

Risk Factors for Displacement of the Abomasum in Dairy Cows and its Relationship with Postpartum Disorders, Milk Yield, and Reproductive Performance

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Abstract : We determined the risk factors for displacement of the abomasum (DA), and the relationships between DA and postpartum disorders, milk yield, and reproductive performance in dairy cows. Initially, we identified the risk factors for DA using data regarding cow health and calving season from 2,208 lactations. Then, we compared the incidence of postpartum disorders, culling, death, and reproductive performance between cows with DA and their control herd-mates (each n = 57). In addition, serum metabolites concentrations and milk yield were compared between cows with DA and controls (each n = 33). Ketosis (odds ratio [OR] = 9.27, $p < 0.0001$) and twin calves ($p = 0.06$) increased the risk of DA. Cows with a parity of three had a higher risk (OR = 5.23, $p < 0.01$) of DA than primiparous cows. Serum total cholesterol concentration was lower but non-esterified fatty acid, β -hydroxybutyrate, and alanine aminotransferase concentrations were higher after calving in cows with DA than in controls ($p < 0.05$). The removal rate from the herd by 2 months after calving was higher ($p < 0.05$) but milk yield 1 and 2 months after calving ($p < 0.01$) and the rate of first insemination by 150 days postpartum were lower (hazard ratio = 0.49, $p < 0.05$) in cows with DA than controls. In conclusion, higher parity, twin calves, and ketosis are risk factors for DA in dairy cows, which is associated with a higher removal rate from the herd, lower milk yield, a longer calving to first insemination interval, and unfavorable levels of metabolites related to energy and liver function.

Key words : dairy cow, abomasal displacement, risk, removal, milk yield, reproductive outcome.

Introduction

Displacement of the abomasum (DA) in a dairy cow is a condition in which the abomasum, which normally lies ventrally in the abdomen, is displaced dorsally. DA usually occurs soon after calving, with a high proportion of cases occurring in the first 14 days postpartum (26). The uterus displaces the abomasum during a normal pregnancy; therefore, the abomasum should move back to its original position after calving. However, if abomasal atony develops at this time, the loss of contractions causes gas to accumulate inside the abomasum, which leads to DA. The majority of cases are treated surgically by a right or left flank laparotomy. However, even after surgical correction of the DA, the outcomes can be disappointing. Thus, once this disorder has occurred, the cost of treatment, reduction in milk production, and greater probability of removal from the herd (by sale or death) all contribute to a substantial economic loss (27,33). The incidence of DA has been reported to be between 0.05% and 7%, and there is great variation (0 to 25%) among individual herds (6,26). The total estimated economic loss resulting from a case of DA is between US\$ 250 and 450 (2). Therefore, it is important to establish strategies to prevent DA to maintain productivity and profit in dairy cow herds,

especially if they are high-yielding.

Numerous studies have attempted to identify the risk factors for DA in dairy cows. Diverse factors, such as the gender and age of the animals, and the presence of peripartum disorders (hypocalcemia, dystocia, twin calves, retained placenta, ketosis, or metritis) have been reported to be risk factors for DA (7,11,22,29). In addition, herd management practices, including nutrition, health, and the environment during transition, may be associated with the incidence of DA (6,30). Severe negative energy balance (NEB) during the transition period, reflected in higher concentrations of specific blood metabolites (non-esterified fatty acids [NEFA] and β -hydroxybutyrate [BHBA]), could also significantly predispose towards DA (14). In addition, hypocalcemia has been shown to be associated with a higher incidence of DA in dairy cows (23,29). Thus, high-yielding dairy cows, which demonstrate severe NEB during early lactation, are particularly vulnerable to DA (15). Therefore, it is important to identify the risk factors for DA in modern high-yielding dairy herds. During recent decades, milk yield has increased dramatically through breeding (genetic gains) and improvement in the nutritional management of Korean dairy herds. Therefore, the first objective of this study was to identify the risk factors for DA in high-yielding dairy herds.

Previous studies have shown that DA is associated with a decrease in milk yield (12,18) and an increase in the incidence of concurrent disorders and/or culling in dairy cows (16,27). However, the relationship between DA and subse-

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quent reproductive performance has not been fully investigated (5). Therefore, the second objective of this study was to characterize the relationships between DA and subsequent reproductive outcomes, postpartum disorders, removal from the herd (by culling or death), and milk production in Korean dairy herds. In addition, the relationship between DA and postpartum serum metabolite concentrations was also evaluated.

Materials and Methods

Animals

This study was conducted on six dairy farms in Chungcheong Province, Korea, with each herd containing approximately 100 cows. The cows were maintained in loose housing systems, fed total mixed rations, and milked twice daily. The mixed rations were composed of brewers' grain, alfalfa hay, cotton seed, beet pulp, corn silage, tall fescue, timothy hay, and mineral and vitamin additives. The mean milk yields for the farms were ~9,500 to 11,000 kg per cow per year.

A total of 2,208 lactations (641 primiparous and 1,567 multiparous, mean [SD] parity 2.6 ± 1.5) were included in the study. Milk yield was measured monthly for each cow during the first 2 months following calving. All cows underwent bi-weekly reproductive health checks by veterinarians on the research team, including an examination of their ovarian structures (follicle and corpus luteum) and uterus by transrectal palpation and ultrasonography.

Health and reproductive management

The definitions of peri- and postpartum health disorders that were used in the present study were similar to those described previously (20,21,31). Calving difficulty was ranked according to the degree of assistance required (1 = no assistance, 2 = minor assistance, 3 = some force required, 4 = significant force required, and 5 = cesarean section). Cows with a calving score > 2 were considered to have dystocia. Retained placenta was defined as the retention of the fetal membranes for longer than 24 h. Puerperal metritis was defined by the presence of fever ($\geq 39.5^\circ\text{C}$) and a watery, fetid uterine discharge during the first 10 days after calving. Ketosis was diagnosed in the presence of the following clinical signs: anorexia, depression, and the odor of acetone on the breath. Abomasal displacement was diagnosed by a "ping" sound during abdominal auscultation by veterinarians in the research team and one case was diagnosed as right DA, whereas the rest of 56 cases were diagnosed as left DA. All cases were corrected by surgery and it was performed through right or left flank laparotomy with ventral abomasopexy under local infiltration anesthesia (26). Clinical endometritis was diagnosed on the basis of the presence of a visible mucopurulent vaginal discharge and/or rectal palpation and ultrasonography 4 weeks after calving. Mastitis was recorded if abnormal milk or signs of inflammation in one or more quarters of the udder were present. Lameness was diagnosed if an abnormal gait or lack of weight-bearing on a limb was observed, and included diagnoses of interdigital and digital dermatitis. "Removal from the herd" was taken to include cows that died or were culled in the first 2 months after calving. The incidence of postpartum disorders was recorded up

to 4 weeks after calving, except in the case of particular disorders, for which the timing of diagnosis is given above. With the exception of dystocia and mastitis, other postpartum disorders were diagnosed by veterinarians in the research team.

The voluntary waiting period from calving to the first artificial insemination (AI) was 40 days. Pregnancy diagnosis was performed 32 to 40 days after AI by transrectal palpation and ultrasonography. Reproductive performance data were collected for a minimum of 210 days after calving, or until pregnancy or culling.

Blood sampling and measurement of serum metabolites

Blood samples were collected from the tail vein of cows 1, 2, and 4 weeks after calving to assay serum metabolite (NEFA, BHBA, total cholesterol [TCH], alanine aminotransferase [ALT], aspartate aminotransferase [AST], calcium, magnesium, glucose, and albumin) concentrations. Ten milliliters of blood were placed into plastic centrifuge tubes, which were immediately placed on ice. The samples were then centrifuged at $2,000 \times g$ for 10 min at 4°C , and the serum was harvested and frozen at -80°C until assayed.

The concentrations of NEFA, BHBA, TCH, ALT, AST, calcium, magnesium, glucose, and albumin in serum samples 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 manufacturer's instructions. The intra- and inter-assay coefficients of variation were $< 5\%$ for each assay.

Study design and statistical analyses

Initially, we identified the risk factors for DA in dairy cows. Calving season, cow parity, dystocia, twins, retained placenta, and ketosis were evaluated. Secondly, we compared the relationships between DA and postpartum disorders (puerperal metritis, clinical endometritis, mastitis, and lameness), removal from the herd (by culling or death), and reproductive performance (hazards of first insemination by 150 days and pregnancy by 210 days after calving) between cows with DA and their control herd-mates (each $n = 57$). In addition, the relationships between DA and serum metabolite (NEFA, BHBA, TCH, ALT, AST, calcium, magnesium, glucose, and albumin) concentrations and milk yield (up to 2 months after calving) were also compared between cows with DA and controls (each $n = 33$).

Data are expressed as the mean \pm SEM. For statistical analysis, calving season was categorized as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February), and cow parity was categorized as 1, 2, 3, or ≥ 4 . Statistical analyses were performed using the SAS program (version 9.4, SAS Inst., Cary, NC, USA).

The risk factors for DA were analyzed by logistic regression using the LOGISTIC procedure. Initially, we evaluated the relationships between DA and single variables (calving season, cow parity, dystocia, twin calves, retained placenta, and ketosis) by performing univariate analysis. Thereafter, the risk factors for DA were analyzed using a multiple logistic regression model. This model included calving season, cow parity, twin calves, ketosis, and the interactions between

these variables. Cow and farm were included in the model as random effects. Backward stepwise regression was used in the multiple regression model, and elimination was performed using the Wald statistic criterion when $p > 0.11$. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by logistic regression. Data are presented as percentages and ORs with their respective 95% CIs.

Concentrations of serum metabolites and milk yield were analyzed using mixed models. The statistical model for the concentrations of serum metabolites included group (cows with DA or control herd-mates), sampling time (1, 2, or 4 weeks after calving), and two-way interactions between group, cow parity, and sampling time. For milk yield, the statistical model included group, time of measurement (1 or 2 months after calving), and two-way interactions between group, cow parity, and time of measurement. Cow and farm were included in the model as random effects. Student's *t*-test was performed when a group effect was observed. The prevalence of postpartum disorders (puerperal metritis, clinical endometritis, mastitis, or lameness) and removal from the herd was compared using chi-square or Fisher's exact tests between cows with DA and control herd-mates.

Cox's proportional hazard model with the PHREG procedure was used to compare the hazard of first insemination by 150 days or pregnancy by 210 days after calving between cows with DA and controls. This estimated the hazard of a cow being inseminated or pregnant at a given time. The time variables used in this model were the intervals in days between calving and first insemination, and between calving and pregnancy. Cows that were sold, died, not inseminated by 150 days, or not pregnant by 210 days after calving were excluded. Cox models included calving season, cow parity, and group (cows with DA or control herd-mates), and cow and farm were included in the model as random effects. The proportional hazard rate was determined on the base of interactions between explanatory variables and time. The median and mean number of days to first insemination and preg-

nancy were determined by survival analysis using the LIFETEST procedure. A p -value ≤ 0.05 was considered to represent statistical significance and $0.05 < p < 0.1$ was considered to indicate a tendency toward a significant difference.

Results

Risk factors for DA

The overall incidence of DA on six dairy farms was 2.58% (57 out of 2,208 births). The logistic regression model revealed that cow parity, twin calves, and ketosis were risk factors for DA (Table 1), while calving season, dystocia, and retained placenta were not associated with DA ($p > 0.05$). Cows with a parity of three had a significantly higher risk (OR = 5.23, $p < 0.01$), and cows with parities of two, or four or greater, tended to have a higher risk ($p = 0.07$) of DA than primiparous cows. Cows giving birth to twins tended to have a higher risk ($p = 0.06$) of DA than cows with single calves.

Table 1. Odds ratios for variables included in the final multiple logistic regression model for the risk of displacement of the abomasum (DA) in dairy cows

Variables	Odds ratio	95% Confidence interval	<i>p</i> -value
Calving season			> 0.05
Cow parity			
1	Reference		
2	2.64	0.939-7.435	0.07
3	5.23	1.929-14.201	< 0.01
≥ 4	2.67	0.948-7.545	0.07
Twins			
No	Reference		
Yes	2.51	0.965-6.501	0.06
Ketosis			
No	Reference		
Yes	9.27	4.853-17.717	< 0.0001

Table 2. Comparison of serum metabolite concentrations between cows with DA and their control herd-mates 1, 2, and 4 weeks after calving

Metabolite ¹	Time after calving (week)					
	1		2		4	
	Control herd-mates	Cows with DA	Control herd-mates	Cows with DA	Control herd-mates	Cows with DA
NEFA (μEq/L)	490.0 ± 72.7	805.4 ± 102.2*	486.3 ± 76.0	597.8 ± 71.2	385.9 ± 69.9	552.0 ± 86.9
BHBA (μmol/L)	1073.8 ± 130.4	2102.3 ± 323.8**	1210.2 ± 192.3	1209.8 ± 181.7	1329.2 ± 240.0	1637.3 ± 379.1
TCH (mg/dL)	119.1 ± 12.4	97.7 ± 5.9	139.6 ± 8.9	100.4 ± 7.0**	194.1 ± 19.3	135.4 ± 8.5*
ALT (IU/L)	26.4 ± 3.9	34.3 ± 4.6	25.0 ± 1.3	30.2 ± 1.9*	25.5 ± 1.9	37.3 ± 3.9*
AST (IU/L)	113.1 ± 11.7	173.7 ± 46.0	122.6 ± 15.0	135.7 ± 12.5	98.8 ± 5.4	111.7 ± 8.2
Calcium (mg/dL)	10.3 ± 0.4	10.0 ± 0.4	10.4 ± 0.4	10.7 ± 0.3	10.7 ± 0.3	10.4 ± 0.3
Magnesium (mg/dL)	2.5 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.1
Glucose (mg/dL)	43.7 ± 4.0	42.2 ± 4.9	46.9 ± 3.6	42.4 ± 5.4	52.2 ± 3.3	42.2 ± 5.2
Albumin (g/dL)	4.1 ± 0.2	4.1 ± 0.1	4.2 ± 0.2	3.9 ± 0.1	4.4 ± 0.2	4.0 ± 0.1

¹NEFA, non-esterified fatty acids; BHBA, β-hydroxybutyrate; TCH, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

*Significant at $p < 0.05$ for comparison between cows with DA and their control herd-mates.

**Significant at $p < 0.01$ for comparison between cows with DA and their control herd-mates.

Table 3. Comparison of the incidence of postpartum disorders and removal from the herd between cows with DA and their control herd-mates

Group	Postpartum disorders % (n)				Removal % (n)
	Puerperal metritis	Clinical endometritis	Mastitis	Lameness	
Control herd-mates	12.3 (7/57)	26.3 (15/57)	10.5 (6/57)	5.3 (3/57)	1.8 (1/57)
Cows with DA	11.3 (6/53)	39.6 (21/53)	3.8 (2/53)	3.8 (2/53)	14.0* (8/57)

Removal included cows that died or were culled.

*Significant at $p < 0.05$ for comparison between cows with DA and their control herd-mates.

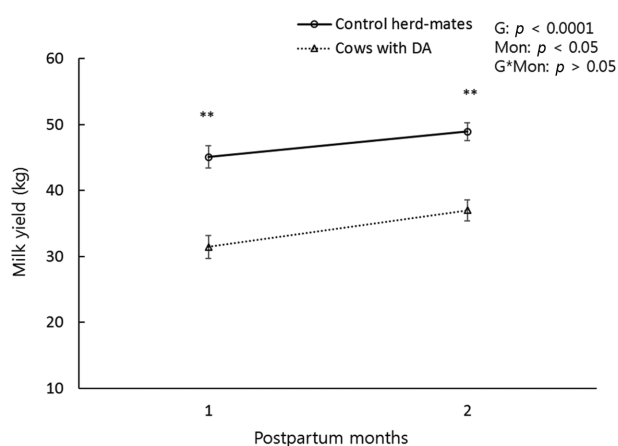


Fig 1. Comparison of milk yield in cows with displacement of the abomasum (DA, $n = 33$) and their control herd-mates ($n = 33$) 1 and 2 months after calving. G, group effect; Mon, measuring time effect; G*Mon, group-by-measuring time effect. There were significant effects of group ($p < 0.0001$) and sampling time ($p < 0.05$), but there was no interaction between group and sampling time ($p > 0.05$). **Significant at $p < 0.01$ for comparison between cows with DA and their control herd-mates.

Finally, cows with ketosis were at a higher risk of DA (OR = 9.27, $p < 0.0001$) than cows without ketosis.

Relationships between DA and serum metabolite concentrations

Table 2 shows a comparison of serum metabolite concentrations between cows with DA and controls 1, 2, and 4 weeks after calving. Cows with DA had higher NEFA ($p < 0.05$) and BHBA ($p < 0.01$) concentrations than their control herd-mates 1 week after calving. Cows with DA had lower serum TCH ($p < 0.01$ to 0.05), but higher ALT ($p < 0.05$) concentrations than their control herd-mates 2 and 4 weeks after calving. However, AST, calcium, magnesium, glucose, and albumin concentrations at the same time points did not differ between cows with DA and controls ($p > 0.05$).

Relationships between DA, postpartum disorders, and removal from the herd

Table 3 shows a comparison of the incidence of postpartum disorders and removal from the herd (by culling or death) between cows with DA and their control herd-mates. The incidences of puerperal metritis, clinical endometritis, mastitis, and lameness did not differ between cows with DA and controls ($p > 0.05$). However, cows with DA were more likely to be removed from the herd (14.0%) than controls

Table 4. Factors affecting the hazard of first insemination by 150 days after calving, analyzed using the PHREG procedure

Variables	Hazard ratio	95% Confidence interval	p -value
Group			
Control herd-mates	Reference		
Cows with DA	0.49	0.265-0.919	< 0.05
Calving season			> 0.05
Cow parity			
1	Reference		
2	0.51	0.175-1.500	> 0.05
3	0.73	0.267-2.004	> 0.05
≥ 4	0.32	0.107-0.972	< 0.05

(1.8%, $p < 0.05$).

Relationships between DA and milk yield and reproductive performance

Fig 1 shows a comparison of the milk yield of cows with DA and their control herd-mates 1 and 2 months after calving. There were significant effects of group ($p < 0.0001$) and sampling time ($p < 0.05$), but there was no interaction between group and sampling time ($p > 0.05$). Milk yield 1 and 2 months after calving was lower ($p < 0.01$) in cows with DA than in controls. Primiparous cows tended to have a lower milk yield than multiparous cows ($p = 0.06$).

Table 4 shows the factors affecting the hazard of first insemination by 150 days after calving, analyzed using the PHREG procedure. This analysis revealed that the hazard of first insemination by this time point was lower (HR = 0.49; $p < 0.05$) in cows with DA than in controls. The median and mean (\pm SEM) number of days to first insemination were 104 and 99.0 ± 4.0 in cows with DA, and 86 and 94.8 ± 5.2 in controls. Cows with parities of four or higher had a lower hazard (HR = 0.32, $p < 0.05$) of first insemination by 150 days after calving than primiparous cows. However, the hazard was not affected by calving season ($p > 0.05$).

The hazard of pregnancy by 210 days after calving did not differ ($p > 0.05$) between cows with DA and controls. In addition, this was not associated with calving season or cow parity ($p > 0.05$). The median and mean (\pm SEM) number of days to pregnancy were 155 and 154.1 ± 7.3 in cows with DA, and 178 and 163.0 ± 7.8 in controls.

Discussion

We determined the risk factors for DA and the relation-

ships between DA and serum metabolites, postpartum disorders, removal from the herd (by culling or death), milk yield, and reproductive performance in dairy cows. Our results indicate that higher parity, twin calves, and ketosis are risk factors for DA in dairy cows, which is associated with higher rates of removal from the herd, lower milk yield, a longer interval between calving and first postpartum insemination, and unfavorable serum concentrations of metabolites related to energy and liver function.

Our observation that multiparous cows, particularly cows with a parity of three, have a higher risk of DA than primiparous cows is consistent with previous studies (12,32). Another study also showed a higher incidence of DA in cows during their second to fourth lactation (25). The higher incidence of DA in cows with higher parity could in part be explained by increasing age, reflecting greater exposure to factors inducing abomasal atony, as previously suggested (8). In addition, it has been suggested that the greater risk of DA in cows with higher parity might be due to their greater milk production (15). Twin calves and ketosis, were also risk factors, whereas dystocia and retained placenta were not associated with DA in our study. Twin calves and ketosis have also been found to be associated with a higher incidence of DA in other studies (9,22,32), and these associations may imply a strong association between DA and peripartum NEB because this a key predisposing factor for ketosis in dairy cows (19). Thus, the association between DA and ketosis has been identified as bidirectional (11,17). However, other studies have shown that dystocia and/or retained placenta are also risk factors for DA (11,19), which is not consistent with our results.

Our observations of high concentrations of NEFA and BHBA 1 week after calving and high concentrations of TCH 2 and 4 weeks after calving in cows with DA are consistent with low feed consumption and severe NEB during the postpartum period in these animals. In addition, the higher concentrations of ALT 2 and 4 weeks after calving in the present study may indicate poorer liver function secondary to hepatic damage in cows with DA. Accordingly, serum BHBA concentrations of $\geq 1,200 \mu\text{mol/L}$ in the first week following calving were associated with a greater risk of DA (13,32). Another study has also reported that increases in the serum concentrations of BHBA and NEFA between 1 and 7 days after calving are associated with a greater risk of DA (19). However, serum concentrations of AST, calcium, magnesium, glucose, and albumin were not associated with the incidence of DA in the present study. Our findings are consistent with one previous study in which serum calcium concentration was found not to be associated with DA (19), although other studies have shown an association between postpartum hypocalcemia and DA (23,29), which was suggested to result from lower contractility of the abomasal wall during hypocalcemia, causing abomasal hypomotility (33). In addition, higher glucose, AST, and urea concentrations (25), and significantly lower serum concentrations of total protein, albumin, TCH, calcium, phosphorus, sodium, potassium, and chloride, have been identified in cows with DA (1). Taking all these metabolite data together, the implication is that maintenance of proper feeding and health management practices to avoid severe NEB, hepatic damage, and other meta-

bolic imbalances during the postpartum period is required to reduce the risk of DA, as reviewed in (30).

Puerperal metritis, clinical endometritis, mastitis, and lameness were not associated with DA in the present study. Some previous studies have also found no association between DA and mastitis (9,27), but others have shown associations between DA and metritis or endometritis (5,22). We found that the risk of removal from the herd (by culling or death) in the first 2 months after calving was higher for cows with DA than for their control herd-mates, but the cumulative incidence did not differ 210 days after calving (data not shown). Consistent with our findings, one previous study showed a lower survival rate in cows with DA (23.4%) than in cows without DA (0.5%) in the first 70 days after calving, after which the difference in survival disappeared (27). By contrast, other studies showed that removal from the herd was more likely at all time points after the diagnosis of DA (16,24). However, it must be noted that the decision to cull is affected by several factors, including a higher risk of death, lower milk production, or the policy of the farmer, as well as incomplete recovery following the treatment of DA (27).

Milk yield 1 and 2 months after calving was lower in cows with DA than in controls, which is consistent with several previous studies (12,16,18). Another study showed that cows with DA produced less milk during their first 4 months of lactation, but subsequently showed similar production levels to cows that had not had DA (27). Similarly, cows affected by DA reached the production level of their matched controls in the third month of lactation in another study (3). All these results indicate that a drop in milk yield resulting from a lack of nutrition and stress occurs when DA develops and continues for part of the lactation period.

Cows with DA were less likely to be inseminated by 150 days after calving than their control herd-mates, whereas the hazard of pregnancy by 210 days did not differ between cows with DA and controls in the present study. The longer calving to insemination interval might be associated with severe NEB in the postpartum period, which is reflected in unfavorable serum metabolites concentrations (NEFA, BHBA, TCH, and ALT) and a higher probability of ketosis and its sequela. Our findings are consistent with previous studies in which cows with DA had a longer calving to insemination interval but a similar calving to conception interval to cows that had not had DA (3,4,27). However, another study reported that the calving to conception interval was significantly longer in cows with DA than in their control herd-mates (5). The reason for the discrepancies among these studies is not clear. However, it is assumed that various confounding factors are likely to be involved, such as the carry-over effect of postpartum disorders, and variations in health and reproductive management (10,28). Thus, a larger scale study of the impact of DA on reproductive outcomes in dairy cows might be merited.

In summary, our data show that higher parity, twin calves, and ketosis are risk factors for DA in Korean dairy cows, and that this condition is associated with a higher probability of removal from the herd, lower milk yield, a longer calving to insemination interval, and unfavorable serum concentrations of metabolites related to energy and liver function. Thus,

proper feeding and health management practices for peripartum dairy cows, especially cows with higher parity, should be maintained to avoid severe NEB, hepatic pathology, and other metabolic imbalances, which should reduce the risk of DA. Moreover, for cows with DA, suitable postoperative treatment and careful health and nutritional management are also necessary to reduce the incidence of removal from the herd, and to prevent lower milk yield and poor reproductive performance in high-yielding dairy herds.

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