

# Postpartum Reproductive Tract Recovery and Prevalence of Health Problems in Dairy Cows

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Abstract : This study estimated the degree of uterine inflammation and resumption of ovarian cyclicity in postpartum dairy cows. In addition, the prevalence of health problems during calving and the postpartum period was investigated. A total of 224 Holstein dairy cows from four dairy farms in Chungcheong Province were used for the study. Uterine discharge was scored on a 0 to 5 rank scale (metricheck score) using a metricheck instrument from 1 to 8 weeks postpartum and uterine cytology (neutrophil level) was examined at 4, 6 and 8 weeks postpartum to evaluate uterine inflammation. Resumption of ovarian cyclicity was evaluated based on progesterone analysis (≥ 1 ng/mL) at 4, 6 and 8 weeks postpartum. Postpartum disorders were diagnosed by veterinarians in the research team. The metricheck score decreased linearly (p < 0.0001) from weeks 1 (3.4 ± 0.1) to 4 (2.0 ± 0.1) postpartum, and then remained at that level through week 8 postpartum ( $1.6 \pm 0.1$ , p > 0.05). The neutrophil levels determined by uterine cytology assays also decreased (p < 0.0001) from week 4 (22.3 ± 1.8%) to 6 (12.1 ± 1.4%) postpartum, and then remained at that level through week 8 postpartum (9.2  $\pm$  1.4%, p > 0.05). The proportion of cows exhibiting the ovarian cyclicity increased linearly (p < 0.0001) from weeks 2 (12.1%) to 8 (74.3%) postpartum. The prevalence of dystocia and retained placenta were 20.5% and 30.4%, respectively. The prevalence of ketosis, milk fever, abomasal displacement, septicemic metritis, endometritis, subclinical endometritis, pyometra, and ovarian cysts were 18.8%, 3.6%, 4.0%, 36.2%, 29.5%, 17.0%, 4.5%, and 14.7%, respectively. The prevalence of digestive problems and mastitis were 5.4% and 9.6%, respectively. The prevalence of culling and death were 4.0% and 3.6%, respectively. The prevalence of dystocia, retained placenta, septicemic metritis, endometritis, ovarian cysts, digestive problems, mastitis, and culling differed among farms (p < 0.05to 0.01). In conclusion, proper examinations for uterine inflammation and ovarian cyclicity during 4 to 8 weeks postpartum are useful clinical tools to maintain herd reproductive health. Dystocia, retained placenta, septicemic metritis, and endometritis were predominant disorders in dairy cows of the survey area.

Key words : dairy cows, uterine inflammation, postpartum cyclicity, health problems.

## Introduction

Milk production, nutritional management, health status, and fertility in dairy cows are closely correlated (1,29,31). Adequate reproductive tract involution and resumption of ovarian cyclicity are important for subsequent fertility in dairy cows (41) and puerperal uterine disease is an important risk factor for delayed uterine involution (16,40). The resumption of postpartum cyclicity is also influenced by uterine inflammation, nutritional status, calving season, and cow parity (21,33,40).

Traditionally, transrectal palpation and ultrasonography have been used to evaluate involution of the reproductive tract (16,25). Uterine location (abdominal or pelvic), contents, tone, mobility, and horn asymmetry have been examined by transrectal palpation, and the existence of intrauterine fluid and endometrial thickness, as well as ovarian structures (corpus luteum and follicles), have been examined by ultrasonography (16,25). Vaginal discharge has been examined and scored, qualitatively, as clear, clear fluid with some

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flakes of pus, mucopurulent (approximately 50% pus and 50% mucus), or purulent (> 50% pus), with or without fetid odor (39). Moreover, uterine cytology has been used to determine endometrial inflammation (subclinical endometritis) (13).

Severe negative energy balance (NEB) during the transition period that lasts from approximately 3 weeks before calving to approximately 3 weeks after calving has been correlated with an imbalance in metabolic status, reduced immunity, and endocrine imbalance, resulting in several postpartum disorders in dairy cows (3,7,14,15,19,24). Uterine diseases (metritis, endometritis, and subclinical endometritis) were the major postpartum diseases that were correlated with reproductive performance and milk production in dairy cows (27). Retained placenta (12.3%), metritis (17.1%), and ketosis (15.8%) were the predominant diseases in some regions of the USA (37). Endometritis (11.7%) and mastitis (22.0%) were the most prevalent clinical diseases, and subclinical ketosis (40.1%) was the most prevalent subclinical disease, in two grazing herds in Florida (32). A study including 10 European countries found that the prevalence of retained placenta (10.4%), metritis (9.6%), and subclinical ketosis (21.8%) were higher than other postpartum diseases in dairy cows (35).

Milk yield has increased dramatically in recent decades

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and has resulted in lower fertility in Korean dairy herds, which may be attributed to increased postpartum disorders (20,28). Nevertheless, investigations regarding the recovery of the postpartum reproductive tract and the prevalence of postpartum diseases in Korean dairy herds have rarely been conducted. Therefore, this study evaluated the degree of uterine inflammation and the resumption of ovarian cyclicity during the postpartum period, and also investigated calving problems and postpartum disorders in dairy cows as a prerequisite for establishing a proper health control regimen.

## **Materials and Methods**

## Animals and health management

This study was conducted on four dairy farms (A-D) in Chungcheong Province during 2012 and 2013. A total of 224 Holstein dairy cows, with  $2.5 \pm 1.4$  lactations (mean  $\pm$  standard deviation; range: 1-7 lactations), were enrolled in this study. The cows were maintained in a loose housing system, fed a total mixed ration, and milked twice daily. The mean milk yields for farms A-D were approximately 12,000, 9,500, 10,000, and 8,500 kg per cow per year. All cows received weekly reproductive health checks by veterinarians in the research team. These included examination of ovarian structures and the uterus *via* transrectal palpation and ultrasonography.

## Evaluation of uterine inflammation

All of the cows were evaluated for vaginal discharges using the metricheck instrument (26) at weeks 4, 6, and 8 postpartum. Briefly, after cleaning the vulva with the disinfectant (chlorhexidine gluconate), the metricheck device was inserted into the vagina until it reached the vaginal fornix and then retracted for evaluation of the vaginal mucus contained in the cup. Any discharge from the cervical ostium or on the floor of the vagina was scored on a ranked scale (0 = no discharge, 1 = clear mucus, 2 = flecks of purulent material within otherwise clear mucus, 3 = mucopurulent but < 50% purulent material and 5 = mucopurulent with > 50% purulent material and a fetid odor).

Tissue samples for uterine cytology were collected at weeks 4, 6, and 8 postpartum (18). Briefly, after cleaning the vulva, a cytobrush and stainless steel rod (which was guarded by a stainless steel sheath and covered with a protective plastic sheath) were introduced into the vagina. At the external ostium of the cervix, the plastic sheath was pulled back, and the stainless steel sheath and stainless steel rod and cytobrush were passed into the body of the uterus. The stainless steel sheath was then retracted to expose the cytobrush. The cytobrush was rotated clockwise to obtain cellular material from the endometrium. After removal from the vagina, the brush was rolled onto a glass slide and the sample was air-dried. All slides were stained using the Diff-Quick stain (Sysmex Inc., Kobe, Japan) according to the manufacturer's guidelines. Each slide was examined microscopically (× 200 magnification) by the same examiner. The numbers of epithelial endometrial cells and neutrophils were counted (up to 200 cells per slide) and the percentage of neutrophils was calculated.

The uterus was also examined via transrectal palpation and

ultrasonography at weeks 4, 6, and 8 postpartum.

#### Estimation of resumption of ovarian cyclicity

Blood samples were collected from the tail vein at weeks 2, 4, 6, and 8 postpartum. Ten milliliters of blood were placed into a plastic centrifuge tube without additives and immediately placed in an ice bath. The samples were then centrifuged at  $2000 \times \text{g}$  for 10 min at 4°C, and the serum was harvested and frozen at  $-80^{\circ}$ C until required. Resumption of ovarian cyclicity was evaluated based on the progesterone concentration ( $\geq 1$  ng/mL).

Serum progesterone concentrations were determined using the Immulite 1000 Immunoassay System (DPC Cirrus Inc., Flanders, NJ, USA), which automates the entire assay process. Briefly, after incubating the Immulite bead with the plasma sample (50  $\mu$ L) and the alkaline phosphatase reagent at 37°C for 30 min, the reaction mixture is separated from the bead by spinning the Test Unit at high speed on its vertical axis. The amount of bound label is then quantitated with a chemiluminescent dioxetane substrate. Light emission is measured using a Photomultiplier Tube, and the results are calculated for each sample. The analysis procedure applied in this study was performed according to the guidelines provided by the manufacturer of the immunoassay system. The intra- and interassay coefficients of variation were < 10% for each assay.

## **Diagnosis of health problems**

The definitions of problems during the calving and postpartum periods used in the present study were similar to those found in previous publications (6,13,23,32,34). Calving difficulty was scored on rank scale (1 = no assistance, 2 =minor assistance, 3 = forceful, 4 = extreme force, and 5 =caesarian section). Cows with calving score > 2 were considered to have dystocia. Retained placenta was defined as the retention of the fetal membrane for longer than 24 h. Septicemic metritis was defined by the presence of fever ( $\geq 39.5^{\circ}$ C) and watery, fetid uterine discharge during the first 10 days postpartum. Ketosis was diagnosed by the following clinical signs: anorexia, depression, and the odor of acetone on the breath. Milk fever was diagnosed by weakness and recumbence after calving. Abomasal displacement was diagnosed by a ping sound during abdominal auscultation. Pyometra was defined by the presence of a distended uterus with pus using ultrasonography. Endometritis was diagnosed by a metricheck score  $\geq$  3 and ultrasonography at postpartum week 4. Subclinical endometritis was evaluated by uterine cytology (neutrophil level > 18%) in the absence of endometritis at postpartum week 4. Ovarian cysts were diagnosed based on the identification of follicles > 25 mm in diameter on the ovaries using ultrasonography and, based on the thickness of the follicle wall, were classified as follicular cysts (< 3 mm) or luteal cysts ( $\geq$  3 mm). Digestive problems were characterized by diarrhea or bloat. Mastitis was characterized by the presence of abnormal milk or by signs of inflammation in one or more of the four quarters of the udder. With the exception of dystocia and mastitis, all other diseases were diagnosed by veterinarians in the research team, and postpartum diseases not mentioned above, including culling and death, were recorded for 8 weeks postpartum.

#### Statistical analysis

Results were expressed as the means  $\pm$  standard error of the means (SEM). Data on the metricheck score, proportion of neutrophils, and percentages of cows with cyclicity during the postpartum period were analyzed by ANOVA, and means were compared by Duncan's multiple range test. The prevalence of dystocia, retained placenta, and other postpartum disorders was evaluated by the Chi-square test or Fisher's exact test (when some of the frequencies were too low (< 5) to use Chi-square). Differences with  $p \le 0.05$  were considered significant.

### Results

The metricheck score decreased linearly (p < 0.0001) from weeks 1 (3.4 ± 0.1) to 4 (2.0 ± 0.1) postpartum, and then remained at a similar level through postpartum week 8 (1.6 ± 0.1, p > 0.05, Fig 1). The neutrophil level counted in the uterine cytology assays also decreased (p < 0.0001) from weeks 4 (22.3 ± 1.8%) to 6 (12.1 ± 1.4%) postpartum, and then remained at a similar level through postpartum week 8 (9.2 ± 1.4%, p > 0.05, Fig 2). The proportion of cows with the ovarian cyclicity, based on serum progesterone levels, increased linearly (p < 0.0001) from weeks 2 (12.1%) to 8 (74.3%)

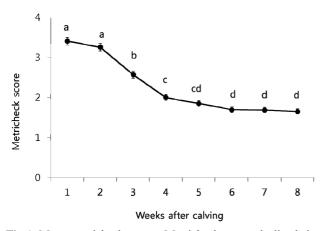


Fig 1. Mean metricheck scores. Metricheck scores declined significantly after calving and reached stable levels by week 5 postpartum. Means with different superscripts (a, b, c, d) differ significantly (p < 0.0001).

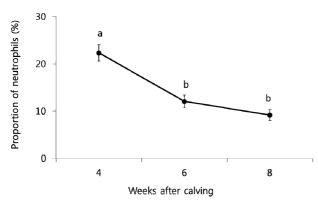


Fig 2. Neutrophil levels. The fraction of neutrophils in the uterine cytology assays declined significantly after calving. Means with different superscripts (a, b) differ significantly (p < 0.0001).

postpartum (Fig 3). Table 1 presents the prevalence of peripartum events, postpartum disorders, culling, and death. The prevalence of dystocia and retained placenta were 20.5% and 30.4%, respectively. The prevalence of ketosis, milk fever, abomasal displacement, septicemic metritis, endometritis, subclinical endometritis, pyometra, and ovarian cysts were 18.8%, 3.6%, 4.0%, 36.2%, 29.5%, 17.0%, 4.5%, and 14.7%, respectively. The prevalence of digestive problems and mastitis were 5.4% and 9.6%, respectively. The prevalence of culling and death were 4.0% and 3.6%, respectively. The prevalence of dystocia, retained placenta, septicemic metritis, endometritis, ovarian cysts, digestive problems, mastitis, and culling differed among the farms included in this study (p < 0.05 to 0.01, Table 1).

## Discussion

This study evaluated the degree of uterine inflammation and resumption of ovarian cyclicity during the postpartum stage, as well as the prevalence of calving problems and postpartum disorders, in dairy cows of four dairy farms in the Chungcheong area. Involution of the uterus based on the levels of inflammatory response through postpartum week 6, and the proportion of cows with resumption of postpartum cyclicity through postpartum week 8, both exhibited a continuous increase. Dystocia, retained placenta, septicemic metritis, and endometritis were the predominant disorders in dairy cows of the survey area.

The uterine inflammatory response was based on the metricheck method and declined continuously over weeks 1 to 4 postpartum in the present study. By contrast, a previous study in New Zealand dairy herds showed a decline in endometritis through postpartum week 3 using the metricheck tool (26). However, a previous report on US dairy herds indicated that the prevalence of cytologically diagnosed endometritis declined from 2 through 8 weeks postpartum (13). The discrepancies in the length of time of uterine inflammation using the same diagnostic tools among these studies may reflect different farm management practices, environments, and other herd conditions.

Several studies have emphasized the negative effects of

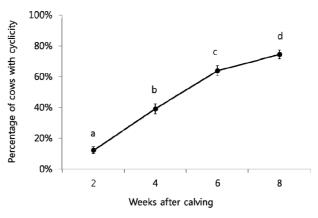


Fig 3. Rates of ovarian cyclicity. The percentage of cows exhibiting ovarian cyclicity increased significantly with postpartum time. Means with different superscripts (a, b, c, d) differ significantly (p < 0.0001).

Disorders	Farm					
	Overall prevalence, %	А	В	С	D	- p
n	-	56	39	35	94	-
Dystocia	20.5	14.3ª	7.7ª	11.4ª	33.0 <sup>b</sup>	< 0.01
Retained placenta	30.4	10.7 <sup>a</sup>	41.0 <sup>c</sup>	20.0 <sup>ab</sup>	41.5°	< 0.01
Ketosis	18.8	12.5	17.9	11.4	25.5	> 0.05
Milk fever	3.6	3.6	7.7	2.9	2.1	> 0.05
Abomasal displacement	4.0	1.8	10.3	5.7	2.1	> 0.05
Septicemic metritis	36.2	14.3ª	33.3 <sup>b</sup>	28.6 <sup>ab</sup>	53.2°	< 0.01
Endometritis	29.5	14.3 <sup>ab</sup>	28.2 <sup>b</sup>	5.7ª	47.9 <sup>c</sup>	< 0.01
Subclinical endometritis	17.0	23.2	15.4	11.4	16.0	> 0.05
Pyometra	4.5	3.6	2.6	2.9	6.4	> 0.05
Ovarian cysts	14.7	14.3 <sup>ab</sup>	25.6 <sup>b</sup>	20.0 <sup>ab</sup>	8.5ª	< 0.01
Digestive problems	5.4	12.5 <sup>b</sup>	5.1 <sup>ab</sup>	2.9 <sup>ab</sup>	2.1ª	< 0.05
Mastitis	9.4	$0^{\mathrm{a}}$	$0^{a}$	34.3°	9.6 <sup>b</sup>	< 0.01
Culling	4.0	14.3 <sup>b</sup>	2.6 <sup>ab</sup>	0ª	$0^{a}$	< 0.01
Death	3.6	1.8	2.6	8.6	3.2	> 0.05

Table 1. Prevalence of peripartum events, postpartum disorders, culling, and death

<sup>a,b,c</sup>Values with different superscripts within the same row differed significantly among farms.

delayed resumption of postpartum cyclicity on subsequent reproductive performance in dairy cattle (10,11,21). The resumption of cyclicity depends on the frequency of luteinizing hormone pulses from the anterior pituitary and may be associated with energy balance (4). In the present study, the proportion of cows with ovarian cyclicity increased continuously from 2 to 8 weeks postpartum, similar to a previous report in which the interval from calving to resumption of ovarian activity ranged from 13 to 64 days (40). Another study demonstrated that the interval from calving to first ovulation was longer in primiparous cows ( $31.8 \pm 10.1$  days) than in multiparous cows  $(21.3 \pm 5.0 \text{ days})$  (41); theses intervals were shorter than the mean interval in the present study  $(34.3 \pm 13.3 \text{ days}, \text{data not})$ shown). The differences in timing of the postpartum resumption of ovarian cyclicity among these studies may be influenced by uterine inflammation, nutritional status, calving season, and cow parity (21,33,40). Taken together, the evaluation of uterine inflammation and the resumption of ovarian cyclicity may indicate the degree of functional recovery of the reproductive tract and may be correlated with subsequent reproductive performance in dairy cows. Thus, routine periodical health examination may be beneficial for dairy cow health during the postpartum period (voluntary waiting period).

Postpartum disorders impact subsequent reproductive performance of dairy cows and result in the need for culling the animals, causing severe economic losses in the dairy industry world-wide (2,19,22,23,38). The prevalence of periparturient disorders, e.g., dystocia (20.5%) and retained placenta (30.4%), was considerably higher in the study than in previous studies reporting 1.4% for dystocia and 3.2% for retained placenta, respectively (32), 6.9% for dystocia (8), and 12.3% for retained placenta (37). Among other metabolic disorders, the prevalence of ketosis (18.8%) was higher than milk fever (3.6%) or abomasal displacement (4.0%) in our study, similar to a previous study where the prevalence of ketosis, milk fever, and abomasal displacement was 15.8%. 1.1%, and 3.8%, respectively (37). In a study of multiparous dairy cows, the prevalence of ketosis, milk fever, and abomasal displacement was 28.1%, 5.7%, and 2.0%, respectively (9), a higher prevalence than our results with the exception of abomasal displacement.

Among uterine diseases, septicemic metritis (36.2%) and endometritis (29.5%) occurred more frequently than subclinical endometritis (17.0%) and pyometra (4.5%) in the present study, and these rates were higher than a recent study in which the prevalence of septicemic metritis, endometritis, and subclinical endometritis was 5.3%, 15.0%, and 13.4%, respectively (32). Other studies report the prevalence of septicemic metritis in the range of 9.6% to 17.1% (35,37), which is also lower than our results. The prevalence of ovarian cysts in our study (14.7%) was similar with two previous studies reporting ranges of 15.2% and 15.7% (12,30). By contrast, a prevalence of 30% was reported in a UK study (17), and a prevalence of only 2.7% was found in an Argentine study (5).

The prevalence of digestive problems (5.4%) and mastitis (9.4%) was similar to the results of Ribeiro et al. (32) for the prevalence of digestive problems (4.0%), but they found a much higher prevalence of mastitis (15.3%) than this study. A European study, however, demonstrated a lower prevalence of mastitis (6.1%) (35). The combined rates of culling and death (7.6%) in the present study were slightly higher than the removal rate (5.5%) from herds in a previous study (37). Our observation that the prevalence of dystocia, retained placenta, septicemic metritis, endometritis, ovarian cysts, digestive problems, mastitis, and culling differed among farms, both in this study and in previous reports, may be due to dif-

ferences in farm management, environment, facilities (including stall space), and other herd conditions among farms.

Taken together, the present study revealed that there was a higher prevalence of the periparturient disorders (dystocia and retained placenta) and uterine disease (septicemic metritis and endometritis) in dairy cows of the survey area. Therefore, veterinary practitioners and dairy owners should consider methods to prevent these disorders through careful health controls and proper nutritional management during the prepartum, calving, and postpartum periods. Moreover, proper checkups for uterine inflammation and resumption of ovarian cyclicity during weeks 4 to 8 postpartum may be useful clinical tools to monitor herd reproductive health.

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## 젖소의 분만 후 생식기의 회복 및 질병 발생 조사

## 정재관·최인수·강현구·정영훈\*·허태영\*·김일화<sup>1</sup>

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**요** 약 : 본 연구는 젖소에서 산후 자궁염증 및 난소주기 회복 정도를 평가하였으며, 또한 분만 사고 및 산후 기간 질 병 발생에 대한 조사를 위하여 실시하였다. 충청 지역 4개 젖소 목장의 사육 젖소 224두를 이용하였다. 자궁의 염증 상태를 평가하기 위하여, 분만 후 8주까지 매주 메트리체크 기구를 이용하여 자궁분비물 중 농의 비율에 따른 성상을 접수화(메트리체크 점수; 0-5점) 하였으며, 분만 후 4, 6, 8주에는 백혈구 비율 산정을 위한 자궁세포 검사를 실시 하 였다. 분만 후 난소주기 재개 여부 확인을 위하여 4, 6, 8주에 프로게스테론 농도(≥ 1 ng/mL)를 분석하였다. 분만 후 데트리체크 점수는 1주(3.4±0.1)부터 4주(2.0±0.1)까지 연속적으로 감소하였으며(*p* < 0.0001), 이 후 8주까지 비슷한 점수를 유지하였다(*p* > 0.05). 자궁세포 검사를 이용한 호중구 비율은 분만 후 4주(22.3±1.8%)에서 6주(12.1±1.4%)까 지 감소하였으며(*p* < 0.0001), 이 후 8주(9.2±1.4%)까지 비슷한 비율을 유지하였다(*p* > 0.05). 난소주기 재개는 분만 후 2주(12.1%)에서 8주(74.3%)까지 연속적으로 증가하였다(*p* < 0.0001). 난산과 후산정체 발생 비율은 각각 20.5%, 30.4% 였다. 분만 후 케토시스, 유열, 4위전위증, 패혈성 자궁염, 자궁내막염, 준임상형 자궁내막염, 자궁축농증 및 난소낭종 발생율은 각각 18.8%, 3.6%, 4.0%, 36.2%, 29.5%, 17.0%, 4.5%, 14.7%였다. 소화기 질병 및 유방염 발생율은 각각 5.4%, 9.6%였다. 도태 및 폐사율은 각각 4.0%, 3.6%였다. 난산, 후산정체, 패혈성 자궁염, 자궁내막염, 난소낭종, 소화 기 질병, 유방염 및 도태율은 목장간 차이가 있었다(*p* < 0.05). 결론적으로, 분만 후 4주에서 8주까지 자궁염 중 상태 및 난소주기 재개 확인은 축군의 번식위생 유지에 도움을 줄 수 있는 임상적인 수단으로 사용될 수 있으며, 조사 지역에서의 젖소에서 난산, 후산정체, 패혈성 자궁염 및 자궁내막염의 발생율이 높았음을 보여 주었다.

주요어 : 젖소, 자궁 염증, 난소 주기, 질병 발생