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Relationships between Biological Factors during the Voluntary Waiting Period and Reproductive Performance in Dairy Cows

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Abstract : We aimed to identify the biological factors during the voluntary waiting period (VWP) that are associated with subsequent reproductive performance in dairy cows. Two hundred and one pregnant dairy cows had their body condition score (BCS) evaluated and blood samples taken 4 weeks before calving, at calving, and 1, 2, 4, 6, and 8 weeks after calving, to measure serum metabolite and progesterone concentrations. In addition, any peri- and postpartum disorders within 4 weeks of calving, and the mean milk yields 4 and 8 weeks postpartum, were recorded. The cows were allocated to two groups according to whether they had become pregnant or not by 120 days after calving: a pregnant group (n = 50) and a non-pregnant group (n = 151). Analyses of serum metabolite concentrations showed that the aspartate aminotransferase and γ-glutamyltransferase activities 2 weeks ($p < 0.05$) and the non-esterified fatty acid concentration 6 weeks ($p < 0.01$) postpartum were higher in the non-pregnant than in the pregnant group. However, the albumin concentrations between 2 and 8 weeks and the total cholesterol and magnesium concentrations between 4 and 8 weeks postpartum were lower in the non-pregnant group ($p < 0.05$). The BCS ($p < 0.05$) and percentages of cows that had resumed cyclicity ($p < 0.001$) by 6 and 8 weeks postpartum were higher in the pregnant than in the non-pregnant group, whereas the incidence of peri- and postpartum disorders was higher in the non-pregnant group ($p < 0.05$). In conclusion, high serum enzyme activities, which imply liver damage, imbalances in energy metabolites and magnesium, and a higher incidence of peri- and postpartum disorders, are associated with poorer reproductive performance, whereas high BCS and early resumption of cyclicity during the VWP are associated with superior reproductive performance in dairy cows.

***Key words* :** dairy cow, voluntary waiting period, reproductive performance.

Introduction

Severe metabolic, endocrine, and immunologic changes during the transition period can subject dairy cows to excessive stress, make them more susceptible to infection, and cause reductions in production and reproductive performance (16,29). Furthermore, as genetic modifications have improved milk production, reproductive performance has deteriorated, which has resulted in severe economic losses in high-yielding dairy herds (3). This is a common problem under both intensive and extensive production systems (18). Therefore, there is a great deal of interest in the identification of biological factors during the transition period that influence subsequent fertility in dairy cows (24).

Blood metabolites and body condition score (BCS) have been used to evaluate the relationships between nutritional, health, and endocrine statuses, as well as reproductive performance in dairy cows (25). Previous studies have shown that total cholesterol (TCH), non-esterified fatty acids (NEFAs), β-hydroxybutyrate (BHBA), and/or aspartate aminotransferase (AST) are associated with subsequent reproductive performance in lactating dairy cows (1,17). In addition, a severe loss of BCS during the dry period or early lactation is also associated with poorer reproductive performance (4). Postpartum disorders, such as metabolic disorders (ketosis, abomasal displacement, and hypocalcemia), retained placenta, septicemic metritis, and endometritis have been shown to have significant influences on reproductive performance (11,23), whereas the relationship between the level of milk production and reproductive performance in dairy cows remains controversial (12). Furthermore, the time taken to resume postpartum cyclicity is associated with subsequent fertility in dairy cows, and is affected by energy balance and the incidence of postpartum disorders during the early lactation period, and parity (30). Dairy cow fertility is influenced by a number of biological factors during the transition period and any additional voluntary waiting period (VWP), and these biological factors and subsequent fertility demonstrate complex causal relationships.

Previous studies have shown associations between a small number of biological factors and subsequent reproductive performance (15). Moreover, the VWP is an important period, during which the reproductive organs of the animal return to their pre-calving status to prepare them for the next pregnancy and lactation. Thus, determination of the relationships between the diverse biological factors during the VWP and subsequent reproductive performance might assist with the maintenance of fertility in dairy herds. Therefore, in the present study, we aimed to determine the effects of various biological factors, including those that reflect metabolism, health,

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and production, during the VWP, on the reproductive performance of dairy cows.

Materials and Methods

Animals

This study was conducted on four dairy farms in Chungcheong Province, Korea. Each herd contained 40-110 cows. The cows were maintained in loose housing systems and fed total mixed rations. All the cows that had calved were milked twice daily, and milk yield was measured and recorded monthly for each cow during their lactation periods. All the experiments were performed according to the ethical guidelines of the Institutional Animal Care and Use Committee of Chungbuk National University, Korea. A total of 201 lactations (59 primiparous and 142 multiparous) in 176 cows were studied. All the cows underwent biweekly reproductive health checks by veterinarians on the research team; these included an examination of their ovarian structures (follicle and corpus luteum [CL]) and uterus by ultrasonography.

Evaluation of BCS and blood sampling

BCS was evaluated on a 5-point scale (with quarter-point divisions) using a visual technique (6) 4 weeks before calving, just after calving (1.0 ± 0.1 h), and 1, 2, 4, 6, and 8 weeks after calving. Blood samples were also collected from the tail vein at the same time points to determine the serum concentrations of TCH, glucose, total protein, albumin, urea nitrogen, calcium, magnesium, NEFA, BHBA, and progesterone, and the serum AST and γ-glutamyltransferase (γGT) activities. Ten milliliters of blood were collected into plain plastic centrifuge tubes and immediately placed in an ice bath. The samples were then centrifuged at 2,000 × g for 10 min at 4°C, and the serum was harvested and frozen at −80°C until assayed.

Case definitions

The definitions of postpartum health disorders that were used in the present study were similar to those described previously (14,28). Dystocia was defined as calving requiring some or significant force, or cesarean section. Retained placenta was defined as the retention of the fetal membranes for longer than 24 h. Milk fever was diagnosed when weakness and recumbency occurred after calving. Subclinical ketosis was diagnosed when the BHBA concentration was ≥ 1,200 μmol/L 1 and/or 2 weeks postpartum. Abomasal displacement was diagnosed by the detection of a 'ping' sound during abdominal auscultation. Digestive disorders included diarrhea and bloat. Septicemic metritis was defined by the presence of fever (≥ 39.5°C) and a watery, fetid uterine discharge during the first 10 days postpartum. Mastitis was recorded if abnormal milk or signs of inflammation in one or more quarters of the udder were present. Clinical endometritis was diagnosed if there was a metricheck score ≥ 3 or using ultrasonography 4 weeks postpartum.

Reproductive management

The VWP between calving and the first artificial insemination (AI) was 50 days. In addition to estrus detection, reproductive programs were employed, which involved the administration of 500 μg of a $PGF_{2\alpha}$ analog, cloprostenol sodium (Estrumate; MSD Animal Health, Seoul, Korea), and an injection of estradiol benzoate (EB) (SY Esrone; Samyang, Seoul, Korea) 36 h later, which was followed by timed AI (TAI) 36 h later (PG-EB protocol). Alternatively, Ovsynch was performed, comprising an injection of 10 μg GnRH analog, buserelin acetate (Gestar; Over, San Vicente, Argentina), $PGF_{2\alpha}$ 7 days later, and another injection of GnRH analog 56 h later, followed by TAI after a further 16 h (20).

Pregnancy diagnosis was performed 32 to 40 days after AI using ultrasonography. Reproductive performance data were collected for a minimum of 120 days postpartum.

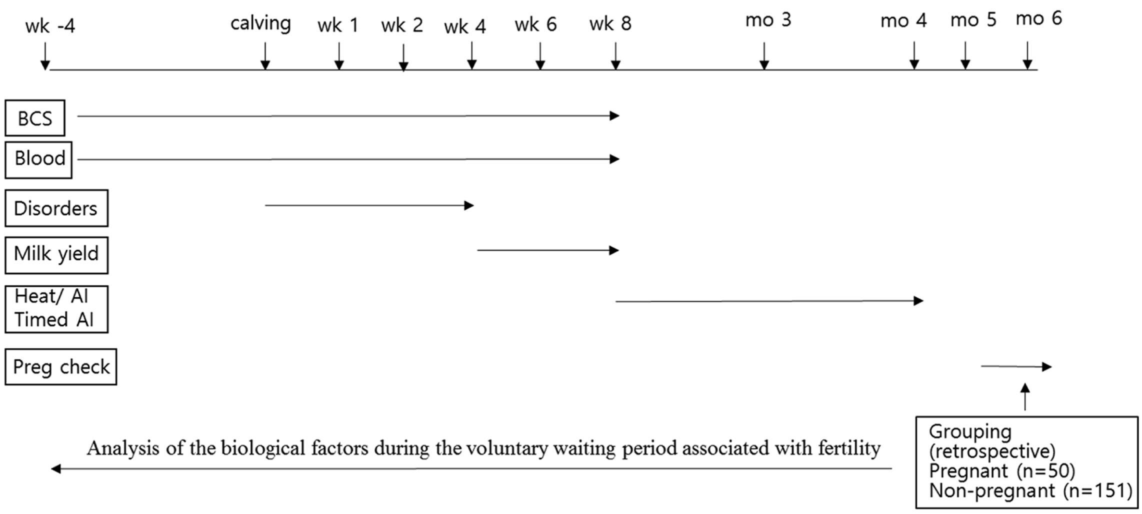

Fig 1. Overall study design. wk = week; mo = month; BCS = body condition score; Blood = blood collection for the measurement of serum metabolite and progesterone concentrations (resumption of postpartum cyclicity was indicated by a progesterone concentration of ≥ 1 ng/mL); Disorders = peri- and postpartum disorders (including dystocia, retained placenta, milk fever, subclinical ketosis, abomasal displacement, digestive disorders, septicemic metritis, mastitis, and clinical endometritis); Heat/AI and Timed AI = cows that exhibited estrus naturally were inseminated according to the am-pm rule, whereas cows that were administered the PG-EB protocol or Ovsynch were subjected to timed artificial insemination; Preg check = pregnancy diagnosis.

Study design

Two hundred and one pregnant dairy cows had their BCS evaluated and blood samples taken 4 weeks before calving, at calving, and 1, 2, 4, 6, and 8 weeks after calving to measure blood metabolite and progesterone concentrations (progesterone ≥ 1 ng/mL implies the resumption of cyclicity). In addition, the occurrence of peri- and postpartum disorders, including dystocia, retained placenta, milk fever, subclinical ketosis, abomasal displacement, digestive disorders, septicemic metritis, mastitis, and clinical endometritis, were recorded for the first 4 weeks after calving. Milk yield records were collected 4 and 8 weeks after calving. The cows were allocated to two groups, according to whether they had become pregnant or not by 120 days after calving: a pregnant group (n = 50) and a non-pregnant group (n = 151). Then, we evaluated which biological factors (serum parameters, BCS, peri- and postpartum disorders, resumption of cyclicity, and milk yield) during the VWP were associated with reproductive performance (conception by 120 days after calving) in the cows. Fig 1 shows the overall study design.

Measurement of serum metabolite and progesterone concentrations and enzyme activities

The serum concentrations of TCH, glucose, total protein, albumin, urea nitrogen, calcium, magnesium, NEFA, and BHBA, and the activities of AST and γGT, were measured using a 7180 Biochemistry Automatic Analyzer 710 (Hitachi, Ltd., Tokyo, Japan) and 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.

The serum concentration of progesterone was determined 2, 4, 6, and 8 weeks postpartum using the Immulite 1000 Immunoassay System (DPC Cirrus Inc., Flanders, NJ, USA),

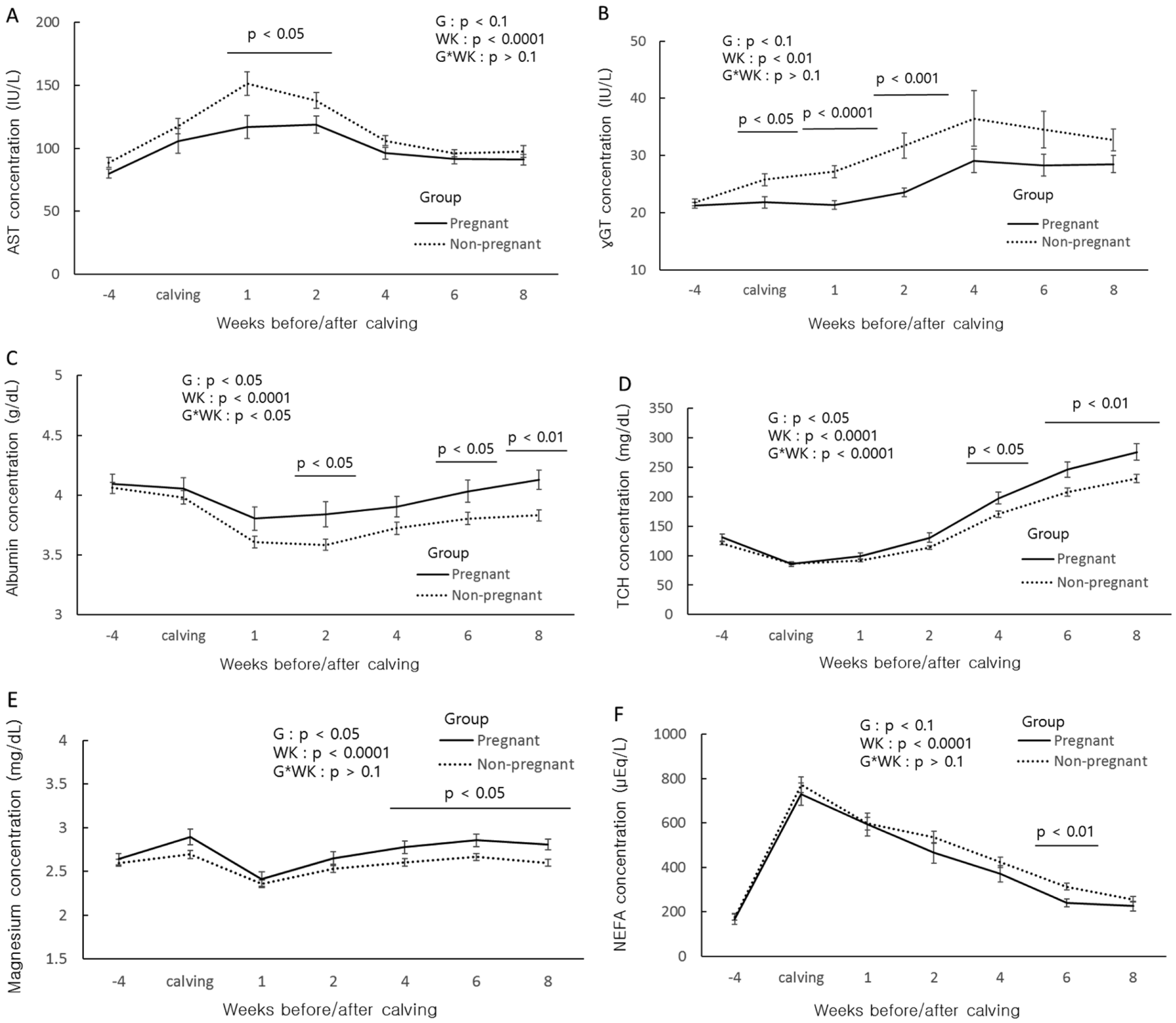

Fig 2. Serum concentrations/activities of key substances at time points around calving. Serum aspartate aminotransferase (AST; A) and γ-glutamyltransferase (γGT; B) activities; and serum albumin (C), total cholesterol (TCH; D), magnesium (E), and non-esterified fatty acids (NEFAs; F) concentrations 4 weeks before calving, at calving, and 1, 2, 4, 6, and 8 weeks after calving in the pregnant (n = 50) and non-pregnant (n = 151) groups. G indicates a group effect, WK indicates a sampling period effect, and G*WK indicates a group-by-sampling period effect.

according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were < 5%. Postpartum resumption of cyclicity was confirmed by the measurement of a progesterone concentration of ≥ 1 ng/mL.

Statistical analyses

Results are expressed as means ± SEMs. For the statistical analyses, the cows were categorized as primiparous or multiparous. Statistical analyses were performed using the SAS program (version 9.4; SAS Inst., Cary, NC, USA).

The serum metabolite concentrations (TCH, glucose, total protein, albumin, urea nitrogen, calcium, magnesium, NEFA, and BHBA), enzyme activities (AST and γGT), and BCS were analyzed using mixed models. The statistical models included group effects (pregnant or non-pregnant), parity (primiparous or multiparous), sampling time (number of weeks pre- and postpartum), and two-way interactions between group, parity, and sampling time. Cows were included in the model as a random effect. Student's *t*-tests were used to identify significant main effects.

The incidences of peri- and postpartum disorders (including dystocia, retained placenta, milk fever, subclinical ketosis, abomasal displacement, digestive disorders, septicemic metritis, mastitis, and clinical endometritis), and the percentage of cows that resumed cyclicity, were compared between the pregnant and non-pregnant groups using chi-square tests.

Comparisons of the mean milk yields 4 and 8 weeks after calving between the groups were performed using a general linear model that included farm, parity, and group (pregnant or non-pregnant) as variables. $p \leq 0.05$ was considered to represent statistical significance, and $0.05 < p < 0.1$ was considered to indicate a trend.

Results

Relationships between serum metabolites and reproductive performance

Fig 2(A-F) shows the comparisons of serum metabolite concentrations/activities between the pregnant and non-pregnant groups. The serum AST activities 1 and 2 weeks postpartum ($p < 0.05$), and the γGT activities after calving ($p < 0.05$) and 1 and 2 weeks postpartum ($p < 0.001$-0.0001), were higher in the non-pregnant than in the pregnant group. The serum albumin concentrations were lower ($p < 0.01$-0.05) 2, 6, and 8 weeks postpartum in the non-pregnant than in the pregnant group. The serum concentrations of TCH and magnesium were higher ($p < 0.01$-0.05) 4, 6, and 8 weeks postpartum in the pregnant than in the non-pregnant group, whereas the serum NEFA concentrations were lower ($p < 0.01$) 6 weeks postpartum in the pregnant than in the non-pregnant group. However, the serum concentrations of glucose, total protein, urea nitrogen, calcium, and BHBA did not differ between the two groups ($p > 0.1$).

Relationships of BCS and peri- and postpartum disorders with reproductive performance

Fig 3 shows comparisons of BCS 4 weeks before calving, after calving, and 1, 2, 4, 6, and 8 weeks after calving between the pregnant and non-pregnant groups. The BCSs were higher 6 ($p < 0.05$) and 8 ($p < 0.001$) weeks postpartum in the pregnant (2.9 ± 0.1 and 2.9 ± 0.1, respectively) than in the non-pregnant (2.8 ± 0.1 and 2.7 ± 0.1) group.

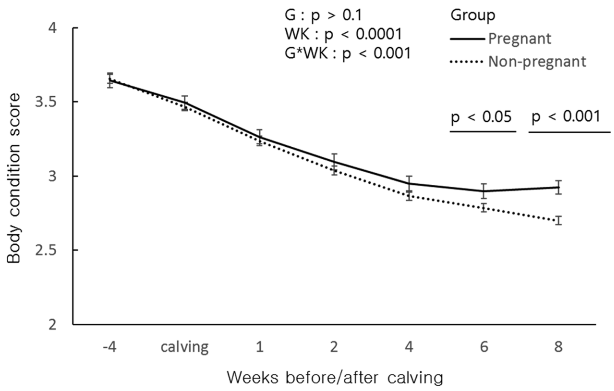

Fig 3. Body condition score at time points around calving. Comparison of the body condition score 4 weeks before calving, at calving, and 1, 2, 4, 6, and 8 weeks after calving in the pregnant (n = 50) and non-pregnant (n = 151) groups. G indicates a group effect, WK indicates a sampling period effect, and G*WK indicates a group-by-sampling period effect.

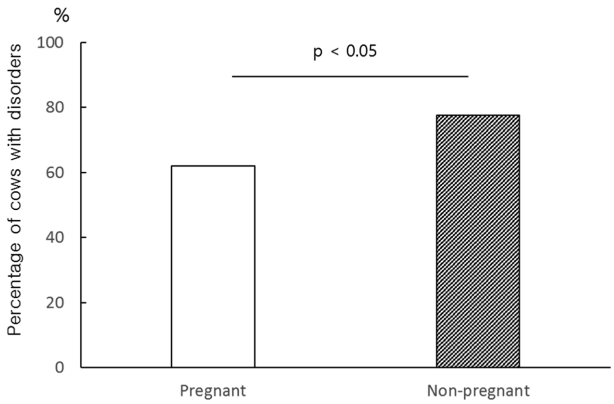

Fig 4. Comparison of the incidences of peri- and postpartum disorders between the groups. The disorders included dystocia, retained placenta, milk fever, subclinical ketosis, abomasal displacement, digestive disorders, septicemic metritis, mastitis, and clinical endometritis. The incidence was compared between the pregnant (n = 50) and non-pregnant (n = 151) groups.

Fig 4 shows a comparison of the incidence of peri- and postpartum disorders between the pregnant and non-pregnant groups. The incidence of the peri- and postpartum disorders (dystocia, retained placenta, milk fever, subclinical ketosis, abomasal displacement, digestive disorders, septicemic metritis, mastitis, and clinical endometritis) was higher ($p < 0.05$) in the non-pregnant than in the pregnant group.

Relationships of the resumption of cyclicity and milk yield with reproductive performance

Fig 5 shows comparisons of the percentage of cows that had resumed cyclicity 2, 4, 6, and 8 weeks after calving between the pregnant and non-pregnant groups. The percentage of cows that had resumed cyclicity was higher 6 ($p < 0.001$) and 8 ($p < 0.0001$) weeks after calving in the pregnant than in the non-pregnant group. However, the least

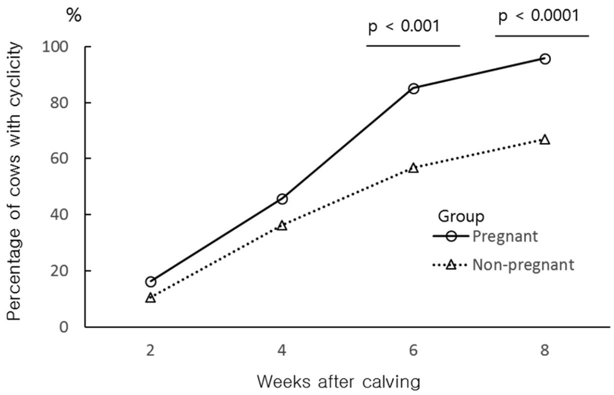

Fig 5. Resumption of cyclicity. Comparison of the percentage of cows that had resumed cyclicity 2, 4, 6, and 8 weeks after calving in the pregnant (n = 50) and non-pregnant (n = 151) groups.

square mean milk yields 4 and 8 weeks after calving did not differ ($p > 0.1$) between the pregnant (38.5 ± 1.0 kg) and non-pregnant (36.7 ± 0.7 kg) groups.

Discussion

In the present study, we have determined the relationships of diverse biological factors, including serum metabolites, enzymes, and minerals; peri- and postpartum disorders; the resumption of cyclicity; and milk yield during the VWP with reproductive performance (conception by 120 days after calving) in dairy cows. High serum activities of AST and γGT, which reflect liver damage, imbalances in energy metabolites (albumin, TCH, and NEFA), low magnesium concentration, and a high incidence of peri- or postpartum disorders, were associated with poorer reproductive performance, whereas a high BCS and early resumption of cyclicity during the VWP were associated with superior reproductive performance in dairy cows.

In the present study, we compared the serum metabolite profiles of cows that did or did not become pregnant by 120 days postpartum. The AST and γGT activities in the non-pregnant group were higher than those in the pregnant group between calving and 2 weeks postpartum, which is consistent with the findings of a previous study (9). These high enzyme activities imply the presence of liver damage related to fatty liver, which arises because of severe negative energy balance (NEB) during early lactation (19,27). The finding of lower serum albumin concentrations 2, 6, and 8 weeks postpartum in the non-pregnant group than in the pregnant group might be associated with greater albumin catabolism, because of an energy deficit (2). Moreover, we found that the non-pregnant group had lower TCH concentrations between 4 and 8 weeks and a higher NEFA concentration 6 weeks postpartum than the pregnant group, which is consistent with the findings of several previous studies (17,21), and is presumably because of lower dry matter intake (DMI) and persistent NEB during the VWP. The findings of a previous study suggested that circulating cholesterol might be used for steroid synthesis in the ovary and luteal function, which would influence subsequent fertility (21). The finding of lower serum magnesium concentrations 4, 6, and 8 weeks postpartum in the non-pregnant group than in the pregnant group is consistent with the findings of a previous study (17), which also showed no associations between calcium concentration and reproductive outcomes, suggesting that poor reproductive performance is not associated with an impairment in calcium mobilization. The low serum magnesium concentration during the postpartum period in the non-pregnant group might indicate low DMI because magnesium is not stored in the body, and therefore the maintenance of its circulating concentration depends on regular dietary intake (8). When the serum metabolite and mineral considerations are considered together, it can be concluded that a low DMI and the consequent severe NEB in the non-pregnant group resulted in an impairment in liver function, imbalances in energy metabolites, and low magnesium concentration, which ultimately leads to poor reproductive performance.

As well as the circulating concentrations of energy metabolites, the energy status of dairy cows is also indicated by the BCS; therefore, this has been widely used to evaluate their nutritional status, especially around the transition period. In the present study, the BCS 6 and 8 weeks postpartum was higher in the pregnant group than in the non-pregnant group, which might be associated with more severe NEB during the VWP. However, the difference in BCS between the pregnant and non-pregnant groups developed later than those in serum metabolites, which is a similar finding to that reported previously (10).

Our findings that the incidence of peri- and postpartum disorders was higher in the non-pregnant than in the pregnant group is consistent with the findings of previous studies, which suggested that the susceptibility to periparturient diseases is affected by severe NEB and impaired innate immunity, and together these lead to lower reproductive performance in dairy cows (13,29). Other previous studies have also shown that the presence of a periparturient disease is associated with a lower pregnancy rate per AI (15,22). The lower reproductive performance that accompanies periparturient diseases might be because of impaired ovarian activity and delayed resumption of ovulation, and other carryover effects on gamete and embryo development (23).

In the present study, the percentage of cows that had resumed cycling by 6 or 8 weeks after calving was higher in the pregnant than in the non-pregnant group. These findings are consistent with those of a previous study (7) in which milk progesterone analyses were used to show that the first ovulation occurring 5 or 7 weeks postpartum were associated with higher pregnancy rates. Similarly, another study showed that cows that demonstrated cyclicity by 65 days postpartum had a higher conception rate than anestrus cows (26). Interestingly, another study showed that non-ovulatory cows had more severe NEB and higher insulin resistance during their early lactation periods (5), which suggests that energy status during early lactation might affect the resumption of cyclicity in dairy cows.

The present finding that the mean milk yields 4 and 8 weeks after calving did not differ between the pregnant and non-pregnant groups is also consistent with the findings of previous studies (9,26). Thus, it is likely that the provision of a favorable environment for high-yielding cows would enable them to maintain good reproductive performance (12).

Conclusion

The higher serum enzyme activities (AST and γGT), implying liver damage, within the first 2 weeks postpartum, the imbalance in energy metabolites (albumin, TCH, and NEFA), and the lower magnesium concentration during the VWP were associated with lower reproductive performance (lack of conception by 120 days postpartum) in dairy cows, as was the higher incidence of peri- or postpartum disorders. However, a high BCS and a resumption of cyclicity by 6 and 8 weeks postpartum were associated with superior reproductive performance. Finally, milk yield was not associated with reproductive performance. Thus, optimal nutritional and health management, to prevent severe NEB and impairments in liver function and innate immunity during the VWP, are important for the maintenance of good reproductive performance in dairy cows.

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