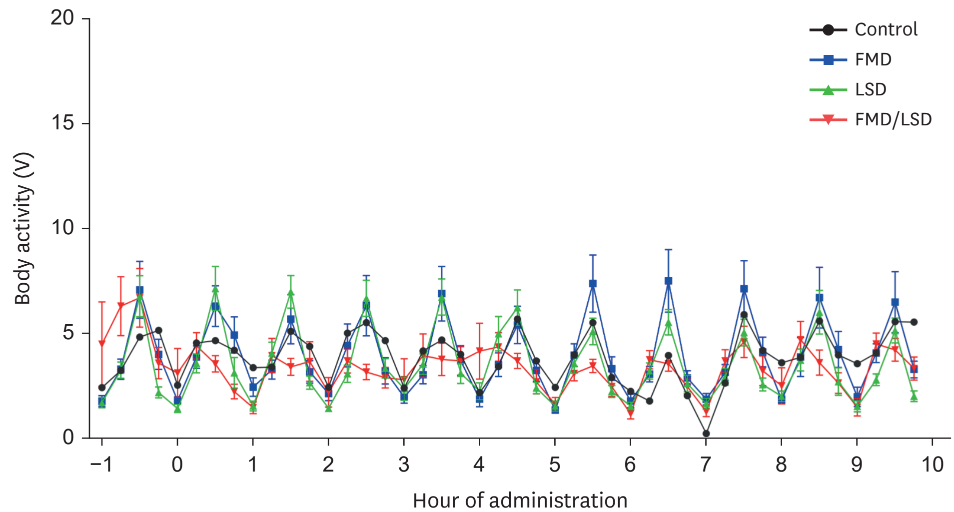

Fig. 3. Change in body activity (V) in pregnant cows. Changes in body activity following FMD and LSD vaccination (n = 23). Figure structure (lines, symbols, color and error bar) and analytical setting are identical to those in **Fig. 1**, and this figure presents data from body activity of pregnant cows. Error bars represent the standard error of the mean. No significant differences were detected among groups.
FMD, foot-and-mouth disease; LSD, lumpy skin disease.

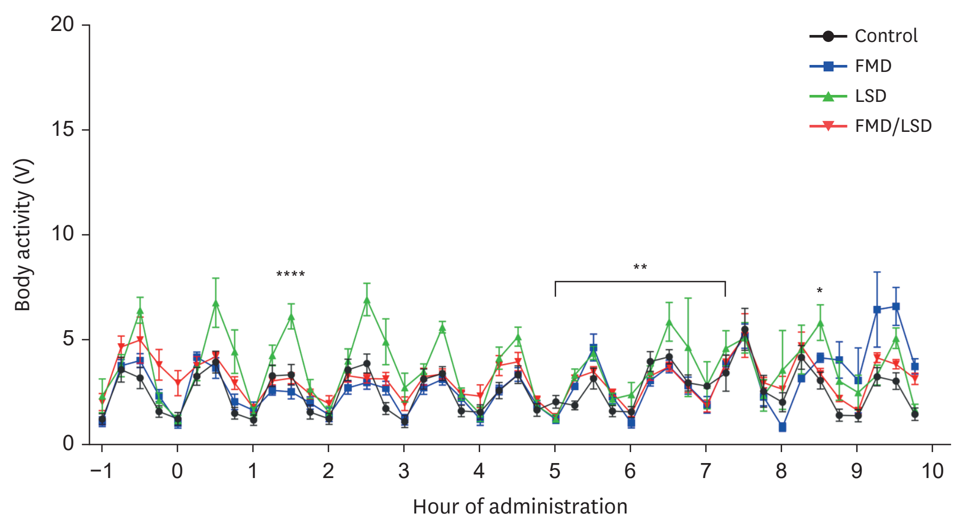

Fig. 4. Change in body activity (V) in nonpregnant cows. Changes in body activity following FMD and LSD vaccination (n = 40). Figure structure (lines, symbols, color and error bar) and analytical setting are identical to those in **Fig. 1**, and this figure presents data from body activity of nonpregnant cows. Error bars represent the standard error of the mean.
FMD, foot-and-mouth disease; LSD, lumpy skin disease.
Statistics: analysis of covariance with age and parity as covariates; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$.

day 4. The FMD/LSD group showed a rapid post-vaccination increase that normalized after day 3 (**Fig. 2A**).

The FMD/LSD group showed elevated ruminoreticular temperature from day 0.5 (12 h) to day 2 post-vaccination. Their temperature was significantly higher than that of the LSD group, with differences ranging from 0.45°C to 0.61°C ($p < 0.05$, $p < 0.01$, and $p < 0.0001$; **Fig. 2B**). More detailed statistical results for group effects are shown in **Supplementary Table 1**.

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Changes in body activity (V) after vaccination

Changes in body activity (V) after vaccination in pregnant cows

No significant difference in body activity was observed between the control and experimental groups of pregnant cows (**Fig. 3**, **Supplementary Table 1**).

Changes in body activity (V) after vaccination in nonpregnant cows

In nonpregnant cows, the FMD/LSD group showed significantly lower body activity than those in the FMD and LSD groups on day 1 and days 5–7 ($p < 0.05$, $p < 0.01$, and $p < 0.0001$). However, no significant difference was observed between the FMD/LSD and control groups (**Fig. 4**, **Supplementary Table 1**).

DISCUSSION

Body temperature is a key physiological indicator of health and reproduction in cows. For example, body temperature can detect estrus, parturition, and body temperature rise after vaccination [8,9,27,33-35]. There have been many reported ways to measure the temperature of cows, including sensors in the rumen, vaginal temperature sensor, rectal temperature probe and infrared skin surface temperature sensor [36-39]. Each sensor shows different body temperatures, and rumen sensors, vaginal sensors, and rectal sensors show a high correlation with each other [40,41]. Therefore, measuring the body temperature of cows using rumen sensors is considered suitable for analyzing physiological indicators.

According to the results of this study, ruminoreticular temperatures tended to rise after vaccination, especially the ruminoreticular temperature increased sharply in cows that were vaccinated simultaneously with FMD and LSD vaccines. A rise in body temperature can lead to early embryonic loss, abortion and stillbirth [42-44]. There is a possibility that vaccination-induced body temperature increases will affect these declines in reproduction. However, the grounds that vaccination directly causes abortion or stillbirth are very limited, so more systematic research is needed, and body temperature monitoring in pregnant cows is still an important factor.

After FMD and LSD vaccination, the body temperature increases [15,16,27] due to fever induced by elevated cytokine levels [45,46]. Studies report that ruminoreticular temperature and acute immune response can increase for up to 3 days after FMD vaccination, peaking during this period [27]. Body temperature increases for up to 8 days post-vaccination [17,47], with acute immune response peaking on day 10 [31]. These findings indicate a possible correlation between post-vaccination changes in body temperature and acute immune response [9,17,27,31,45-47].

In a previous study [31], acute-phase immune protein levels peaked on day 3 post-vaccination in the FMD group and on day 10 in the LSD group. This pattern is similar to the ruminoreticular temperature increases observed in the single-vaccination groups in this study. Kim et al. [31] also report that the acute immune response in the FMD/LSD group remained elevated from days 3–10. As mentioned earlier, we hypothesized that the acute immune response may be correlated with clinical signs such as elevated body temperature after vaccination [9,17,27,31,47]. However, in this study, the ruminoreticular temperature of the FMD/LSD group significantly increased by day 3 and normalized faster than that of the single vaccination groups. Possible factors include vaccination dose, experimental timing, and individual variation [17,47-50]. Therefore, analyzing body temperature changes, immune response, and protective effects after simultaneous vaccination is essential.

In this experiment, there was only one control group for pregnant cows. Due to the limitations of securing pregnant cows with ruminoreticular sensors and the situation in the field, which had not been long after the occurrence of LSD, only one could be used as a control group. The physiological response observed in that cow can only be used as a reference for the normal physiological response of unvaccinated pregnant cow and can only be used to compare the tendency of ruminoreticular temperature change with the vaccination group. However, due to the extremely small and imbalanced sample size in pregnant cows, particularly the inclusion of only a single control animal (n = 1), statistical interpretation and generalization should be approached with caution. Therefore, further studies with larger and more balanced populations are required to validate these findings.

Simultaneous vaccination improves convenience and efficiency for farmers. However, the approach to simultaneous vaccination should be applied cautiously due to potential side effects. Therefore, further studies on reproduction-related side effects and immune effects after simultaneous vaccination are needed, and the findings of this study may serve as foundational data.

SUPPLEMENTARY MATERIAL

Supplementary Table 1

Detailed statistical results for intergroup analysis parameters

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