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Original Article

Recovery of ovarian activity and uterine involution in postpartum primiparous Hanwoo cow

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ABSTRACT

Background: Hanwoo cattle farmers aim to improve calf production and reproductive efficiency. Recovery of the reproductive tract postpartum is a critical factor influencing the postpartum period and conception of breeding cows. This study aimed to precisely analyze the recovery process of the reproductive tract in primiparous Hanwoo postpartum and to establish recovery criteria.

Methods: Ten primiparous Hanwoo cows were used in this study. After parturition, estrus was examined daily using visual observations and estrus detection patches. Ovarian recovery, cervical diameter, and uterine horn diameter were examined using ultrasonography four times per week.

Results: The analysis revealed that the first estrus occurred at 19.1 ± 6.5 days postpartum, the first ovulation at 27.1 ± 4.5 days, and the first normal estrus cycle at 39.2 ± 6.4 days. The ovulation rate during the first estrus was 40%. A normal estrus cycle occurred in 11.1% of patients at the first ovulation. The cervix diameter recovered to 42.0 ± 3.5 mm and the uterine horn diameter to 34.4 ± 7.1 mm by 24 days postpartum, with the difference in uterine horn diameter recovering to 2.6 ± 1.2 mm by 31 days postpartum.

Conclusions: This study can aid in determining the optimal breeding time for postpartum primiparous Hanwoo cow and provide foundational data for Hanwoo breeding studies.

Keywords: Hanwoo, ovarian activity, postpartum, primiparous, uterus involution

INTRODUCTION

Obtaining one calf per cow per year is a significant economic goal for farmers (Bellows et al., 2002; Hess et al., 2005; Severino-Lendechy et al., 2020). For Hanwoo cows, achieving this goal requires successful artificial insemination (AI) within 70-80 days postpartum. Missing the optimal timing for AI or AI failure results in a loss of approximately 180,000 KRW per cow based on a 21-day estrus cycle (KOrean Statistical Information Service, 2022). Over the past 5 years, the slaughter rate of Hanwoo breeding cows has been 57.5%, indicating that more than half of the breeding cows are managed with more than two parities (Ministry of Agriculture, Food and Rural Affairs, 2024). Further studies are needed to accurately determine the recovery period of the reproductive tract postpartum in primiparous Hanwoo cows and establish the optimal timing for AI.

Recovery of the reproductive tract postpartum in breeding cows is a critical factor affecting the postpartum days open and conception (Spicer et al., 1986; Short et al., 1990; Ciccioli et al., 2003; Forde et al., 2011). Assessments of ovarian activity and uterine recovery are performed to measure the recovery of the reproductive tract postpartum. The recovery of ovarian activity is characterized by regular cycles of 18-24 days. In suckling beef cows, recovery of ovarian activity is observed at 30-130 days postpartum. In dairy cows, it is observed at 15-45 days postpartum (Forde et al., 2011). In beef cows, ovarian recovery first occurs approximately 30 days postpartum and requires 3.2 ± 0.2 dominant follicles for the first ovulation. In the first estrus, they do not exhibit signs of estrus and have a short luteal phase of 8-10 days. The second ovulation, which occurs with a regular cycle, occurs 9-11 days after the first ovulation (Murphy et al., 1990; Crowe, 2008).

Cows with recovered ovarian activity have a functional corpus luteum (CL) that maintains progesterone (P_4) concentrations above 1 ng/mL for approximately 2 weeks, ensuring regular estrus cycles (Sirois and Fortune, 1988; Yang et al., 1999; Ghanem et al., 2008). The peak concentration of estradiol (E_2) influences the intensity signs of estrus and follicle size (Perry et al., 2014). A dominant follicle must exceed 10 mm in diameter to acquire ovulatory capacity (López-Gatius et al., 2022). Larger dominant follicles are associated with larger corpora lutea and higher P_4 concentrations (Pfeifer et al., 2012). The recipients with a CL larger than 1.5 cm showed statistically higher pregnancy rates, the pregnancy rate was higher when the plasma P_4 levels were between 2.0 and 4.0 ng/mL (Choi et al., 2023). In the case of embryo transfer, when P_4 concentrations are ≥2.5 ng/mL and E_2 concentrations are ≤1.5 pg/mL on days 6-7 after estrus, conception rates reach 73.3%, compared to 37.2% in other recipients. Additionally, conception rates increase when the E_2/P_4 ratio less than 1.0 (Nishigai et al., 2000).

Uterus involution postpartum can affect conception rates and the length of the postpartum days open (Spicer et al., 1986; Short et al., 1990). In beef cows, the diameters of the uterus horn and cervix recover 20-40 days postpartum (Spicer et al., 1986; Short et al., 1990). In addition, histological recovery was observed 40 days postpartum (Okano and Tomizuka, 1996).

In Hanwoo cow, studies have been conducted on the number of services per conception postpartum (Kim et al., 2014) and on reproductive traits (Baek et al., 1998a; 1998b; Yang et al., 1999; Kim et al., 2002; Jung et al., 2004; Kim et al., 2014; Cho et al., 2015), but studies on the recovery of the reproductive tract postpartum are lacking. Without established criteria for the morphological and endocrine recovery process of the reproductive tract, suggesting an optimal AI timing based solely on the return to the first estrus postpartum and the number of services per conception has limitations. A well-organized study on the recovery of the reproductive tract postpartum is needed to understand the recovery process in primiparous Hanwoo cows and establish the necessary basic data for determining the optimal timing for AI.

Thus, this study aimed to precisely analyze the recovery process of the reproductive tract postpartum in primiparous Hanwoo cows and establish recovery criteria. To achieve this, ovarian activity and uterine involution were measured using ultrasonography and blood hormone concentrations were analyzed.

MATERIALS AND METHODS

Animals and management

The study was performed using 10 primiparous Hanwoo cows (average age at calving: 24.4 ± 2.2 months, average parity: 1.0 ± 0.0, average weight: 366.9 ± 42.5 kg, and average body condition score (BCS, score 1-5): 2.9 ± 0.2) raised at the Hanwoo Research Institute of the National Institute of Animal Science, Rural Development Administration. Each cow was fed 3.0–3.5 kg of commercial formulated concentrates and 4.0–4.5 kg of hay (tall fescue) according to the Korean Feeding Standard for Hanwoo (National Institute of Animal Science, 2017), with mineral blocks and water provided *ad libitum* throughout the experimental period. This study was performed from January to April 2024, approximately 10–90 days postpartum. During the study period, estrus detection was performed daily, rectal ultrasonography was performed four times a week (Monday, Wednesday, Friday, and Saturday), and blood sampling for hormone analysis was performed three times a week (Monday, Wednesday, and Friday). This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Institute of Animal Science, Republic of Korea (Approval Number: NIAS2023-0606).

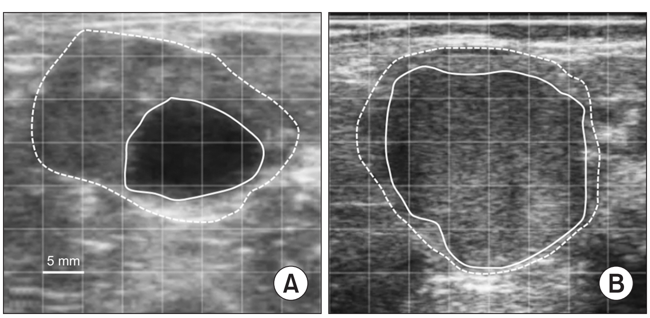

Fig. 1. Ultrasound images of the ovaries taken using an ultrasound device. (A) Shows an ovary with a dominant follicle, and (B) shows an ovary with a corpus luteum. In (A), the white dotted line indicates the ovary, and the white solid line indicates the dominant follicle. In (B), the white dotted line indicates the ovary, and the white solid line indicates the corpus luteum. The cross-sectional area was calculated using the horizontal and vertical diameters of the dominant follicle and corpus luteum within the ovary.

Estrus detection

Estrus detection was performed every morning for 1 h. To detect estrus, estrus detection patches (Estrotect™, Rockway, Spring Valley, WI, USA) were applied at the midpoint of the rump and tail head. To accurately confirm estrus, a 4.5–8.5 MHz ultrasound device (IMV imaging, Easi-scan: Go bovine ultrasound scanner, L'Agle, France) was used to examine the dominant follicles, ovulation, and CL in the ovaries. A dominant follicle was defined as a follicle larger than 8 mm with signs of estrus, and ovulation was defined as the disappearance of the dominant follicle within two days (Perry et al., 2007).

Measurement of ovarian activity and uterine recovery

Ultrasonography for primiparous Hanwoo cows was initiated on day 10 postpartum, and ovarian activity and uterine recovery were measured every 2 days. To assess ovarian activity recovery, the size of follicles, ovulation, and the formation and duration of the corpus luteum (CL) were measured during estrus (Fig. 1). The size of the follicles and CL was calculated as the average of the horizontal and vertical diameters, expressed as (horizontal diameter + vertical diameter, mm)/2 (Perry et al., 2007). If the formed CL was maintained for less than 10 days, it was classified as a short estrus cycle, and if it was maintained for approximately 2 weeks, it was classified as a normal estrus cycle (Sirois and Fortune, 1988; Yang et al., 1999; Ghanem et al., 2008). Ovarian activity was analyzed based on the first estrus, first ovulation, first normal estrus cycle postpartum, and sequence of estrus. The diameters of the cervix and the horns of the uterus were measured (Fig. 2). The cervical diameter was measured at the thickest part, and the diameter of each uterine horn was measured approximately 2 cm from the cranial end of the uterine body. Uterine involution was considered when no significant differences were observed in the measured diameters

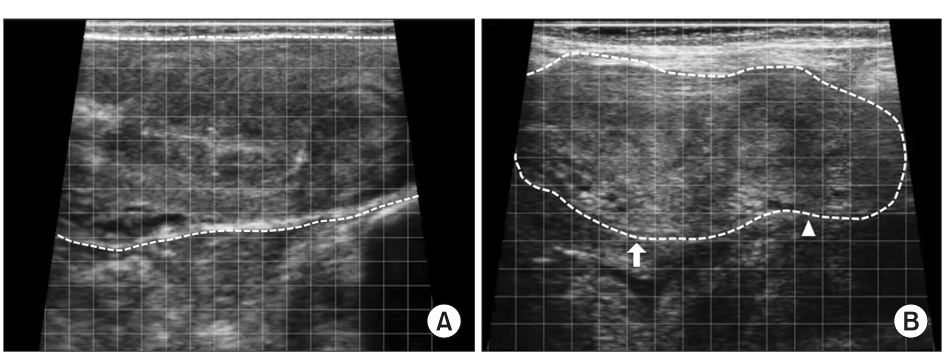

Fig. 2. Ultrasound images of the uterus taken using an ultrasound device. The white dotted line in (A) indicates the cervix, and the white dotted line in (B) indicates the uterine horn. The white arrow indicates the right uterine horn, and the white triangle indicates the left uterine horn (B).

(Zhang et al., 2010; Lin et al., 2021).

Blood sampling and measurement of serum hormones

To analyze the recovery of ovarian activity, serum hormone concentrations were measured. Blood samples were collected at 9 AM, 2 h after feeding at 7 AM. Blood (10 mL) was collected from the jugular vein of the cows using a serum vacutainer (BD Vacutainer Serum REF 367820; Plymouth, UK) and transported to the laboratory. The blood samples were centrifuged at 3,000 rpm for 20 min at 4℃. The serum was stored at -80℃ until analysis. The concentration of P_4 and E_2 were evaluated using the electric chemiluminescence immunoassay method (Roche, Cobas e411, Mannheim, Germany) as previously described (Kim et al., 2021). The hormones analyzed were estradiol (E_2, pg/mL) and progesterone (P_4, ng/mL), with detection limits of 5.0 pg/mL for E_2 and 0.05 ng/mL for P_4. The peak concentration of E_2 was analyzed before estrus, and the concentration of P_4 was analyzed at 7 and 14 days after estrus, as well as the time during which maintained $P_4 \geq 1$ ng/mL.

Statistical analysis

Differences in ovarian activity according to estrus were analyzed using the chi-square test, ANOVA, and Tukey's post hoc test. The diameters of the cervical and uterine horns were analyzed using the *t*-test to verify significance ($p < 0.05$). All analyses were performed using the statistical analysis software (IBM, SPSS Statistics version 26, NY, USA).

RESULTS

This study evaluated the recovery rate of the reproductive tract in primiparous Hanwoo cows postpartum over time by assessing estrus expression, morphological changes in the ovaries and uterus, and serum hormone concentrations. Table 1 shows the estrus patterns according to the first estrus, first ovulation, and first normal estrus cycle groups. The postpartum estrus days of the first estrus, first ovulation, and first normal estrus cycle were significantly increased on the first estrus, first ovulation, and first normal estrus cycle (first estrus, first ovulation, and first normal estrus cycle = 19.1 ± 6.5^a, 27.1 ± 4.5^b, and 39.2 ± 6.4 day^c, respectively, $p < 0.05$). The number of estrus cycles was significantly increased on the first estrus, first ovulation, and first normal estrus cycle (first estrus, first ovulation, and first normal estrus cycle = 1.0 ± 0^a, 1.7 ± 0.6^b, and 2.8 ± 0.6 cycle^c, respectively, $p < 0.05$). The estrus detection rate using the patch was 80-90%, and the first estrus, first ovulation, and first normal estrus cycle groups were not significantly different. The estrus detection rate based on observed signs of estrus was significantly lower in the first estrus group compared to the first normal estrus cycle group (first estrus, first ovulation, and first normal estrus cycle = 10^a, 20^{ab}, and $60\%^b$, respectively, $p < 0.05$). The frequency of simultaneous detection of the patch and the observed signs of estrus were significantly lower in the first estrus and first ovulation groups compared to the first normal estrus cycle group (first estrus, first ovulation, and first normal estrus cycle = 0^a, 0^a, and $40\%^b$, respectively, $p < 0.05$). The ovulation rate was significantly lower in the first estrus group compared to the first ovulation and first normal estrus cycle groups (first estrus, first ovulation, and first normal estrus cycle = 40^a, 100^b, and $100\%^b$, respectively, $p < 0.05$). The CL formation rate was also significantly lower in the first estrus group compared to the first ovulation and first normal estrus cycle groups (first estrus, first ovulation, and first normal estrus cycle = 40^a, 90^b, and $100\%^b$, respectively, $p < 0.05$).

Table 2 shows the follicle size, normal CL formation

Table 1. Analysis of estrus patterns according to first estrus, first ovulation, and first normal estrus cycle

Group	Postparturm (days)	No. of estrus cycles	Estrus detection rate (%)			Ovulation rate (%)	CL formation rate (%)
			Patch	Signs of estrus	Simultaneous detection		
First estrus	19.1 ± 6.5^a	1.0 ± 0^a	90^a	10^a	0^a	40^a	40^a
First ovulation	27.1 ± 4.5^b	1.7 ± 0.6^b	80^a	20^{ab}	0^a	100^b	90^b
First normal estrus cycle	39.2 ± 6.4^c	2.8 ± 0.6^c	80^a	60^b	40^b	100^b	100^b

The value on postpartum days and number of estrus cycles are indicated as the mean ± standard deviation. [a,b,c]Values with different superscripts within a column ($p < 0.05$). Estrus detection rate as a percentage of total estrus observed by different detection methods. Patch = estrus detection using color change in the estrus detection patch. Signs of estrus = estrus detection based on mounting, standing, and mucus discharge. Simultaneous detection = estrus detected simultaneously by both the patch and signs of estrus.

Table 2. Analysis of follicle size, E_2 peak concentration, corpus luteum, and P_4 concentration according to first estrus, first ovulation, and first normal estrus cycle

Group	Follicle size (mm)	E_2 peak conc. (pg/mL)	Normal CL formation rate (%)	CL maintenance period (day)	P_4 conc. (ng/mL)		$P_4 \geq 1$ ng/mL (day)
					After 7 days estrus	After 14 days estrus	
First estrus	11.3 ± 1.3[a]	9.9 ± 3.3[a]	0[a]	5.5 ± 3.2[a]	0.1 ± 0.0[a]	0.2 ± 0.3[a]	0.3 ± 0.4[a]
First ovulation	11.4 ± 1.5[a]	11.7 ± 2.2[a]	11.1[a]	6.7 ± 3.8[a]	0.3 ± 0.3[a]	0.6 ± 1.4[a]	1.8 ± 3.7[a]
First normal estrus cycle	11.6 ± 2.1[a]	10.7 ± 4.7[a]	100[b]	17.0 ± 1.4[b]	1.2 ± 0.9[b]	3.7 ± 2.1[b]	9.9 ± 4.1[b]

All values except for normal CL formation rate, are mean as mean ± standard deviation. [a,b,c]Values with different superscripts within a column ($p < 0.05$). Follicle size = (horizontal diameter + vertical diameter, mm)/2. E_2 peak concentration = the highest E_2 concentration measured before estrus. Normal CL formation rate = the rate of corpus luteum formation that was maintained for approximately 14 to 18 days. $P_4 \geq 1$ ng/mL = the period during which the P_4 concentration remained above 1 ng/mL.

Table 3. Analysis of estrus patterns, ovulation rate, corpus luteum formation rate according to the first, second, third, and fourth estrus cycle postpartum

Estrus cycle	Estrus day	Estrus interval	Estrus detection rate (%)			Ovulation rate (%)	CL formation rate (%)
			Patch	Signs of estrus	Simultaneous detection		
1st	19.1 ± 6.5[a]	11.2 ± 4.3[a]	90[a]	10[a]	0[a]	40[a]	40[a]
2nd	30.3 ± 5.8[a]	14.4 ± 5.7[ab]	90[a]	30[ab]	20[ab]	90[b]	80[ab]
3rd	44.7 ± 9.8[b]	19.9 ± 5.1[bc]	80[a]	60[b]	40[b]	100[b]	90[b]
4th	64.4 ± 12.0[c]	21.0 ± 1.5[c]	90[a]	60[b]	50[b]	100[b]	100[b]

The estrus day and estrus interval are indicated as the mean ± standard deviation. [a,b,c]Values with different superscripts within a column ($p < 0.05$). Estrus detection rate = the proportion of total estrus detected by each method. Patch = estrus detected by color change in the estrus detection patch. Signs of estrus = estrus detected by observing mounting, standing to be mounted, and mucus discharge. Simultaneous detection = estrus detected simultaneously by both the patch and signs of estrus.

rate, CL maintenance period, and serum hormone concentrations according to the first estrus, first ovulation, and first normal estrus cycle groups. The follicle size and E_2 peak concentration were not significantly different among the first estrus, first ovulation, and first normal estrus cycle groups. The rate of normal CL formation was significantly lower in the first estrus and first ovulation groups compared to the first normal estrus cycle group (first estrus, first ovulation, and first normal estrus cycle = 0[a], 11.1[a], and 100%[b], respectively, $p < 0.05$). The CL maintenance period was also significantly lower in the first estrus and first ovulation groups compared to the first normal estrus cycle group (first estrus, first ovulation, and first normal estrus cycle = 5.5 ± 3.2[a], 6.7 ± 3.8[a], and 17.0 ± 1.4 day[b], respectively, $p < 0.05$). The P_4 concentration at 7 and 14 days after estrus was significantly lower in the first estrus and first ovulation groups compared to the first normal estrus cycle group (P_4 concentration 7 days after estrus; first estrus, first ovulation, and first normal estrus cycle = 0.1 ± 0.0[a], 0.3 ± 0.3[a], and 1.2 ± 0.9 ng/mL[b], respectively, $p < 0.05$, P_4 concentration 14 days after estrus; first estrus, first ovulation, and first normal estrus cycle = 0.2 ± 0.3[a], 0.6 ± 1.4[a], and 3.7 ± 2.1 ng/mL[b], respectively, $p < 0.05$). The duration for which $P_4 \geq 1$ ng/mL was maintained and was also significantly shorter in the first estrus and first ovulation groups compared to the first normal estrus cycle group (first estrus, first ovulation, and first normal estrus cycle = 0.3 ± 0.4[a], 1.8 ± 3.7[a], and 9.9 ± 4.1 day[b], respectively, $p < 0.05$).

Table 3 shows the estrus patterns according to the first, second, third, and fourth estrus postpartum groups. The postpartum estrus day significantly increased as the number of estrus cycles increased (first, second, third, and fourth = 19.1 ± 6.5[a], 30.3 ± 5.8[a], 44.7 ± 9.8[b], and 64.4 ± 12.0 day[c], respectively, $p < 0.05$). The interval between the estrus cycles significantly increased as the number of estrus cycles increased (first, second, third, and fourth estrus = 11.2 ± 4.3[a], 14.4 ± 5.7[ab], 19.9 ± 5.1[bc], and 21.0 ± 1.5 day[c], respectively, $p < 0.05$). The estrus detection rate using the patch was 80–90%, and no significant differences were observed among the first, second, third, and fourth estrus groups. The estrus detection rate based on observed signs of estrus significantly increased from the first to the third estrus, with no significant difference between the third and fourth estrus (first, second, third, and fourth estrus = 10^{a}, 30^{ab}, 60^{b}, and $60\%^{b}$, respectively, $p < 0.05$). The frequency of simultaneous detection using the patch and observed signs of estrus also significantly increased from the first to the third estrus, with no significant difference between the third and fourth estrus (first, second, third, and fourth estrus = 0^{a}, 20^{ab}, 40^{b}, and $50\%^{b}$, respectively, $p < 0.05$). The ovulation rate significantly increased from the first to the second estrus, with no significant difference between the second and fourth estrus (first, second, third, and fourth estrus = 40^{a}, 90^{b}, 100^{b}, and $100\%^{b}$, respectively, $p < 0.05$). The CL formation rate significantly increased from the first to the third estrus, with no significant difference between the third and fourth estrus (first, second, third, and fourth estrus = 40^{a}, 80^{ab}, 90^{b}, and $100\%^{b}$, respectively, $p < 0.05$).

Table 4 shows the follicle size, normal CL formation rate, CL maintenance period, and serum hormone concentrations according to the first, second, third, and fourth estrus cycles postpartum. No significant differences were observed in the follicle size and E_2 peak concentration among the number of estrus cycles. The normal CL formation rate significantly increased from the first to the third estrus, with no significant difference between the third and fourth estrus (first, second, third, and fourth estrus = 0^{a}, 50^{b}, 100^{c}, and $83.3\%^{c}$, respectively, $p < 0.05$). The CL maintenance period was not significantly different between the first and second estrus, but was significantly shorter in the first compared to those in the third and fourth estrus (first, second, third, and fourth estrus = 5.5 ± 3.2^{a}, 11.0 ± 5.5^{ab}, 17.0 ± 2.1^{b}, and 15.5 ± 2.3 day^{b}, respectively, $p < 0.05$). The CL size 7 days after estrus was significantly smaller in the first estrus compared to those in the second, third, and fourth estrus (first, second, third, and fourth estrus = 5.9 ± 5.9^{a}, 16.8 ± 5.0^{b}, 19.1 ± 3.7^{b}, and 19.0 ± 4.0 mm^{b}, respectively, $p < 0.05$). Similarly, the CL size 14 days after estrus was significantly smaller in the first estrus compared to the second, third, and fourth estrus (first, second, third, and fourth estrus = 0^{a}, 11.3 ± 10.0^{b}, 20.7 ± 2.4^{b}, and 19.3 ± 3.9 mm^{b}, respectively, $p < 0.05$). The P_4 concentration at 7 days after estrus was not significantly different among the first, second, and third estrus, but it was significantly lower in the first estrus compared to that in the fourth estrus (first, second, third, and fourth estrus = 0.1 ± 0.0^{a}, 0.7 ± 0.6^{ab}, 1.3 ± 0.9^{ab}, and 2.1 ± 1.5 ng/mL^{b}, respectively, $p < 0.05$). The P_4 concentration at 14 days after estrus was not significantly different between the first and second estrus, but it was significantly lower in the first estrus compared to the third and fourth estrus (first, second, third, and fourth estrus = 0.8^{a}, 3.0 ± 2.0^{ab}, 3.6 ± 2.1^{b}, and 4.4 ± 1.8 ng/mL^{b}, respectively, $p < 0.05$). The duration for which the P_4 remained ≥ 1 ng/mL was not significantly different between the first and second estrus, but it was significantly shorter in the first estrus compared with those in the third and fourth estrus (0.3 ± 0.5^{a}, 4.4 ± 4.8^{ab}, 10.1 ± 4.6^{b}, and 11.2 ± 3.3 day^{b}, respectively, $p < 0.05$).

Table 5 shows the measurements of the cervical diameter, uterine horn diameter, and the difference in the uterine horn diameter at 7 day intervals starting at 10 days postpartum. The uterine horn diameter significantly decreased from 10 to 24 days postpartum (10, 17, and 24 days = 53.1 ± 2.7^{a}, 46.8 ± 4.5^{ab}, and 42.0 ± 3.5 mm^{bc}, respectively, $p < 0.05$). After 24 days, at 42.0 ± 3.5 mm, no significant decrease was observed. The cervix diameter

Table 4. Analysis of follicle size, corpus luteum, E_2 peak concentration, and progesterone concentration according to the first, second, third, and fourth estrus cycles postpartum

Estrus cycle	Follicle size (mm)	E_2 peak conc. (pg/mL)	Normal CL formation rate (%)	CL maintenance period (day)	CL size (mm)		P_4 conc. (ng/mL)		$P_4 \geq$ 1ng/mL (day)
					After 7 days estrus	After 14 days estrus	After 7 days estrus	After 14 days estrus	
1st	11.3 ± 1.3	9.9 ± 3.3^{a}	0^{a}	5.5 ± 3.2^{a}	5.9 ± 5.9^{a}	0^{a}	0.1 ± 0.0^{a}	0.8^{a}	0.3 ± 0.5^{a}
2nd	11.1 ± 2.1	10.8 ± 4.4^{a}	50^{b}	11.0 ± 5.5^{ab}	16.8 ± 5.0^{b}	11.3 ± 10.0^{b}	0.7 ± 0.6^{ab}	3.0 ± 2.0^{ab}	4.4 ± 4.8^{ab}
3rd	11.7 ± 1.7	11.0 ± 4.9^{a}	100^{c}	17.0 ± 2.1^{b}	19.1 ± 3.7^{b}	20.7 ± 2.4^{b}	1.3 ± 0.9^{ab}	3.6 ± 2.1^{b}	10.1 ± 4.6^{b}
4th	10.5 ± 1.7	11.3 ± 3.8^{a}	83.3^{c}	15.5 ± 2.3^{b}	19.0 ± 4.0^{b}	19.3 ± 3.9^{b}	2.1 ± 1.5^{b}	4.4 ± 1.8^{b}	11.2 ± 3.3^{b}

All values except normal CL formation rate are expressed as the mean ± standard deviation. ^{a,b,c}Values with different superscripts within a column ($p < 0.05$). Follicle size, and CL size = (horizontal diameter + vertical diameter, mm)/2. E_2 peak concentration = highest E_2 concentration measured before estrus. E_2 peak interval = interval between E_2 peak and estrus. Normal CL formation rate = percentage of CL maintained for approximately 14 to 18 days. $P_4 \geq 1$ ng/mL = duration for which P_4 concentration remained above 1 ng/mL.

Table 5. Changes in cervix diameter, uterine horn diameter, and the difference in uterine horn diameter by days postpartum

Group	Postparturm (days)									
	10	17	24	31	38	45	52	59	66	73
Cervix diameter (mm)	53.1 ± 2.7[a]	46.8 ± 4.5[ab]	42.0 ± 3.5[bc]	41.4 ± 5.2[bc]	38.1 ± 4.3[c]	40.3 ± 4.9[bc]	36.7 ± 5.0[c]	39.1 ± 7.1[bc]	37.7 ± 4.2[c]	39.3 ± 3.9[bc]
Uterine horn diameter (mm)	45.2 ± 3.3[a]	37.1 ± 5.3[ab]	34.4 ± 7.1[bc]	28.4 ± 4.6[bc]	27.4 ± 3.5[c]	28.3 ± 4.3[bc]	26.8 ± 3.8[c]	30.1 ± 4.4[bc]	28.9 ± 2.7[bc]	30.3 ± 2.7[bc]
Difference in uterine horn diameter (mm)	11.0 ± 7.5[a]	7.5 ± 5.2[ab]	8.6 ± 5.2[ab]	2.6 ± 1.2[b]	3.5 ± 2.4[b]	1.7 ± 1.7[b]	3.9 ± 3.3[b]	3.0 ± 1.6[b]	2.9 ± 1.7[b]	3.2 ± 2.3[b]

All values are expressed as the mean ± standard deviation. [a,b,c]Values with different superscripts within a row ($p < 0.05$). Cervix diameter (mm) = diameter at the thickest part of the cervix. Uterine horn diameter (mm) = mean diameter of both uterine horns measured 2 cm from the uterine body bifurcation. Difference in uterine horn diameter (mm) = difference between the diameters of both uterine horns measured 2 cm from the uterine body bifurcation.

Table 6. Comparison of estradiol and progesterone concentrations at estrus time according to the first, second, third, and fourth estrus cycles postpartum

	After estrus (day)	Estrus cycle			
		1st	2nd	3rd	4th
Estradiol (pg/mL)	From −2 to 0	11.0 ± 3.5	11.0 ± 4.0	11.6 ± 4.2	11.6 ± 3.9
	0	5.4 ± 0.9	5.0 ± 0.0	9.3 ± 4.9	8.3 ± 4.8
	7	6.3 ± 1.8	5.2 ± 0.3	6.4 ± 3.8	6.6 ± 2.6
	14	5.0 ± 0.0	5.0 ± 0.0	5.9 ± 2.5	5.7 ± 1.7
Progesterone (ng/mL)	From −2 to 0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
	0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
	7	0.1 ± 0.0[a]	0.5 ± 0.7[a]	1.3 ± 1.1[ab]	2.2 ± 1.6[b]
	14	0.1 ± 0.0	3.5 ± 2.1	3.3 ± 2.3	3.8 ± 2.2

All values are expressed as the mean ± standard deviation. [a,b]Values with different superscripts within a row ($p < 0.05$). After estrus (day) = represents the date after estrus on the basis of 0 days of estrus. From −2 to 0 = hormones on the days peak E_2 between −2 and 0 days before estrus were analyzed.

also significantly decreases from 10 to 24 days postpartum (10, 17, and 24 days = 45.2 ± 3.3[a], 37.1 ± 5.3[ab], and 34.4 ± 7.1 mm[bc], respectively, $p < 0.05$), with no significant decrease observed after 24 days, at 34.4 ± 7.1 mm. The difference in uterine horn diameter did not significantly decrease from 10 to 24 days postpartum, but the difference was significantly decreased on day 31 compared to that on day 10 (10 and 31 days = 11.0 ± 7.5[a] and 2.6 ± 1.2 mm[b], respectively, $p < 0.05$). No significant decrease in the difference in uterine horn diameter was observed after 17 days, at 7.5 ± 5.2 mm.

Table 6 shows the concentrations of E_2 and P_4 as the time progressed to estrus according to the first, second, third, and fourth estrus cycle groups postpartum. From -2 to 0 days before estrus, there were no significant differences in E_2 and P_4 concentrations between the estrus cycle groups. On day 0 of estrus, there were no significant differences in E_2 concentrations between the estrus cycle groups. P_4 concentrations on day 0 of estrus showed no significant differences between the estrus cycle groups. On day 7 after estrus, E_2 concentrations showed no significant differences between the estrus cycles. However, P_4 concentrations on day 7 were significantly higher in the fourth estrus cycle compared to the first and second cycles (first, second, third, and fourth = 0.1 ± 0.0[a], 0.5 ± 0.7[a], 1.3 ± 1.1[ab], and 2.2 ± 1.6 ng/mL[b], respectively, $p < 0.05$). On day 14 after estrus, E_2 concentrations showed no significant differences. P_4 concentrations on day 14 after estrus showed no significant differences.

Table 7 shows the concentrations of E_2 and P_4 as the

Table 7. Comparison of estradiol and progesterone concentrations at estrus time according to the estrus types

	After estrus (day)	Types of estrus cycle		
		Short	P_4 low	Normal
Estradiol (pg/mL)	From −2 to 0	9.9 ± 4.1[a]	16.7 ± 2.1[b]	10.6 ± 2.8[a]
	0	5.2 ± 0.6[a]	10.9 ± 6.0[b]	6.5 ± 3.1[ab]
	7	5.9 ± 1.5	7.6 ± 4.2	5.6 ± 1.8
	14	5.0 ± 0.0	7.1 ± 3.1	5.0 ± 0.0
Progesterone (ng/mL)	From −2 to 0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
	0	0.1 ± 0.6	0.1 ± 0.0	0.1 ± 3.1
	7	0.1 ± 0.1[a]	0.7 ± 0.9[a]	2.1 ± 1.3[b]
	14	0.1 ± 0.0[a]	2.0 ± 1.5[a]	4.5 ± 2.0[b]

All values are expressed as the mean ± standard deviation. [a,b]Values with different superscripts within a row ($p < 0.05$). After estrus (day) = represents the date after estrus on the basis of 0 days of estrus. From −2 to 0 = hormones on the days peak E_2 between −2 and 0 days before estrus were analyzed. Short estrus cycle = the estrus cycle with an estrus cycle interval ≤ 17 days. P_4 low estrus cycle = the estrus cycle with an estrus interval of 18–24 days and a period of P_4 > 1 ng/mL of 10 days or less. Normal estrus cycle = the estrus cycle with an estrus interval of 18–24 days and a period of P_4 > 1 ng/mL of about 2 weeks.

time progressed to estrus according to the short estrus cycle, P_4 low estrus cycle, and normal estrus cycle groups. From −2 to 0 days before estrus, E_2 concentrations were significantly higher in the P_4 low estrus cycle groups compared to the short and normal estrus cycle groups (short estrus cycle, P_4 low estrus cycle, and normal estrus cycle = 9.9 ± 4.1[a], 16.7 ± 2.1[b], and 10.6 ± 2.8 pg/mL[a], respectively, $p < 0.05$). P_4 concentrations from −2 to 0 days before estrus showed no significant differences between estrus cycle groups. On day 0 of estrus, E_2 concentrations were significantly higher in the P_4 low estrus cycle group compared to the short estrus cycle group (short estrus cycle, P_4 low estrus cycle, and normal estrus cycle = 5.2 ± 0.6[a], 10.9 ± 6.0[b], and 6.5 ± 3.1 pg/mL[ab], respectively, $p < 0.05$). P_4 concentrations on day 0 of estrus showed no significant differences between the estrus cycle groups. On day 7 after estrus, E_2 concentrations showed no significant differences between the estrus cycle groups. P_4 concentrations on day 7 were significantly higher in the normal estrus cycle group compared to the short and P_4 low estrus cycle groups (short estrus cycle, P_4 low estrus cycle, and normal estrus cycle = 0.1 ± 0.1[a], 0.7 ± 0.9[a], and 2.1 ± 1.3 ng/mL[b], respectively, $p < 0.05$). On day 14 after estrus, E_2 concentrations showed no significant differences between the estrus cycle groups. P_4 concentrations on day 14 were significantly higher in the normal estrus cycle group compared to the short and P_4 low estrus cycle groups (short estrus cycle, P_4 low estrus cycle, and normal estrus cycle = 0.1 ± 0.0[a], 2.0 ± 1.5[a], and 4.5 ± 2.0 ng/mL[b], respectively, $p < 0.05$).

DISCUSSION

In the present study, we established the recovery of ovarian activity and uterine involution and established diagnostic criteria for primiparous Hanwoo cow using estrus detection, ultrasonography, and hormone analysis. The recovery of the reproductive tract is a critical factor that influences subsequent pregnancies in postpartum cows. To the best of our knowledge, this is the first study to observe reproductive tract recovery in primiparous Hanwoo cows by monitoring the changes in ovarian activity and uterine diameter. In the present study, we observed the first estrus at 19.1 ± 6.5 days, the first ovulation at 27.1 ± 4.5 days, and the first normal estrus cycle at 39.2 ± 6.4 days in the postpartum primiparous Hanwoo cows (Table 1). In previous studies on postpartum Hanwoo cow, Baek et al. (1998a) reported the first estrus at 70.1 ± 45.8 days, and Park et al. (2022) reported it at 55.4 ± 8.7 days. The difference in the results of Baek et al. (1998a) was thought to be due to the use of data from a farm survey. Park et al. (2022) used the same estrus detection method, but the difference in their data was thought to have appeared because their study started observing estrus after 30 days and used cows with an average parity of 2 to 3, which have different reproductive ability compared to primiparous cow.

In the present study, the ovulation rate during the first estrus was 40% (Table 1). Murphy et al. (1990) observed the first estrus in beef cattle using an estrus detection method and reported it at 54.2 ± 3.2 days, with an ovulation rate of 11%. The difference in these results may be due to use of multiparous cows with lower fertility compared to primiparous cows (Baek et al., 1998a). The difference in species may have also been a contributing factor. The first ovulation postpartum in beef cattle occurs between approximately 30 and 70 days (Murphy et al., 1990), which is consistent with the findings of the present study. Following Forde et al. (2011), ovarian activity in beef cattle recovers between 30 and 130 days. As shown in Table 1, the ovarian activity recovery period observed in the present study, 39.2 ± 6.4 days, is consistent with these findings.

As shown in Table 2, a normal estrus cycle occurred in 11.1% of cows at the first ovulation. In beef cattle, a normal estrus cycle has been reported in over 30% of the first ovulations (Crowe, 2008). Murphy et al. (1990) reported a normal estrus cycle of 22.2%. As shown in Table 4, the first estrus had a short CL maintenance period and a smaller CL size than the third estrus, which had a normal estrus cycle of 100%. These results suggest that the CL maintenance period and CL size may be related to the period of the estrus cycle. The normal estrus cycle in bovines is reported to occur at regular intervals of 18 to 24 days, with a CL phase of approximately 2 weeks (Crowe, 2008; Forde et al., 2011). The first normal estrus cycle occurs between 30 and 130 days in beef cattle. In the present study, the first normal estrus cycle was observed at 39.2 ± 6.4 days, an average of 2.8 ± 0.6 estrus postpartum. This finding was in agreement with those of Seong and Lee, (2002), who reported that the normal estrus cycle occurs at approximately 40 days postpartum in Hanwoo cow.

As shown in Table 3, the second estrus occurred at 30.3 ± 5.8 days postpartum, with an ovulation rate of 90% and a CL formation rate of 80%. However, the estrus interval and CL maintenance period were 14.4 ± 5.7 days and 11.0 ± 5.5 days, which were shorter compared to those of a normal estrus cycle (Yang et al., 1999; Crowe, 2008; Forde et al., 2011). Additionally, in terms of CL activity, the period for which the P_4 concentration remained over 1 ng/mL was shorter compared to that in normal estrus cycles at 4.4 ± 4.8 days. The third estrus occurred at 44.7 ± 9.8 days postpartum, with an ovulation rate of 100% and a CL formation rate of 90%, similar to the second estrus. However, the estrus interval and CL maintenance period were at 19.9 ± 5.1 and 17.0 ± 2.1 days, respectively, which were within the normal estrus cycle range. The period for which the P_4 concentration remained over 1 ng/mL was still shorter compared with that of the normal estrus cycle, at 10.1 ± 4.6 days in the third estrus. These results suggest that hormonal recovery of regular estrus cycles may be incomplete depending on the individual, and it was confirmed that hormone concentration in the estrus cycle of several individuals did not completely return to normal levels. It was reported that first estrus is no signs of estrus and short estrus interval (Murphy et al., 1990; Crowe, 2008). On the other hand, in the present study, we detected estrus using estrus detection patch and visual observation, confirming only the estrus cycles when signs of estrus were observed. Therefore, it is judged that the E_2 concentration from −2 to 0 days before estrus was similar (Table 6). Although no significant differences were observed that the E_2 concentration was higher on day 7 after estrus in the third and fourth estrus cycle groups (Table 6). The estrus detection rates based on signs of estrus observation in the 3rd and 4th estrus were significantly higher at 60% and 90% compared to the 1st estrus group at 10% (Table 3). This result supports the findings of Perry et al. (2014), which suggest that E_2 concentration during estrus is related to intensity signs.

In the group with P_4 low estrus cycle, the E_2 concentration from −2 to 0 days before estrus was significantly higher at 16.7 ± 2.1 pg/mL, while the P_4 concentration on day 7 after estrus was significantly lower at 0.7 ± 0.9 ng/mL (Table 7). This estrus pattern aligns with Nishigai et al. (2000), who reported a low pregnancy rate after embryo transfer. It suggests that although estrus cycles are regular, ovarian activity may not completely recover. The hormonal range of normal estrus cycles differs from Nishigai et al. (2000), who reported that pregnancy rates increase when P_4 concentration is ≥ 2.5 ng/mL and the E_2/P_4 ratio is < 1.0 at 7 after estrus. However, Yang et al. (1999) reported that P_4 concentration increased between 4 and 5 days after ovulation, and reached a maximum P_4 concentration of 2.6 to 6.2 ng/mL on 17 days after ovulation in Hanwoo cows. In the present study, P_4 concentration on day 7 after estrus in the normal estrus cycle was significantly higher at 2.1 ± 1.3 ng/mL and the P_4 concentration 14 days after estrus in normal estrus cycles corresponded to the normal range at 4.5 ± 2.0 ng/mL. This suggests that the rate of increase and decrease in E_2 and P_4 concentrations may be associated with the recovery of the estrus cycle. It was confirmed that the decrease in E_2 concentration is slow and the increase in P_4 concentration is insufficient in short and P_4 low estrus cycle groups. Therefore, estimating of hormone concentrations during the early postpartum estrus cycle needed to assess the recovery of the estrus cycle in each individual.

Uterine involution is a critical factor influencing subsequent pregnancy on postpartum (Spicer et al., 1986; Short et al., 1990). During uterine involution, the prostaglandin $F_2\alpha$ concentration is higher than normal quantities, leading to premature regression of the CL before maternal recognition of pregnancy (Crowe, 2008). As shown in Table 5, the cervix recovered to 42.0 ± 3.5 mm and the diameter of the uterine horn to 34.4 ± 7.1 mm by 24 days postpartum, with the difference in uterine horn diameter recovering to 2.6 ± 1.2 mm by 31 days postpartum. Recovery of uterine size in primiparous Hanwoo cow was completed within 31 days. In beef cattle, uterine size recovery occurs between 20 and 40 days postpartum (Spicer et al., 1986; Short et al., 1990), and Spicer et al. (1986) reported that the uterus recovers by 28 days postpartum in beef cattle. The endometrium returns to its original state at 40 days postpartum (Okano and Tomizuka, 1996).

CONCLUSION

In the present study, we investigated the period and criteria for the recovery of ovarian activity and uterine involution in primiparous Hanwoo cows. The first estrus was observed at 19.1 ± 6.5 days postpartum, but a normal estrus cycle was observed at 39.2 ± 6.4 days postpartum. The size of the cervix and uterine horn recovered to 42.0 ± 3.0 mm and 34.4 ± 7.1 mm by 24 days postpartum and the difference in uterine horn diameter returned to 2.6 ± 1.2 mm by 31 days postpartum. Taken together, the recovery of the reproductive tract was complete at approximately 40 days postpartum in primiparous Hanwoo cows. This study can aid in determining the optimal period for artificial insemination and provides foundational data for postpartum Hanwoo studies.

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