

# Improvement of conception rate on Hanwoo; The key hormones and novel estrus detector

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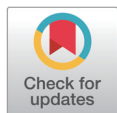
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Received: Jul 28, 2021

Revised: Oct 13, 2021

Accepted: Oct 25, 2021

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## Abstract

Two field experiments were conducted to improve the conception rate of Hanwoo cow. The first experiment aimed to investigate the physiological condition of Hanwoo cows on estrus, including metabolic profiles and body condition score (BCS). The second experiment investigated the effect of a novel estrus detector on the artificial insemination (AI) conception rate for Hanwoo cows. For the first experiment, 80 Hanwoo cows ( $2.5 \pm 0.10$  of parity), approximately one month before estrus, were housed in 16 pens and offered the experimental diets twice daily with free water access. The BCS were recorded, and blood was collected from the jugular veins just before AI. The collected blood was used to measure physiological conditions, such as metabolite and hormone levels. For the second experiment, each cow was equipped with a neck-mounted estrus detector collar, which had a sensor connected through the internet. Approximately one month before estrus, three hundred sixty Hanwoo cows ( $2.4 \pm 0.21$  of parity) were assigned into groups with or without W-Tag collar treatments. The animals were managed the same as in the first experiment. The pregnancy rate reached 55% in the first experiment. The concentration of luteinizing hormone (LH) was higher ( $p < 0.012$ ; 1.56 vs. 1.08 ng/mL) in cows that were not pregnant (NPG) than in cows that were pregnant (PG) after AI. The BCS and other concentrations of metabolites and hormones in the blood were not different in both NPG and PG cows. The ranges of estrogen, LH, and follicle-stimulating hormone for PG cows were 11.9 to 39.0 pg/mL,  $< 0.25$  to 1.98 ng/mL, and  $< 0.50$  to 0.82 ng/mL, respectively. In the second experiment, cows with the estrus detector had lower days open ( $p < 0.001$ ; 78.1 vs. 84.8 d), insemination frequency ( $p < 0.001$ ; 1.26 vs. 2.52), and return of estrus ( $p < 0.001$ ; 70.9 vs. 79.1 d) than those in cows without the estrus detector. In conclusion, the present study indicated that lower LH concentration just before AI potentially increased the pregnancy rate of Hanwoo cows. Furthermore, the application of estrus detectors to Hanwoo cows could improve the conception success rate for AI.

**Keywords:** Artificial insemination, Conception rate, Estrus detector, Hanwoo cow, Luteinizing hormone

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#### Competing interests

No potential conflict of interest relevant to this article was reported.

#### Funding sources

This research was supported (Project No. 315019-05-2-SB030) by IPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), and Ministry of Agriculture, Food and Rural Affairs, Korea.

#### Acknowledgements

Not applicable.

#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

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#### Ethics approval and consent to participate

This research has been approved by administration office of Gyeongsang National University, Jinju, Korea under the animal care and use guidelines of the Animal Research Unit (GNU-191011-E0050).

## INTRODUCTION

Artificial insemination (AI) is the most common breeding method for Hanwoo cattle in Korea [1]. In 2019, total of Hanwoo cattle were reported around 3.48 million, which 90% of Hanwoo cows applied AI technique for breeding program [1]. In the field, the breeder usually follows visual symptoms (i.e., animal behavior) to predict the time of ovulation, such as mounting, restlessness, noisiness, pink and swollen vulva, or decrease milk production [2,3]. Thus, the success of AI commonly depends on the breeder's ability to identify the visual symptoms of cows during their estrus period [4]. During estrus, the visual symptoms of cows are caused by an increase of estrogen and a decrease in progesterone [3]. Follicle-stimulating hormones (FSH) and luteinizing hormones (LH), secreted by the pituitary gland, together stimulate the development of follicular growth and trigger ovulation [5]. Estrogen, FSH, and LH are the hormones with the highest concentrations when AI is performed on cows [3,5]. However, the key hormone related to the success rate of AI for Hanwoo cows in the field is unclear. Even though AI can be conducted based on the visual symptoms displayed by Hanwoo cows, it does not always lead to optimal timing. Errors regarding estrus detection could decrease the conception rate from AI, and lead to increased cost [6].

In general, the optimal time for AI is approximately 12–15 hours after the first appearance of estrus symptoms [7,8]. However, the detection of the first estrus symptoms is difficult to ascertain accurately. For this reason, instruments to detect estrus period onset in cows have been developed and tested in previous studies [8,9]. The use of an estrus detector in the field can help breeders detect the first appearance of estrus, which can lead to an increase in conception rate from AI [3,6]. Recently, there are several methods to predict the first appearance of estrus, such as observing body and vaginal temperature, rumination time, lying behavior, mounting and standing-to-be-mounted behavior, and physical activity [8,9,10]. From 2010 to 2018, pedometer and neck mounted collar were the most studied tools of estrus detection by previous researchers [8,9]. In the principle, the differences in physical activity between non-estrus and estrus cows can predict the first appearance of the estrus period [7,8]. However, limited studies have been conducted to evaluate the effect of an estrus detector on AI success rate for Hanwoo cows in the field.

Therefore, two field trials were conducted in the present study to determine the key hormones and the effect of a novel estrus detector on Hanwoo cow AI conception rate.

## MATERIALS AND METHODS

The animal procedures for the first and second experiments were approved by the administration office of Gyeongsang National University, Jinju, South Korea, under the animal care and use guidelines of the Animal Research Unit (GNU-191011-E0050).

### First experiment

#### Animal, diet, and management

This experiment was conducted at Hapcheon Livestock Industry Cooperatives (Hapcheon, Korea). A total of 80 Hanwoo cows ( $2.5 \pm 0.10$  of parity;  $40 \pm 3$  mo of age), approximately a month before estrus, were assigned for this experiment. Five cows were randomly housed in each pen (5 m × 10 m). The pens were installed with a feeder on a slatted floor. The animals were fed a total mixed ration (TMR) that was formulated with iso-nitrogenous and iso-caloric nutrients to supply the nutrient requirements of cows (Table 1), in accordance with the Korean Feeding Standards for Korean Cattle by the National Livestock Research Institute [11]. The ingredients and chemical compositions of TMR are presented in Table 1. During maintenance, the basal diet (3 kg) with rice

**Table 1.** Ingredients and chemical compositions of total mixed ration

Item	Basal diet	Rice straw
Ingredients (%)		
Protein concentrate	8.00	
Corn flakes	6.00	
Soybean meal	3.50	
Soybean hulls	5.00	
Italian ryegrass silage	7.00	
Corn silage	6.00	
Annual rye straw	10.0	
Tall fescue hay	7.00	
Brewers grain	10.0	
Spent mushroom	20.0	
Tofu by-product	4.50	
Rice bran	3.00	
Premix <sup>1)</sup>	0.20	
Microbes	0.20	
Molasses	3.00	
Water	6.60	
Chemical composition (% DM)		
Dry matter	60.8 ± 1.22 <sup>2)</sup>	87.2 ± 0.51
Crude protein	13.1 ± 0.31	4.88 ± 0.24
Ether extract	5.43 ± 0.21	2.02 ± 0.18
Crude ash	10.6 ± 0.76	12.4 ± 0.58
Neutral detergent fiber	33.6 ± 0.38	61.1 ± 0.55
Acid detergent fiber	11.5 ± 0.67	46.5 ± 0.36

<sup>1)</sup>One kilogram contained the following: vitamin A, 450,000 IU; vitamin D<sub>3</sub>, 300,000 IU; vitamin E, 25,000 IU; vitamin K<sub>3</sub>, 500 mg; vitamin B<sub>1</sub>, 200 mg; vitamin B<sub>12</sub>, 13 mg; pantothenic acid, 40 mg; niacin, 30 mg; biotin, 20 mg; folic acid, 10 mg; FeSO<sub>4</sub>, 3,500 mg; CoSO<sub>4</sub>, 150 mg; CuSO<sub>4</sub>, 4,500 mg; MnSO<sub>4</sub>, 2,000 mg; ZnSO<sub>4</sub>, 2,500 mg; I, 400 mg; Se (Na), 150 mg.

<sup>2)</sup>Mean ± SD.

straw (1.5 kg) were fed twice daily at 08:00 h and 17:00 h. Water was offered *ad libitum* during the experimental period. About 500 g of TMR was sub-sampled to analyze its chemical compositions. For conducting AI, the onset of estrus from Hanwoo cows was predicted based on visual appearance such as mounting, restlessness, noisiness, and so on. After detecting the onset of estrus, AI was carried out about 12 to 15 h later. At the onset of the estrus period, just before AI, the body condition score (BCS) of each Hanwoo cow was recorded according to Wildman et al. [12]. Furthermore, 10 mL of blood was collected from the jugular vein 3 h after morning feeding. Blood samples were collected in syringes and injected into tubes containing clot activator. The treated blood samples were placed in a gel separator (Vacuette Z Serum Sep Clot Activator, Greiner Bio-One, Kremsmünster, Austria), and they were immediately stored on ice before delivery to the laboratory. Blood plasma samples were obtained by centrifuging blood at 969×g for 15 min at 4°C (SUPRA21K, Hanil Science Industrial, Incheon, Korea) and stored at -20°C until subsequent analyses. Blood plasma was used for the analyses of blood metabolites and hormones. The pregnancy status of the 80 cows studied was noted 2 months after AI. Non-pregnant cows were labeled as “NPG” and pregnant cows were labeled as “PG”.

### **Laboratory analysis**

The sub-sampled TMR was dried at 65 °C for 48 h and ground fine enough by a cutting mill to pass through a 1-mm screen (Shinmyung Electric, Gimpo, Korea), in accordance with a previous study [13]. The dry matter (DM) concentration was analyzed using a forced-air drying oven at 105 °C for 24 h. Crude ash (CA) was determined by a muffle furnace at 550 °C for 5 h. Crude protein (CP) and ether extract (EE) were measured by the producers of Kjeldahl (method 984.13 of AOAC [14]) and Soxhlet (method 920.39 of AOAC [14]), respectively. Neutral detergent fiber (NDF; method 2002.04 of AOAC [14]) and acid detergent fiber (ADF; method 973.18 of AOAC [14]) were determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY, USA).

Pregnancy diagnosis was determined using the rectal palpation method designed by Cowie [15]. Collected plasma was used to analyze the metabolite parameters consisting of blood urea nitrogen (BUN) and glucose, and the hormone parameters consisting of prolactin, estrogen, LH, FSH, and progesterone. The plasma concentration of BUN was determined using a UREA/BUN kit (Roche, Basel, Swiss). An enzymatic kinetic assay was used to determine the plasma concentrations of glucose (GLU kit; Roche). An electrochemiluminescence immunoassay (ECLIA) was used to determine the concentrations of plasma prolactin, estrogen, LH, FSH, and progesterone (ELISA kit, Roche).

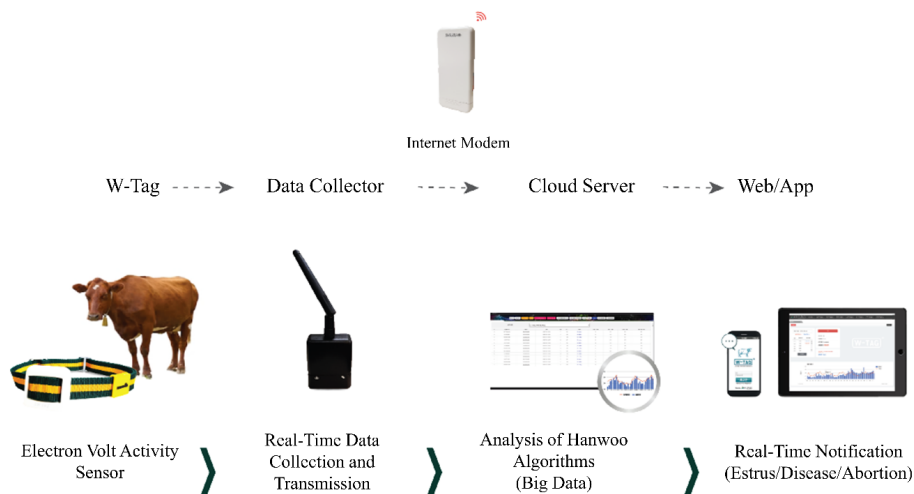
### **Second experiment**

#### **Estrus detector**

A novel estrus detector, the W-Tag estrus detector (Wuyang, Jeonju, Korea), was applied in this study. For preparation, an internet modem was installed in the cattle house. An accelerometer for the W-Tag estrus detector, using an IBSO3 model, (Ingics Technology New Taipei City, Taiwan) was installed into neck-mounted collars to record the physical activity of the cows in real-time. The physical activity of each cow was recorded as an electron volt (eV). Using the internet of things (IoT) approach, every physical activity of the cows paired with W-Tag estrus detectors was recorded and transferred automatically into a data collector through the internet. In principle, a cow has different physical activity during the estrus period, compared to the rest of its herd [16]. Thus, the algorithm data among estrus and non-estrus cows was analyzed by a cloud server to predict the first appearance of the estrus period and the optimal time to conduct AI. Estrus status, and the optimum time for AI can be predicted using W-Tag estrus detectors which can be accessed by devices, such as smartphones or computers. The system of W-Tag estrus detection is presented in Fig. 1. The W-Tag estrus detector is recommended to be applied at -20°C to 60°C, with maximum humidity of 95%.

#### **Animal, diet, and management**

An experiment was conducted from September 2019 to December 2020 at five beef cattle farms in Hapcheon, Korea. A total of 360 Hanwoo cows ( $2.4 \pm 0.21$  of parity), with varied ages and body weights, were used in this second study. Approximately one month before estrus, 100 cows were applied with W-Tag estrus detectors, and 260 cows were not applied with W-Tag estrus detectors, as a control group. Cows with W-Tag estrus detectors were randomly located among five Hanwoo farms. Five Hanwoo cows were placed in each pen (5 m × 10 m). All pens were installed with a feeder on a slatted floor. Animal diet and management followed the same procedure for each beef cattle farm. The diet was formulated based on Korean Feeding Standards to maintain the health of the animals [11]. Water was offered *ad libitum* in all farms during the experimental period.



**Fig. 1.** The module of W-Tag estrus detector system for Hanwoo cows. W-Tag, neck-mounted collars; Web/APP, website or mobile application (Second experiment).

### Laboratory analysis

The days open, insemination frequency, and onset of estrus were evaluated using a novel estrus detector. The days open and insemination frequency were examined from postpartum to the time before insemination. The return of estrus was examined from postpartum to the first appearance of estrus.

### Statistical analysis

Data from first experiment consisting of BCS, metabolite profiles, and hormones were analyzed using an analysis of variance (ANOVA) by PROC ANOVA from Statistical Analysis System (SAS, version 9, Cary, NC, USA) [17]. Data from second experiment consisting of the days open, insemination frequency, and return of estrus were also analyzed using same procedure of SAS in the first experiment. Mean separation was analyzed using Tukey's test and significant differences were declared at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### First experiment

The ingredients and chemical compositions of basal diet and rice straw are shown in Table 1. Concentrations of DM, CP, and NDF from the basal diet and rice straw were 60.8%, 13.1%, and 33.6% and 87.2%, 4.88%, and 61.1%, respectively. Of the 80 Hanwoo cows, 44 cows were reported to be pregnant after AI (Table 2). The pregnancy rate was 55% after AI in the first experiment. Several studies have reported that the pregnancy rate of a beef cow from AI is around 40% to 60% [5,18]. Thus, the result of the pregnancy rate in the present study was in the range of the average rate. A varied pregnancy rate for Hanwoo cows in the field could be caused by the different competences of farmers for detecting the estrus period and different methods of estrus synchronization [5,18].

Just before AI, the concentration of LH was higher in NPG cows than in PG cows (1.09 vs. 1.56 ng/mL; respectively,  $p = 0.012$ ). Similar LH, estrogen, and FSH also reached their peaks during the estrus period [2,3,5,6]. However, the concentrations of both hormones before AI did

**Table 2.** Body condition score, and the physiological condition of metabolites and hormones from Hanwoo cows on just before artificial insemination in the first experiment (n=80)

Item	Hanwoo cow <sup>1)</sup>		SEM	p-value
	NPG	PG		
Pregnant number (head)	36	44	-	-
Body condition score	2.74	2.73	0.381	0.899
Metabolites (ng/mL)				
Blood urea nitrogen	11.1	12.9	3.157	0.237
Glucose	46.3	45.8	12.60	0.866
Hormones				
Prolactin (ng/mL)	0.05	0.05	-	-
Estrogen (pg/mL)	23.7	19.9	10.90	0.183
LH (ng/mL)	1.56 <sup>a</sup>	1.08 <sup>b</sup>	0.794	0.012
FSH (ng/mL)	1.00	0.99	0.058	0.234
Progesterone (ng/mL)	23.0	27.9	5.915	0.146

<sup>1)</sup>NPG, cows with non-pregnancy status after AI; PG, cows with pregnancy status after AI. The pregnancy status was determined after 2 mo of AI.

<sup>a,b</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).

LH, luteinizing hormone; FSH, follicle-stimulating hormone; AI, artificial insemination.

not influence the conception rate in the present study. There were no differences in estrogen and FSH between NPG and PG cows. Generally, FSH stimulates the development of follicle growth and increases estrogen levels, which cause estrus [3,5]. LH is responsible for follicle maturation and the releasing of follicles from the ovary [3,5]. This present study revealed that a lower concentration of LH just before AI could increase the conception rate of Hanwoo cows. According to Lamb et al. [5], the presence of LH promotes ovulation and stimulates the corpus luteum to release progesterone. The concentration of LH is likely to decrease after ovulation. Then the progesterone concentration will show the opposite trend. In another breed, such as Shorthorns and Angus, the peak concentration of LH could reach 42 ng/mL, while the concentration would decline between 1.70 to 0.5 ng/mL after ovulation [19]. In Holstein cows, the peak concentration of LH was approximately 17.07 to 18.85 ng/mL and after 12 h was approximately 1.15 to 0.90 ng/mL [20]. Higher LH concentration just before AI in NPG cows showed that the time for semen injection might be earlier than the optimal time for AI. The injection of semen should be conducted after LH concentration has decreased from its peak. A follicle has been released from the ovary in this situation. This study indicated that low LH concentration in the blood of cows in estrus could increase the conception rate from AI. The other metabolites and hormones were not different among NPG and PG cows. The means of blood urea nitrogen, glucose, prolactin, estrogen, FSH, and progesterone for all Hanwoo cows in the first experiment were 12.0 ng/mL, 46.1 ng/mL, 0.05 ng/mL, 21.8 pg/mL, 1.32 ng/mL, 0.99 ng/mL, and 25.5 ng/mL, respectively. The mean of BCS for all Hanwoo cows was 2.74. LH was the key hormone related to the success of AI, while other blood metabolites such as BUN and glucose were not related.

The ranges of BCS, BUN, glucose, estrogen, LH, FSH, and progesterone concentrations in the PG cows were 2.00 to 4.00, 5.80-19.3 mg/dL, 22.0 to 66.0 mg/dL, 11.9 to 39.0 ng/dL, < 0.25 to 1.98 ng/dL, < 0.50 to 0.82 Ng/dL, and 1.72 to 30.0 ng/dL, respectively (Table 3). This data was generated for each metabolite and hormone parameter in the PG cows to understand the minimum and maximum concentrations better. In general, NPG cows have a BUN range at 12.5 to 17.1 mg/dL and a glucose range at 49.3 to 60.0 mg/dL, respectively, in their blood [21,22]. Moreover, Kwon et al. [21] reported that the concentrations of BUN and glucose in blood showed

**Table 3.** Range value of body condition score and physiological condition of metabolites and hormones from Hanwoo cows with pregnancy status in the first experiment (n=44)

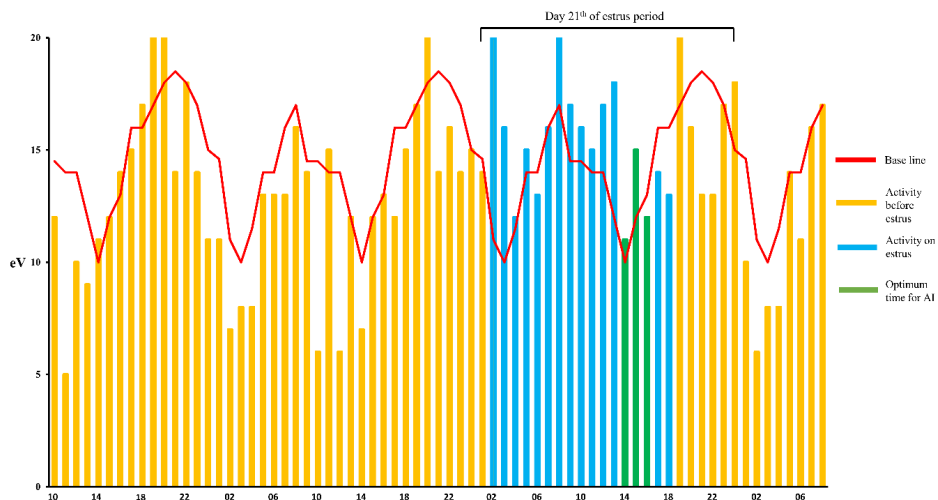
Item	Minimum	Maximum	Standard deviation
Body condition score	2.00	4.00	0.337
Metabolites (ng/mL)			
Blood urea nitrogen	5.80	19.3	3.376
Glucose	22.0	66.0	11.83
Hormones			
Prolactin (ng/mL)	< 0.05	< 0.05	-
Estrogen (pg/mL)	11.9	39.0	7.49
LH (ng/mL)	< 0.25	1.98	0.497
FSH (ng/mL)	< 0.50	0.82	0.049
Progesteron (ng/mL)	1.72	30.0	11.72

LH, luteinizing hormone; FSH, follicle-stimulating hormone.

no differences between pregnant and non-pregnant Hanwoo cows. In another study, the blood concentrations of LH, FSH, and progesterone on Hanwoo cows during estrus varied depending on seasons. Especially, the FSH and LH concentrations were lower in the summer season, while the progesterone concentration was higher [23]. The PG cows before AI had similar concentrations of LH and FSH with other heifer breeds, such as Holstein [20]. Furthermore, in Holstein heifers, the LH concentration was around  $1.10 \pm 0.03$  ng/mL and the FSH concentration was around  $0.50 \pm 0.01$  ng/mL [20]. Furthermore, the concentrations of LH and FSH were around  $1.10 \pm 0.03$  ng/mL and  $0.50 \pm 0.01$  ng/mL, respectively [20]. In another beef cattle breed, LH concentration was around 1 to 1.5 ng/mL during prepartum and periodically increases up to 3 ng/mL after 2 weeks postpartum until period of estrus [19].

### Second experiment

According to the first experiment, a failure in predicting the optimal time for AI could occur even if the breeder had pinpointed the visual symptoms of estrus. From a practical point of view, the first appearance of estrus is difficult to identify accurately. Though the concentration of LH in blood is a good indicator for proper timing for AI, collecting blood samples from a hypersensitive cow should neither be recommended nor be encouraged. Therefore, synchronizing the appropriate time for AI by obtaining information on LH concentration will be an extremely challenging task for farmers. For this reason, the second experiment was developed with the concept that recent technological advances may provide more accurate information on estrus-related animal behavior. In the field, an estrus detector could be applied to predict the first appearance of estrus more accurately than observing visual symptoms [5,8,24]. Physical activity among estrus cows and non-estrus cows could be used as an indicator for estrus [7,8]. By using a W-Tag estrus detector, the physical activity of the cow can be recorded in real-time. For example, an estrus cow presents different physical activity compared to a non-estrus cow. This can be recorded by the application of a W-Tag estrus detector (Fig. 2). The algorithm analysis of physical activity from cows from the cloud server showed that the first appearance of estrus occurred at the second hour of the 21st day. Therefore, the optimum time for AI, using W-Tag estrus detection, could be predicted to be from the 14 to 16th h of the 21st d. The prediction time for optimal AI by W-Tag estrus detection is supported by the results of previous studies, which reported an optimum time for AI at 12 to 15 h after the first appearance of estrus [7,8]. The application of the W-Tag estrus detector as a novel instrument presented beneficial effects for the breeding program of Hanwoo in the present study. The days open ( $p < 0.001$ ; 84.8



**Fig. 2.** Physical activity of Hanwoo cows on 19 to 21 of estrus cycle using W-Tag estrus detector. The red line as base line indicated a general physical activity of Hanwoo cows (herd). The blue bar indicated the duration of estrus in Hanwoo cows, while the green bar indicated an optimum time to conduct artificial insemination (AI) (Second experiment).

vs. 78.1 d), insemination frequency ( $p < 0.01$ ; 2.52 vs. 1.26), and return of estrus ( $p < 0.001$ ; 79.1 vs. 70.9 d) of Hanwoo were lower with application of the estrus detector than without application of the estrus detector (Table 4). The days open, insemination frequency, and return of estrus were improved by the application of a W-Tag estrus detector. This improved the overall conception rate of AI for Hanwoo cows.

In several cases, visual symptoms of a cow on estrus are not clearly apparent. This makes it difficult for breeders to predict when estrus begins [6,25]. Compared to observing temperature and behavior changes, observation of physical activity changes using an accelerometer had higher accuracy for predicting the first appearance of estrus [9]. The physical activity of Hanwoo cows on estrus was reported in real-time, which helped to predict precisely the first appearance of estrus and the optimum time for AI. At-Taras and Spahr [24] also reported that the application of an accelerometer to observe the physical activity of cows had a higher efficiency rate than a heat-mount detector for estrus period prediction. This provides a convincing reason for the use of an accelerometer as an estrus detector. As an upgrade, we attached a wireless sensor to the accelerometer with an IoT system to help farmers easily view the estrus status of their cows any time, from a variety of devices, as fast as possible.

**Table 4.** Effects of W-Tag estrus detector application on days open, insemination frequency, and return of estrus day of Hanwoo cows in the second experiment

Item	Hanwoo cow <sup>1)</sup>		SEM	p-value
	Not use	Use		
Farm (n)	5	5	-	-
Animal (head)	260	100	-	-
Days open (d)	84.8 <sup>a</sup>	78.1 <sup>b</sup>	1.209	< 0.001
Insemination frequency	2.52 <sup>a</sup>	1.26 <sup>b</sup>	0.266	< 0.001
Return of estrus (d)	79.1 <sup>a</sup>	70.9 <sup>b</sup>	0.648	< 0.001

<sup>1)</sup>Not use, cows without estrus detector; Use, cows with estrus detector.

<sup>a,b)</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).



## CONCLUSION

The first experiment concluded that the concentration of LH just before AI influenced the conception rate. Therefore, a lower concentration of LH just before AI could improve the conception rate of Hanwoo cows. The second experiment concluded that the application of a W-Tag estrus detector could decrease the days open, increase the insemination frequency, and speed the return of estrus for Hanwoo cows and increase the successful conception rate of AI.

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