

Relationship between Incidence of Endometritis and Metabolic Status during Peri- and Postpartum Periods in Dairy Cows

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Abstract : This study compared blood metabolites during peri- and postpartum periods among cows with clinical or subclinical endometritis and cows without endometritis. Blood samples from 207 Holstein dairy cows were collected at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum to measure serum concentrations of calcium, magnesium, non-esterified fatty acids (NEFAs), total cholesterol, albumin, urea nitrogen, β -hydroxybutyrate (BHBA), aspartate aminotransferase (AST), γ -glutamyltransferase, glucose, and phosphorus. Clinical endometritis was diagnosed by the observation of vaginal discharge ($>50\%$ pus) and subclinical endometritis was diagnosed by the evaluation of uterine cytology ($>18\%$ neutrophils) at 4 weeks postpartum. Cows were divided into three groups based on the presence or absence of clinical or subclinical endometritis: the control group ($n = 104$), the clinical endometritis group ($n = 66$), and the subclinical endometritis group ($n = 37$). Calcium and magnesium concentrations were lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the study period ($p < 0.05$ to 0.0001), whereas the NEFAs concentration was higher in the clinical endometritis group than in the control group throughout the study period ($p < 0.01$). The total cholesterol concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the pre- and postpartum periods ($p < 0.05$ to 0.001). The albumin concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups during the postpartum period ($p < 0.05$ to 0.001). The urea nitrogen concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups at 4 and 6 weeks postpartum ($p < 0.01$). At 1 week postpartum, the BHBA concentration was higher in the clinical endometritis group than in the control group ($p < 0.05$), whereas the AST concentration was higher in the clinical endometritis and subclinical endometritis groups than in the control group ($p < 0.05$). In conclusion, lower serum concentrations of calcium, magnesium, total cholesterol, albumin, and urea nitrogen, but higher concentrations of NEFAs, BHBA, and AST during the postpartum period were associated with the incidence of clinical endometritis, indicating the importance of balanced nutrition during the transition period.

Key words : dairy cows, clinical endometritis, subclinical endometritis, blood metabolites.

Introduction

Postpartum uterine disease is a common reproductive disease that results in a reduced reproductive performance leading to substantial economic losses (9,17,27). Although most cows are exposed to bacterial infection after calving (8), more than 70% remove the uterine bacteria *via* innate immune responses. Thus, 15-20% of cows develop clinical endometritis, and about 30% develop subclinical endometritis (28). Clinical endometritis is represented the presence of purulent ($>50\%$ pus) uterine discharge 21 days or more after parturition, or mucopurulent (approximately 50% pus and 50% mucus) discharge 26 days postpartum (19,26). Subclinical endometritis, defined as endometrial inflammation of the uterus in the absence of purulent material in the vagina, is diagnosed by uterine cytology to determine the proportion of neutrophils in samples (9). Likewise, subclinical endometritis is defined as the presence of $>18\%$ neutrophils in uterine

cytology samples collected 21-33 days postpartum or $>10\%$ neutrophils in samples collected 34-47 days postpartum (16).

Postpartum uterine infection and development of the disease depend on the bacterial load, pathogenicity of the microorganism, and defense mechanisms of the uterus (27). Thus, proper regulation of immune responses during the weeks after calving is important for subsequent uterine health (31). However, during the transition period, reduced immune function due to decreased dry matter intake leading to a negative energy balance influences uterine disease (13). Recent studies reported that the cytokine profile in uterine tissue and uterine flushing is associated with the severity (clinical and subclinical endometritis) and persistency of uterine inflammation (18,20). Moreover, Dubuc *et al.* (6) demonstrated that the risk factors for purulent vaginal discharge (clinical endometritis) and cytological endometritis (subclinical endometritis) are different. Purulent vaginal discharge is more likely to occur in cows with dystocia, twins, and metritis, which might increase uterine trauma and bacterial contamination. However, cytological endometritis is a greater risk in cows experiencing hyperketonemia during 1 week postpartum or with a

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thin body condition score at parturition, which might reflect the effect of immune suppression or metabolic imbalance in the peripartum period. Moreover, purulent vaginal discharge and cytological endometritis both result in a reduced risk of pregnancy; however, the effects are cumulative, suggesting that they have different origins (7). In this respect, understanding the mechanism underlying uterine inflammation in clinical vs. subclinical endometritis might be required to cope with these diseases.

Several blood metabolic profiles including β -hydroxybutyrate (BHBA), non-esterified fatty acids (NEFAs), albumin, and calcium change during peri- and postpartum periods, and the resultant changes in metabolic parameters during these periods may provide useful information related to postpartum uterine health (4,22). However, the levels of blood metabolites have rarely been determined separately for cows with clinical or subclinical endometritis in individual studies. Determining how clinical and subclinical endometritis are related to metabolic changes might be important to understand the mechanism underlying the severity of uterine inflammation and might provide a valuable plan to prepare an effective uterine health management strategy in dairy herds. Therefore, the objective of this study was to compare blood metabolites among cows with clinical or subclinical endometritis and those without endometritis during peri- and postpartum periods.

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 207 Holstein dairy cows with 2.4 ± 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 were approximately 8,500-12,000 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.

Study design

At 4 weeks postpartum, cows were grouped based on the presence or absence of clinical or subclinical endometritis: the control group ($n = 104$), the clinical endometritis group ($n = 66$), and the subclinical endometritis group ($n = 37$). This study compared blood metabolites among cows with clinical or subclinical endometritis and those without endometritis during 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum.

Evaluation of uterine disease

All the cows were evaluated for vaginal discharges using the Metricheck instrument (23) at 4 weeks postpartum. Briefly, after cleaning the vulva with disinfectant (chlorhexidine gluconate), the Metricheck device (Metricheck, Simcrotech, Hamilton, New Zealand) was inserted into the vagina until it reached the vaginal fornix and was then retracted to evaluate 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 with <50% purulent material, 4 = mucopurulent with >50% purulent material, and 5 = mucopurulent with >50% purulent material and a fetid odor). Clinical endometritis was diagnosed by a Metricheck score of ≥ 3 .

To diagnose subclinical endometritis, tissue samples for uterine cytology were collected at 4 weeks postpartum (16). Briefly, after cleaning the vulva, a cytobrush and stainless steel rod (Aries Surgical, Davis, CA, USA), 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, 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 ($\times 200$ 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. Subclinical endometritis was evaluated by uterine cytology ($> 18\%$ neutrophils) in the absence of clinical endometritis. Cows not diagnosed with clinical or subclinical endometritis were classified as controls.

Measurement of metabolite levels in serum samples

The concentrations of calcium, magnesium, NEFAs, total cholesterol, albumin, urea nitrogen, BHBA, aspartate aminotransferase (AST), γ -glutamyltransferase (γ GT), glucose, and phosphorus were measured in serum samples with a 7180 Biochemistry Automatic Analyzer 710 (Hitachi Ltd., Tokyo, Japan) using commercial enzyme assay kits (Wako Pure Chemical Ltd., Osaka, Japan), according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were $< 5\%$ for each assay.

Statistical analysis

Results were expressed as the means \pm standard error of the means. For statistical analyses, cow parity was grouped as either primiparous or multiparous. Statistical analyses were performed using the SAS program (version 9.2, SAS Inst., Cary, NC, USA).

The effects of group (control, clinical endometritis, or subclinical endometritis), cow parity (primiparous or multiparous), sampling time (number of weeks pre- and postpartum), and two-way interactions between group, cow parity, and sampling time on serum metabolite concentrations were determined using the mixed model. Cows were included in the model as a random effect. Duncan's multiple range tests were used to identify significant main effects. A p -value ≤ 0.05 was considered significant, and $0.05 < p < 0.1$ was considered to indicate a tendency toward significance.

Results

Table 1 presents the distribution of cows with clinical or subclinical endometritis, or control cows among 4 farms.

There were significant effects of group ($p < 0.05$ to 0.0001) and sampling time ($p < 0.0001$), but no interaction between group and sampling time ($p > 0.05$) on the calcium, magne-

Table 1. Distribution of cows with clinical or subclinical endometritis, or control cows among 4 farms

| Farm (No. cows) | Group | | |
|--------------------|-------------|------------------------------|---------------------------------|
| | Control (%) | Clinical endometritis (%) | Subclinical endometritis (%) |
| A (36) | 52.8 | 30.5 | 16.7 |
| B (33) | 81.8 | 6.1 | 12.1 |
| C (47) | 57.5 | 17.0 | 25.5 |
| D (91) | 34.1 | 49.4 | 16.5 |

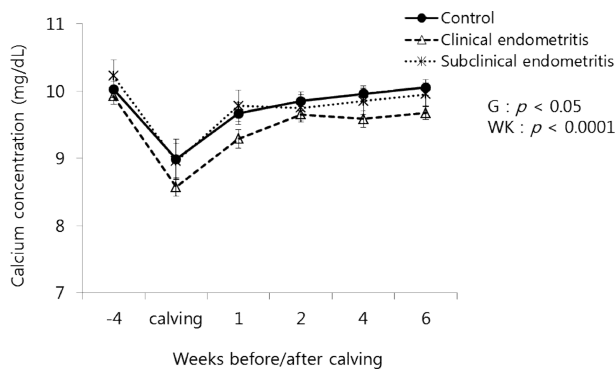


Fig 1. Calcium concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect. The calcium concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the study period ($p < 0.05$).

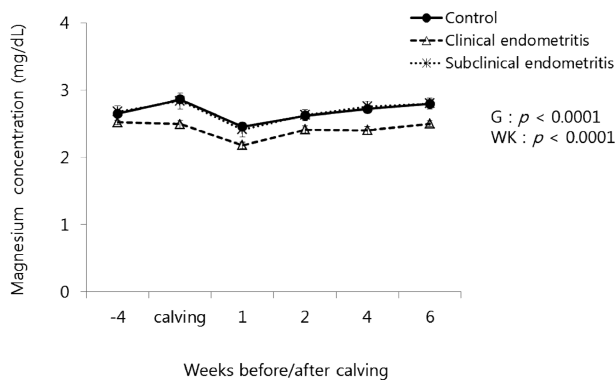


Fig 2. Magnesium concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect. The magnesium concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the study period ($p < 0.0001$).

sium, and NEFA concentrations. Calcium and magnesium concentrations were significantly lower in the clinical endometritis group than in the control and subclinical endometritis groups ($p < 0.05$ to 0.0001 , Figs 1 and 2). The NEFA concentration was higher in the clinical endometritis group than in the control group throughout the study period ($p < 0.01$), whereas it tended to be higher in the clinical endometritis group than in the subclinical endometritis group ($p < 0.1$, Fig 3). Multiparous cows had a higher serum NEFA concentration than primiparous cows ($p < 0.001$), whereas calcium and magnesium concentrations were not affected by cow parity.

There were significant effects of group ($p < 0.05$ to 0.0001), sampling time ($p < 0.0001$), and interaction between group and sampling time ($p < 0.05$ to 0.0001) on the total cholesterol, albumin, and urea nitrogen concentrations. The total cholesterol concentration was lower in the clinical endometri-

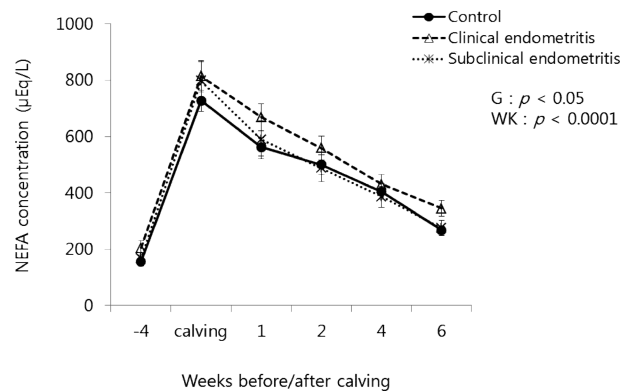


Fig 3. NEFA concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect. The NEFA concentration was higher in the clinical endometritis group than in the control group throughout the study period ($p < 0.01$), whereas it tended to be higher in the clinical endometritis group than in the subclinical endometritis group ($p < 0.1$).

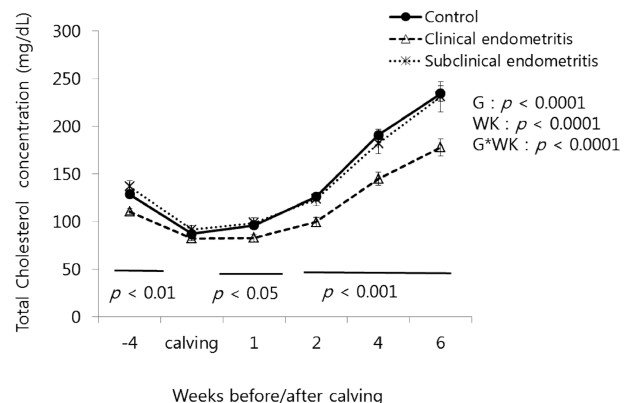


Fig 4. Total cholesterol concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect, $G*WK$: group-by-sampling period effect. The total cholesterol concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the pre- and postpartum periods ($p < 0.05$ to 0.001).

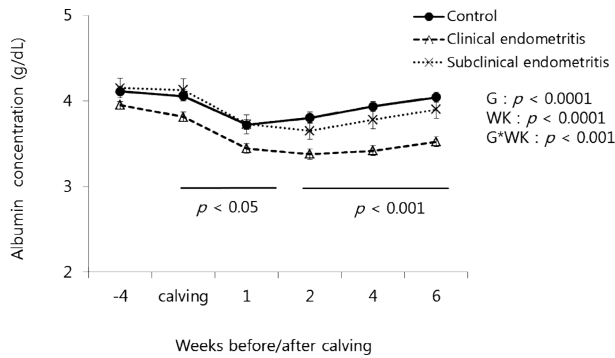


Fig 5. Albumin concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect. The albumin concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups during the postpartum period ($p < 0.05$ to 0.001).

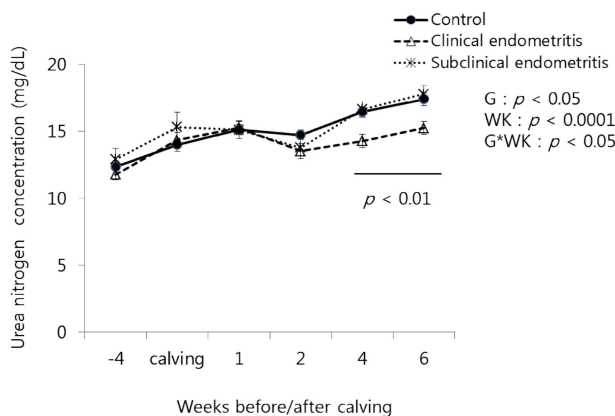


Fig 6. Urea nitrogen concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect. The urea nitrogen concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups at 4 and 6 weeks postpartum ($p < 0.01$).

tis group than in the control and subclinical endometritis groups throughout the pre- and postpartum periods ($p < 0.05$ to 0.001, Fig 4). The albumin concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups during the postpartum period ($p < 0.05$ to 0.001, Fig 5). The urea nitrogen concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups at 4 and 6 weeks postpartum ($p < 0.01$, Fig 6). Multiparous cows had a higher serum albumin concentration than primiparous cows ($p < 0.05$), whereas parity did not affect total cholesterol or urea nitrogen concentrations.

There was no significant effect of group ($p > 0.05$), but significant effects of sampling time ($p < 0.0001$) and the interaction between group and sampling time ($p < 0.05$ to 0.01) on the BHBA and AST concentrations. The BHBA concentration was higher in the clinical endometritis group than in the

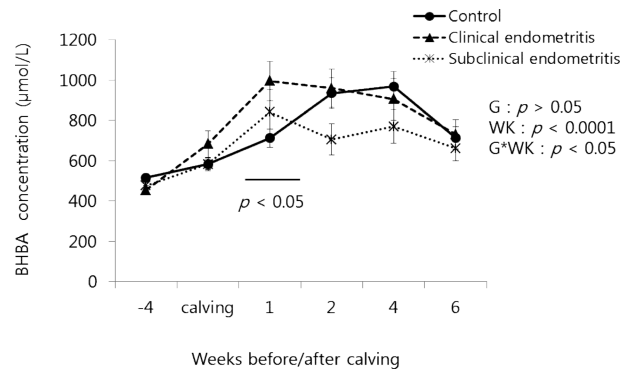


Fig 7. BHBA concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect. The BHBA concentration was higher in the clinical endometritis group than in the control group at 1 week postpartum ($p < 0.05$).

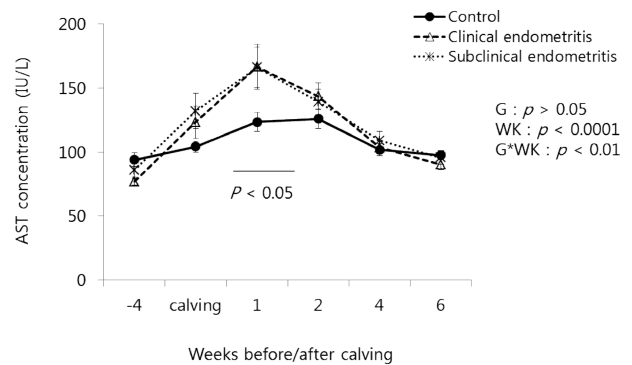


Fig 8. AST concentrations in the control, clinical endometritis, and subclinical endometritis groups at 4 weeks prepartum, just after calving, and at 1, 2, 4, and 6 weeks postpartum. G: group effect, WK: sampling period effect, G*WK: group-by-sampling period effect. The AST concentration was higher in the clinical endometritis and subclinical endometritis groups than in the control group at 1 week postpartum ($p < 0.05$).

control group at 1 week postpartum ($p < 0.05$, Fig 7), whereas the AST concentration was higher in the clinical endometritis and subclinical endometritis groups than in the control group ($p < 0.05$, Fig 8) at the same time point. Multiparous cows had a higher serum BHBA concentration than primiparous cows ($p < 0.01$), whereas parity did not affect the AST concentration. Although serum glucose, γ GT, and phosphorus concentrations differed across sample times ($p < 0.001$), none of these measurements differed among the control, clinical endometritis, and subclinical endometritis groups ($p > 0.05$).

Discussion

This study compared blood metabolites among cows with clinical or subclinical endometritis and those without endometritis during peri- and postpartum periods to prepare preventive and treatment regimens for coping with the diseases. Lower serum concentrations of calcium, magnesium, total cholesterol, albumin, and urea nitrogen, but higher concentrations of NEFAs, BHBA, and AST during the postpartum

period were associated with the incidence of clinical endometritis, whereas none of these measurements except for the AST concentration were associated with the incidence of subclinical endometritis. These results indicate that cows with clinical endometritis experience severe metabolic imbalance during the postpartum period and that the importance of balanced nutrition during the transition period should be emphasized to cope with the disease.

Calcium and magnesium concentrations were lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the study period, similar to the study of Martinez *et al.* (22) in which cows with subclinical hypocalcemia had a greater risk of clinical and subclinical endometritis than normocalcemic cows. The serum concentration of calcium was previously reported to be lower in cows with clinical endometritis or subclinical endometritis than in those without uterine diseases (24), which supports our results. A previous study in which cows with clinical endometritis were not separated from cows with subclinical endometritis reported that the calcium concentration was not associated with endometritis, but that the magnesium concentration was lower in cows with endometritis than in the healthy cows at 2 weeks postpartum (4). Another study reported that there was a decrease in the calcium concentration, but not the magnesium concentration, in cows with clinical endometritis in comparison to cows without endometritis (3). This discrepancy regarding the concentrations of calcium and/or magnesium among studies is unclear and should be investigated further. The decrease in the calcium concentration may be associated with immune function impairment (3). Likewise, hypocalcemic cows not only have a decreased level of circulatory neutrophils, but also a decreased neutrophil oxidative burst capacity and a decreased risk of uterine infection (22). Magnesium is an important mineral involved in calcium homeostasis, and a decrease in the magnesium concentration can induce hypocalcemia (10). Moreover, Bertoni *et al.* (2) reported that the magnesium level is reduced in cows with impaired liver function. The NEFA concentration increases when the energy supply is insufficient to cope with energy demand during the peripartum period. In the present study, the NEFA concentration was higher in the clinical endometritis group than in the control group throughout the study period, whereas it tended to be higher in the clinical endometritis group than in the subclinical endometritis group. Our observations infer that the degree of uterine inflammation is associated with the energy deficit status during the postpartum period. Similarly, a previous study, in which cows with clinical endometritis were not separated from cows with subclinical endometritis, showed that cows with endometritis had a higher NEFA concentration than healthy cows during the periparturient period (13). On the other hand, Ribeiro *et al.* (24) reported that the NEFA concentration was higher in cows with subclinical endometritis, but not in cows with clinical endometritis, than in cows without the diseases, which is discordant with our results. An elevated NEFA concentration is detrimental to neutrophil functions and uterine health (13), due to immunosuppressive effect on uterine health associated with increased lipid mobilization during the 7 days before parturition (7).

The total cholesterol concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups throughout the pre- and postpartum periods in the present study. Discordant with our observations, a previous study demonstrated that the total cholesterol concentration tended to be lower in cows with subclinical endometritis than in healthy animals at 4 weeks postpartum, but not at any other time point postpartum (25). The albumin concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups during the postpartum period in the present study. Accordingly, Burke *et al.* (4) reported that the albumin concentration was lower in cows with endometritis (clinical or subclinical) than in cows without endometritis. Furthermore, Green *et al.* (12) reported that the albumin concentration was lower in pasture-grazed cows with clinical or subclinical endometritis than in cows unaffected by endometritis. A lower serum albumin concentration may indicate increased albumin catabolism due to an energy deficit (1) and impaired liver function (4). The urea nitrogen concentration was lower in the clinical endometritis group than in the control and subclinical endometritis groups at 4 and 6 weeks postpartum in our study. This is inconsistent with a previous report (25) that the urea nitrogen concentration was lower in cows with subclinical endometritis than in non-diseased animals at 2 and 4 weeks postpartum. This discrepancy among studies is unclear. The lower urea nitrogen concentration that we detected in cows with clinical endometritis might be due to reduced dietary protein intake and/or reduced ureagenesis in the liver (21,30).

BHBA is an indicator of complete fat oxidization in the liver and is thus used to assess the adaptive response to energy imbalance postpartum (15). The BHBA concentration was higher in the clinical endometritis group than in the control group at only 1 week postpartum. A previous study reported that cows with endometritis (clinical or subclinical) had a higher BHBA concentration than healthy animals during the postpartum period (13), whereas Bicalho *et al.* (3) found no difference in the BHBA or NEFA concentration between cows with and without clinical endometritis. AST and γ GT have been used to assess liver function associated with hepatic lipidosis during the postpartum period (11,29). In the present study, the AST concentration was higher in the clinical and subclinical endometritis groups than in the control group at 1 week postpartum, whereas the γ GT concentration was not associated with the incidence of clinical or subclinical endometritis at any time point. Our results are similar to those of a previous study (4) in which the AST concentration tended to be higher in cows with endometritis (clinical or subclinical) than in cows without endometritis. An elevated AST concentration during the postpartum period in cows with endometritis indicates liver dysfunction and, potentially, hepatic tissue damage (2). The concentrations of glucose and phosphorus were not associated with the incidence of clinical or subclinical endometritis in the present study, which suggests that homeostasis of these metabolites was maintained (5,14). Accordingly, Burke *et al.* (4) reported that the glucose concentration was not associated with incidence of clinical or subclinical endometritis, whereas another study reported that the glucose concentration was lower in

cows with subclinical endometritis than in uninfected animals at 4 and 6 weeks postpartum (25).

Taken together, lower serum concentrations of calcium, magnesium, total cholesterol, albumin, and urea nitrogen, but higher concentrations of NEFAs, BHBA, and AST during the postpartum period were associated with the incidence of clinical endometritis in dairy cows, although none of these measurements except for the AST concentration were associated with the incidence of subclinical endometritis. These results indicate that cows with clinical endometritis experience more severe negative energy balance, impaired liver function, reduced immune responses, and imbalances in several blood metabolites than cows with subclinical endometritis and cows without clinical or subclinical endometritis. Therefore, the importance of balanced nutrition during the transition period should be emphasized to cope with uterine disease.

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젖소의 자궁내막염 발생과 분만 전·후 대사 상태와의 상관관계

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요약 : 본 연구는 임상형 혹은 준임상형 자궁내막염에 이환된 젖소와 자궁내막염이 발생되지 않은 젖소 사이에 분만 전·후 기간 동안 혈액 대사물질의 농도를 비교하였다. 분만 전 4주, 분만 후, 분만 1, 2, 4, 6주 후 혈액을 채취하였으며, 혈청 분리 후 calcium, magnesium, non-esterified fatty acids (NEFAs), total cholesterol, albumin, urea nitrogen, β -hydroxybutyrate (BHBA), aspartate aminotransferase (AST), γ -glutamyltransferase, glucose 및 phosphorus 농도를 측정하였다. 분만 후 4주에, 임상형 혹은 준임상형 자궁내막염 발생 유무에 따라 대조군(n=104), 임상형 자궁내막염군(n=66) 및 준임상형 자궁내막염군(n=37)으로 구분하였다. Calcium과 magnesium 농도는 실험 전 기간에 걸쳐 임상형 자궁내막염군이 대조군과 준임상형 자궁내막염군에 비해 낮았으나($p < 0.05$ to 0.0001), NEFAs 농도는 실험 전 기간에 걸쳐 임상형 자궁내막염군이 대조군에 비해 높았다($p < 0.01$). Total cholesterol 농도는 분만 전 및 분만 후 기간 중 임상형 자궁내막염군이 대조군과 준임상형 자궁내막염군에 비해 낮았다($p < 0.05$ to 0.001). Albumin 농도는 분만 후 기간 동안 임상형 자궁내막염군이 대조군과 준임상형 자궁내막염군에 비해 낮았다($p < 0.05$ to 0.001). Urea nitrogen 농도는 분만 후 4주 및 6주에 임상형 자궁내막염군이 대조군과 준임상형 자궁내막염군에 비해 낮았다($p < 0.01$). 분만 후 1주에, BHBA 농도는 임상형 자궁내막염군이 대조군에 비해 높았으나($p < 0.01$), AST 농도는 임상형 자궁내막염군과 준임상형 자궁내막염군이 대조군에 비해 높았다($p < 0.05$). 결론적으로, 분만 후 혈청 calcium, magnesium, total cholesterol, albumin 및 urea nitrogen 농도의 감소와 NEFAs, BHBA 및 AST 농도의 증가가 임상형 자궁내막염의 발생과 관련되었으며, 이것은 전환기 중의 균형 잡힌 영양의 중요성을 제시한다.

주요어 : 젖소, 임상형 자궁내막염, 준임상형 자궁내막염, 혈액 대사물질