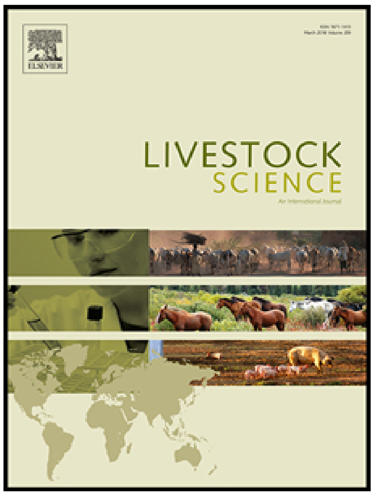
Accepted Manuscript



Relationship between serum magnesium concentration during the transition period, peri- and postpartum disorders, and reproductive performance in dairy cows

J.K. Jeong , I.S. Choi , S.H. Moon , H.G. Kang , I.H. Kim

PII: S1871-1413(18)30109-4
DOI: 10.1016/j.livsci.2018.04.013
Reference: LIVSCI 3443

To appear in: *Livestock Science*

Received date: 2 December 2017
Revised date: 21 April 2018
Accepted date: 21 April 2018

Please cite this article as: J.K. Jeong , I.S. Choi , S.H. Moon , H.G. Kang , I.H. Kim , Relationship between serum magnesium concentration during the transition period, peri- and postpartum disorders, and reproductive performance in dairy cows, *Livestock Science* (2018), doi: 10.1016/j.livsci.2018.04.013

Highlights

- The relationship between serum magnesium and reproductive performance was studied.
- High serum magnesium is associated with a lower incidence of peripartum disorders.
- High serum magnesium is associated with improved reproductive performance.
- Serum magnesium may be useful for predicting health and reproductive performance.

Relationship between serum magnesium concentration during the transition period, peri- and postpartum disorders, and reproductive performance in dairy cows

J.K. Jeong, I.S. Choi, S.H. Moon, H.G. Kang, I.H. Kim*

Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, 28644, South Korea

*Corresponding author: I.H. Kim, Tel.: +82 43 2612571; Fax: +82 43 2673150.

E-mail address: illhwa@cbu.ac.kr (I.H. Kim).

ABSTRACT

This study evaluated the relationship between serum magnesium concentration, peri- and postpartum disorders, and reproductive performance in dairy cows. Blood samples were collected from 198 Holstein cows 4 weeks prepartum, after calving, and 1, 2, 4, 6, and 8 weeks postpartum to measure serum magnesium and/or progesterone concentrations. To determine the relationship between serum magnesium concentration and peri- or postpartum disorders, cows were divided into two groups based on their mean serum magnesium concentration 4 weeks prepartum and just after calving: a Low-Mg group (1.45–2.65 mg/dL, n = 110) and a High-Mg group (2.70–5.40 mg/dL, n = 88). Analysis of these groups showed that the incidences of dystocia, retained placenta, endometritis, and lameness were lower (P < 0.05–0.01) in the High-Mg than in the Low-Mg groups. To determine the relationship between serum magnesium concentration during the transition period and reproductive performance, cows were divided into two groups based on their mean serum magnesium concentration from 4 weeks prepartum to 4 weeks postpartum: a Low-Mg group (1.80–2.59 mg/dL, n = 116) and a High-Mg group (2.60–4.17 mg/dL, n = 82). The probability of cyclicity resuming (shown by a progesterone concentration ≥ 1 ng/mL) by 8 weeks postpartum was higher in the High-Mg group (hazard ratio [HR]: 1.70; $P < 0.01$) than that in the Low-Mg group. Moreover, the probabilities of first insemination by 150 days and pregnancy by 210 days postpartum were higher (HRs: 2.16 and 2.36, respectively; $P \leq 0.0001$) in the High-Mg than in the Low-Mg groups, resulting in the mean calving-to-conception interval being 25 days shorter in the High-Mg group. In conclusion, high serum magnesium concentration during the transition period is associated with lower incidences of peri- and postpartum disorders and improved reproductive performance in dairy cows.

Keywords: Magnesium, Dairy cow, Disorder, Reproductive performance

1. Introduction

Dairy cows face challenges during the transition period between ~3 weeks before and 3 weeks after calving. Unsuccessful adaptation to these has been associated with altered immune function, occurrence of postpartum diseases, and lower milk production and reproductive performance (Caixeta et al., 2017; Chapinal et al., 2011; Jonsson et al., 2013; Kim and Suh, 2003; LeBlanc et al., 2005). Severe body condition loss due to a negative energy balance (NEB) during the transition period may be the major manifestation in cows that do not successfully adapt, and this is associated with several peripartum complications, poorer productivity, and reproductive defects in dairy cows (Carvalho et al., 2014; Roche et al., 2007; Sheehy et al., 2017). In addition, macromineral-related disorders arising from abnormal calcium, magnesium, phosphorus, or potassium concentrations may also lead to clinical or subclinical complications during the transition period (Goff, 2006). Any decline in their blood levels below the normal physiological limit, or unfavorable ratios, may result in adverse effects on health and subsequent reproductive performance (Chapinal et al., 2012; Lean et al., 2006; Liesegang et al., 2007). Thus, the identification of useful biomarkers of abnormal peripartum metabolism that could be used to predict peri- and postpartum health and reproductive performance may improve the economic viability of dairy herds.

Magnesium, which has a normal concentration of 1.8–2.4 mg/dL in the blood, maintains the electrical potential across nerve and muscle membranes and aids nerve impulse transmission (National Research Council, 1996). The concentration of magnesium in colostrum is about three times higher than that of regular milk (Tsioulpas et al., 2007), and milk production in lactating cows can rapidly deplete extracellular magnesium, resulting in hypomagnesemia if it is not replaced (Goff, 2006). Magnesium deficiency leads to hyperexcitability, caused by a reduction in resting nerve membrane potential (National Research Council, 2005). Insufficient levels of serum magnesium during the transition period are likely to be associated with unfavorable metabolic status and possibly higher incidences of peri- and postpartum complications, leading to poor reproductive performance. Many studies have shown that insufficient blood calcium (clinical or subclinical hypocalcemia) results in higher incidences of peri- and postpartum health problems and impaired fertility (Caixeta et al., 2017; Martinez et al., 2012; Rodriguez et al., 2017). However, information regarding the relationships between serum magnesium concentration and peri- or postpartum health and subsequent reproductive performance is lacking for dairy cows.

We hypothesized that low serum magnesium levels might be associated with dystocia and a higher incidence of postpartum complications, possibly leading to subsequent decreases in the reproductive performance of dairy cows. This would likely be the result of poor maintenance of the electrical potential across nerve and muscle membranes. Therefore, this study evaluated the relationships between serum magnesium concentration, the incidences of the peri- and postpartum disorders, and reproductive performance in dairy cows.

2. Materials and Methods

2.1. Experimental animals

This study was performed on four Holstein dairy farms in Chungcheong Province. The cows were maintained in loose housing systems, fed total mixed rations, and milked twice daily. The ration was based on brewers' grain, alfalfa hay, cotton seed, beet pulp, corn silage, tall fescue, timothy hay, minerals, and vitamins, and was designed to contain 17–20% crude protein, 4.8–5.0% crude fat, and 28–33% neutral detergent fiber. The macro-mineral component included 0.45–0.80% calcium, 0.25–0.40% magnesium, 0.32–0.43% phosphorus, 1.20–1.49% potassium, 0.20–0.35% sodium, and 0.25–0.42% chloride.

A total of 198 pregnant Holstein dairy cows, each with a total of 1–7 lactations (56 primiparous and 142 multiparous), were included in the study. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Korea.

2.2. Study design

To determine the relationship between serum magnesium concentration and peri- or postpartum disorders, cows were divided into two groups based on their mean serum magnesium concentration 4 weeks prepartum and just after calving: a Low-Mg group (1.45–2.65 mg/dL, n = 110) and a High-Mg group (2.70–5.40 mg/dL, n = 88). To determine the relationship between serum magnesium concentration during the transition period and reproductive performance, cows were divided into two groups based on their mean serum magnesium concentration between 4 weeks prepartum and 4 weeks postpartum: a Low-Mg group (1.80–2.59 mg/dL, n = 116) and a High-Mg group (2.60–4.17 mg/dL, n = 82).

The incidences of the following peri- and postpartum health problems and reproductive outcomes were compared between the Low-Mg and High-Mg groups: (i) dystocia, retained placenta, metritis, endometritis, metabolic disorders (ketosis, milk fever, and abomasal displacement), digestive disease, mastitis, lameness, and removal from the herds (by culling and death); (ii) probability of cyclicity resuming by 8 weeks postpartum; (iii) probability of first insemination by 150 days postpartum; and (iv) probability of conception by 210 days postpartum.

2.3. Blood sampling and measurement of serum magnesium and progesterone concentrations

Blood samples were collected from the tail vein of cows 4 weeks prepartum, just after calving (1.0 ± 0.1 h), and 1, 2, 4, 6, and 8 weeks postpartum, to assay serum magnesium and/or progesterone concentrations. Ten milliliters of blood were collected from each cow into plain plastic centrifuge tubes, which were immediately placed on ice. 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.

Serum magnesium concentration was measured using a 7180 Biochemistry Automatic Analyzer 710 (Hitachi Ltd., Tokyo, Japan) and commercial enzyme assay kits (Magnesium-HR II; Wako Pure Chemical Ltd., Osaka, Japan), according to the manufacturer's instructions.

Serum progesterone was determined using the Immulite 1000 Immunoassay System (DPC Cirrus Inc., Flanders, NJ, USA), according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation were <5% for each assay.

Resumption of postpartum cyclicity was determined by measuring serum progesterone (a concentration of ≥ 1 ng/mL was taken as indicating cyclicity had recommenced) at 2, 4, 6, and 8 weeks postpartum.

2.4. Definitions of disorders and reproductive management

The definitions used for the peri- and postpartum disorders that were diagnosed in this study were similar to those published previously (Cook et al., 2006; López de Maturana et al., 2007; Sheldon et al., 2006). 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 difficulty score > 2 were considered to have dystocia. Retained placenta was defined as the retention of the fetal membranes for > 24 h. Metritis was defined by the presence of fever (≥39.5°C) and a watery, fetid uterine discharge during the first 10 days after calving. Endometritis was diagnosed at 4 weeks postpartum by examining any vaginal discharge using the Metricheck tool (McDougall et al., 2007). Cows with a mucopurulent discharge (< 50% pus) were diagnosed with endometritis.

Ketosis was diagnosed by the following clinical signs: anorexia, depression, and an acetone odor on the breath. Milk fever was diagnosed by the presence of weakness, recumbency, and a favorable response to calcium therapy after calving. Abomasal displacement was diagnosed by a ‘ping’ sound during abdominal auscultation. Digestive disease was diagnosed by the presence of diarrhea or bloat. Mastitis was diagnosed by the production of abnormal milk or signs of inflammation in one or more quarters of the udder. Lameness was diagnosed if an abnormal gait or lack of weight bearing on a limb were observed, and included diagnoses of interdigital and digital dermatitis. ‘Removal from the herds’ was taken to include cows that died or were culled. The incidences of postpartum disorders and removal from herds were finalized by 4 weeks postpartum, except for particular disorders for which the timing of diagnosis is given above. With the exception of dystocia and mastitis, all other peri- and postpartum disorders were diagnosed by veterinarians in the research team.

The voluntary waiting period from calving to the first artificial insemination was 40 days. Pregnancy was diagnosed 35–40 days after insemination by transrectal ultrasonography. Data regarding reproductive performance were collected for a minimum of 210 days postpartum or until pregnancy or culling.

2.5. Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM). For statistical analyses, cows were defined as either primiparous or multiparous, and the calving season was defined as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February). Statistical analyses were performed using SAS software (version 9.4, SAS Inst., Cary, NC, USA).

The mean serum magnesium concentrations were compared between primiparous and multiparous cows during the pre- and postpartum periods using Student's *t*-test. The incidences of peri- and postpartum disorders, such as dystocia, retained placenta, metritis, endometritis, metabolic disorders (ketosis, milk fever, and abomasal displacement), digestive disease, mastitis, lameness, and removal from the herds, were compared between the groups using chi-square or Fisher's exact tests.

Cox's proportional hazard model with the PHREG procedure was used to compare the probabilities of cyclicity resuming by 8 weeks, of the first insemination taking place by 150 days, and of pregnancy by 210 days postpartum between the groups. This analysis estimated the probability of a cow having resumed cycling, been inseminated or become pregnant at a given time. The time variables used in this model were the interval between calving and the resumption of cyclicity in weeks, and between calving and first insemination and between calving and pregnancy in days. Cows that died, were sold, had not resumed cycling by 8 weeks, had not been inseminated by 150 days, or were not pregnant by 210 days postpartum were not included in the analysis. Cox models included calving season, cow parity (primiparous or multiparous), and group (Low-Mg and High-Mg). Cow and farm were included in the model as random effects. The proportional hazard rate was determined based on interactions between explanatory variables and time, and by evaluating Kaplan–Meier curves. The median and mean number of weeks between calving and resumption of cyclicity, and of days between calving and first insemination or pregnancy, were determined by survival analysis using the Kaplan–Meier model and the LIFETEST procedure within the SAS software. A survival plot was generated using the survival option within MedCalc software (11.1, MedCalc Software, Mariakerke, Belgium). $P < 0.05$ was considered to be significant.

3. Results

3.1. Descriptive statistics

The mean serum magnesium concentration between 4 weeks prepartum and just after calving was 2.7 ± 0.1 mg/dL (range: 1.45–5.40 mg/dL), while the mean serum magnesium concentration between 4 weeks prepartum and 4 weeks postpartum was 2.6 ± 0.1 mg/dL (range: 1.80–4.17 mg/dL). The mean magnesium concentrations in both analyses were higher ($P < 0.05$–0.01) in multiparous (2.7 ± 0.1 and 2.6 ± 0.1 mg/dL) than primiparous cows (2.5 ± 0.1 mg/dL).

3.2. Associations between serum magnesium concentration prepartum and around calving with peri- and postpartum disorders and removal from the herds

Table 1 shows a comparison of the incidences of peri- and postpartum disorders and removal from herds between the two groups. The incidences of dystocia, retained placenta, endometritis, and lameness were lower ($P < 0.05$–0.01) in the High-Mg than in the Low-Mg group, whereas the incidences of metritis, metabolic disorders, digestive disease, mastitis, and removal from the herds did not differ between the two groups ($P > 0.05$).

3.3. Association of serum magnesium concentration during the transition period with reproductive performance

Table 2 shows the factors affecting the probability of resuming cyclicity by 8 weeks postpartum, analyzed using the PHREG procedure. This analysis revealed that the probability of resuming cyclicity by 8 weeks postpartum was higher in the High-Mg group (hazard ratio [HR]: 1.70; $P < 0.01$) than in the Low-Mg group, as shown by the survival curves (**Fig. 1**). However, this probability was not affected by calving season or cow parity ($P > 0.05$).

Table 3 shows the factors affecting the probability of insemination by 150 days postpartum, analyzed using the PHREG procedure. This analysis revealed that the probability of first insemination by 150 days postpartum was higher (HR: 2.16; $P = 0.0001$) in the High-Mg than in the Low-Mg group, but this probability was not affected by calving season or cow parity ($P > 0.05$). The High-Mg group was inseminated a mean 20 days sooner than the Low-Mg group, as indicated by the survival curves (**Fig. 2**).

Table 4 shows the factors affecting the probability of pregnancy by 210 days postpartum. The probability of pregnancy by 210 days postpartum was higher (HR: 2.36; $P < 0.0001$) in the High-Mg than in the Low-Mg group, but was not affected by calving season or cow parity ($P > 0.05$). The High-Mg group took a mean 25 days fewer to conceive than the Low-Mg group, as shown by the survival curves (**Fig. 3**).

4. Discussion

This study aimed to determine the relationships between serum magnesium concentration, the incidences of peri- and postpartum disorders, and reproductive performance in dairy cows. Our results reveal that high serum magnesium concentrations during the transition period were associated with lower incidences of peri- and postpartum disorders and improved reproductive performance in dairy cows. Thus, serum magnesium concentration during the transition period could be a useful biomarker of peri- and postpartum health and subsequent reproductive performance in dairy cows.

The mean serum magnesium concentration (2.6 mg/dL) measured in the study herds during the pre- and postpartum periods in the present study was similar to that previously reported (Djokovic et al., 2014), but higher than in other previous reports (2.1–2.3 mg/dL, Cozzi et al., 2011; Jonsson et al., 2013). The inconsistency in the serum magnesium concentrations among these studies might be due to the differing management strategies adopted among the farms for cow nutrition (diet composition and the level of milk production) (Moretti et al., 2017).

Serum magnesium concentrations above the mean in the study herds between 4 weeks prepartum and just after calving were associated with lower incidences of dystocia, retained placenta, endometritis, and lameness. These observations are consistent with a previous study (Qu et al., 2014), in which low serum magnesium concentrations during the last week before calving were associated with a greater incidence of retained placenta. Another study also showed that low serum magnesium during the first 8 days postpartum was associated with a higher incidence of retained placenta (Tsiamadis et al., 2016). The definitive mechanism whereby low serum magnesium might cause a higher incidence of dystocia and retained placenta has not been established by the current or previous studies. However, one previous study did show that magnesium plays an important intracellular role in decreasing the excitability of smooth muscle, leading to a reduction in smooth muscle tone (Griffin, 2003). Thus, it is likely that low serum magnesium during the peripartum period might increase the risk of retention of the fetus and placenta because it has also been shown that magnesium deficiency leads to hyperexcitability caused by a reduction in the nerve resting membrane potential (National Research Council, 2005).

We have shown in this study that hypomagnesemia was associated with a higher incidence of endometritis, which is consistent with a previous study in which endometritis was associated with both low blood magnesium concentration and hepatocellular damage (Burke et al., 2010). It is known that impaired immune function during the transition period is responsible for some postpartum disorders, including uterine disease (Kim et al., 2005), and is strongly associated with severe NEB during this time (Esposito et al., 2014). Because serum magnesium concentration usually reflects dietary magnesium intake (Goff, 2008), it could be concluded that low serum magnesium arises as a result of low feed intake. However, we found no differences in body condition score during the pre- and postpartum periods between the groups (data not shown). Therefore, we speculate that greater excitability of uterine muscle resulting from low serum magnesium might predispose towards uterine disease in dairy cows.

Our finding of a higher incidence of lameness in the Low-Mg than in the High-Mg group is consistent with data from a previous study (Gerloff and Swensen, 1996), in which cows that developed severe lameness had lower magnesium concentrations than healthy cattle. However, another study demonstrated no obvious difference in serum magnesium concentration between sound and lame cows (Zhao et al., 2015). Many factors influence the susceptibility of cattle to lameness in the peripartum period, including nutrition, and these may lead to a reduction in keratinization (Wilde, 2006). In addition, a previous study showed that the degree of body condition loss during the prepartum period, attributed to lower feed consumption, was associated with a higher incidence of lameness (Hoedemaker et al., 2009). More comprehensive investigations are required to better define the relationship between serum magnesium and lameness after calving.

Our findings that there were no differences in the incidences of metritis, metabolic disorders, digestive disease, mastitis, and removal from the herds between groups were consistent with some previous studies in which serum magnesium was not associated with the incidences of ketosis, abomasal displacement, mastitis, culling, and death (Mokhber Dezfouli et al., 2013; Tsiamadis et al., 2016).

We have also shown a higher probability of cyclicity resuming postpartum in the High-Mg group compared to the Low-Mg group, and this finding is also consistent with that of a previous study (Aungier et al., 2014), which showed that high serum magnesium at 4 weeks postpartum was associated with a higher probability of ovulation at any given time point postpartum. The higher probabilities of first insemination by 150 days and pregnancy by 210 days postpartum in the High-Mg than in the Low-Mg groups were also mirrored by a previous study, which reported that a high prepartum magnesium concentration was associated with a decrease in days open (Ingraham et al., 1987). Another previous study showed that a high blood magnesium concentration (approximately 2.2 mg/dL) at the time of first service was associated with a better first service conception rate (Tillard et al., 2008). Conversely, it is likely that lower incidences of peri- and postpartum disorders and a swifter resumption of postpartum cyclicity in the High-Mg group might have a favorable effect on subsequent reproductive performance compared to the Low-Mg group. However, it is unknown whether the serum magnesium concentration during the transition period directly affected reproductive performance in the present study. Thus, further investigations are required to define the links between serum magnesium and the reproductive performance of dairy cows.

5. Conclusion

Our data demonstrate that high serum magnesium concentrations during the transition period are associated with lower incidences of peri- and postpartum disorders (dystocia, retained placenta, endometritis, and lameness) and improved reproductive performance (higher probabilities of resumption of cyclicity by 8 weeks, first insemination by 150 days, and pregnancy by 210 days postpartum) in dairy cows. Thus, measurement of serum magnesium during the transition period could be useful to predict peripartum health and subsequent reproductive performance in dairy cows. However, further fundamental research is required to determine the exact mechanism whereby circulating magnesium concentration influences peri- and postpartum health and subsequent reproductive performance in dairy cows.

Conflict of interest statement

None.

Acknowledgments

This work was carried out with the support of the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ010818)” Rural Development Administration, Republic of Korea.

References

Aungier, S.P., Roche, J.F., Diskin, M.G., Crowe, M.A., 2014. Risk factors that affect reproductive target achievement in fertile dairy cows. J. Dairy Sci. 97, 3472–3487.

Burke, C.R., Meier, S., McDougall, S., Compton, C., Mitchell, M., Roche, J.R., 2010.

Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J. Dairy Sci. 93, 5363–5373.

Caixeta, I.S., Ospina, P.A., Capel, M.B., Nydam, D.D., 2017. Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. Theriogenology 94, 1–7.

Carvalho, P.D., Souza, A.H., Amundson, M.C., Hackbart, K.S., Fuenzalida, M.J., Herlihy, M.M., Ayres, H., Dresch, A.R., Vieira, L.M., Guenther, J.N., Grummer, R.R., Fricke, P.M., Shaver, R.D., Wiltbank, M.C., 2014. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. J. Dairy Sci. 97, 3666–3683.

Chapinal, N., Carson, M., Duffield, T.F., Capel, M., Godden, S., Overton, M., Santos, J.E.P., 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94, 4897–4903.

Chapinal, N., LeBlanc, S.J., Carson, M.E., Leslie, K.E., Godden, S., Capel, M., Santos, J.E.P., Overton, M.W., Duffield, T.F., 2012. Herd-level association of serum metabolites in the transition period with disease, milk production, and early laction reproduction performance. J. Dairy Sci. 95, 5676–5682.

Cook, N., Oetzel, G., Nordlund, K., 2006. Modern techniques for monitoring high-producing dairy cows. 1. Principles of herd-level diagnoses. In Pract. 28, 510–515.

Cozzi, G., Ravarotto, L., Gottardo, F., Stefani, A.L., Contiero, B., Moro, L., Brscic, M., Dalvit, P., 2011. Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. J. Dairy Sci. 94, 3895–3901.

Djokovic, R.D., Kurcubic, V.S., Ilic, Z.Z., 2014. Blood serum levels of macro- and micronutrients in transition and full lactation cows. Bulg. J. Agric. Sci. (BJAS) 20, 715–720.

Esposito, G., Irons, P.C., Webb, E.C., Chapwanya, A., 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144, 60–71.

Gerloff, B.J., Swensen, E.P., 1996. Acute recumbency and marginal phosphorus deficiency in dairy cattle. J. Am. Vet. Med. Assoc. 208, 716–719.

Goff, J.P., 2006. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. Anim. Feed Sci. Technol. 126, 237–257.

Goff, J.P., 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176, 50–57.

Griffin, I.J., 2003. Encyclopedia of Food Sciences and Nutrition (Second Edition) Academic Press Pages 3641–3646 https://doi.org/10.1016/B0-12-227055-X/00723-9.

Hoedemaker, M., Prange, D., Gundelach, Y., 2009. Body condition change ante- and postpartum, health and reproductive performance in German Holstein cows. Reprod. Domest. Anim. 44, 167–173.

Ingraham, R.H., Kappel, L.C., Morgan, E.B,. Srikandakumar, A., 1987. Correction of subnormal fertility with copper and magnesium supplementation. J. Dairy Sci. 70, 167–180.

Jonsson N.N., Fortes, M.R.S., Piper, E.K., Vankan, D.M., de Cisnero,s J.P.J., Wittek, T., 2013. Comparison of metabolic, hematological, and peripheral blood leukocyte cytokine profiles of dairy cows and heifers during the periparturient period. J. Dairy Sci. 96, 2283–2292.

Kim, I.H., Suh, G.H., 2003. Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. Theriogenology 60, 1445–1456.

Kim, I.H., Na, K.J., Yang, M.O., 2005. Immune responses during the peripartum period in dairy cows with postpartum endometritis. J. Reprod. Dev. 51, 757–764.

Lean, I.J., DeGaris, P.J., McNeil, D.M., Block, E., 2006. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. J. Dairy Sci. 89, 669–684.

LeBlanc, S.J., Leslie, K.E., Duffield, T.F., 2005. Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88, 159–170.

Liesegang, A., Chiappi, C., Risteli, J., Kessler, J., Hess, H.D., 2007. Influence of different calcium contents in diets supplemented with anionic salts on bone metabolism in periparturient dairy cows. J. Anim. Physiol. Anim. Nutr. 91, 120–129.

López de Maturana, E., Legarra, A., Varona, L., Ugarte, E., 2007. Analysis of fertility and dystocia in Holsteins using recursive models to handle censored and categorical data. J. Dairy Sci. 90, 2012–2024.

Martinez, N., Risco, C.A., Lima, F.S., Bisinotto, R.S., Greco, L.F., Ribeiro, E.S., Maunsell, F., Galvão, K., Santos, J.E.P., 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95, 7158–7172.

McDougall, S., Macaulay, R., Compton, C., 2007. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. Anim. Reprod. Sci. 99, 9–23.

Mokhber Dezfouli, M., Eftekhari, Z., Sadeghian, S., Bahounar, A., Jeloudari, M., 2013. Evaluation of hematological and biochemical profiles in dairy cows with left displacement of the abomasum. Comp. Clin. Path. 22, 175–179.

Moretti, P., Paltrinieri, S., Trevisi, E., Probo, M., Ferrari, A., Minuti, A., Giordano, A., 2017. Reference intervals for hematological and biochemical parameters, acute phase proteins and markers of oxidation in Holstein dairy cows around 3 and 30 days after calving. Res. Vet. Sci. 114, 322–331.

National Research Council, 1996. Nutrient requirements of beef cattle, 7th ed. National Academy Press, Washington, DC.

National Research Council, 2005. Mineral tolerance of animals, seventh ed. National Academy Press, Washington, DC.

Qu, Y., Fadden, A.N., Traber, M.G., Bobe, G., 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 97, 4151–4165.

Roche, J.R., Lee, J.M., Macdonald, K.A., Berry, D.P., 2007. Relationships among body condition score, body weight, and milk production variables in pasture-based dairy cows. J. Dairy Sci. 90, 3802–3815.

Rodriguez, E.M., Aris, A., Bach, A., 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. J. Dairy Sci. 100, 7427–7434.

Sheehy, M.R., Fahey, A.G., Aungier, S.P., Carter, F., Crowe, M.A., Mulligan, F.J., 2017. A comparison of serum metabolic and production profiles of dairy cows that maintained or lost body condition 15 days before calving. J. Dairy Sci. 100, 536–547.

Sheldon, I.M., Lewis, G.S., LeBlanc, S., Gilbert, R.O., 2006. Defining postpartum uterine disease in cattle. Theriogenology 65, 1516–1530.

Tillard, E., Humblot, P., Faye, B., Lecomte, P., Dohoo, I., Bocquier, F., 2008. Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions. Theriogenology 69, 443–457.

Tsiamadis, V., Banos, G., Panousis, N., Kritsepi-Konstantinou, M., Arsenos, G., Valergakis, G.E., 2016. Genetic parameters of subclinical macromineral disorders and major clinical diseases in postparturient Holstein cows. J. Dairy Sci. 99, 8901–8914.

Tsioulpas, A., Grandison, A.S., Lewis, M.J., 2007. Change in physical properties of bovine milk from the colostrum period to early lactation. J. Dairy Sci. 90, 5012–5017.

Wilde, D., 2006. Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. Anim. Reprod. Sci. 96, 240–249.

Zhao, X.J., Wang, X.Y., Wang, J.H., Wang, Z.Y., Wang, L., Wang, Z.H., 2015. Oxidative stress and imbalance of mineral metabolism contribute to lameness in dairy cows. Biol. Trace Elem. Res. 164, 43–49.

Figure Captions

Figure 1

Survival curves for the interval from calving to resumption of cyclicity in the Low-Mg (1.80–2.59 mg/dL, n = 116) and High-Mg (2.60–4.17 mg/dL, n = 82) groups. The probability of cyclicity resuming by 8 weeks postpartum was higher (hazard ratio = 1.96; confidence interval = 1.366–2.803; $P < 0.0001$) in the High-Mg than in the Low-Mg group.

Figure 2

Survival curves for the interval from calving to first insemination in the Low-Mg (1.80–2.59 mg/dL, n = 116) and High-Mg (2.60–4.17 mg/dL, n = 82) groups. The probability of first insemination by 150 days postpartum was higher (hazard ratio = 2.00; confidence interval = 1.409–2.846; $P < 0.0001$) in the High-Mg than in the Low-Mg group.

Figure 3

Survival curves for the interval between calving and pregnancy in the Low-Mg (1.80–2.59 mg/dL, n = 116) and High-Mg (2.60–4.17 mg/dL, n = 82) groups. The probability of pregnancy by 210 days postpartum was higher (hazard ratio = 2.21; confidence interval = 1.456–3.344; $P = 0.0001$) in the High-Mg than in the Low-Mg group.

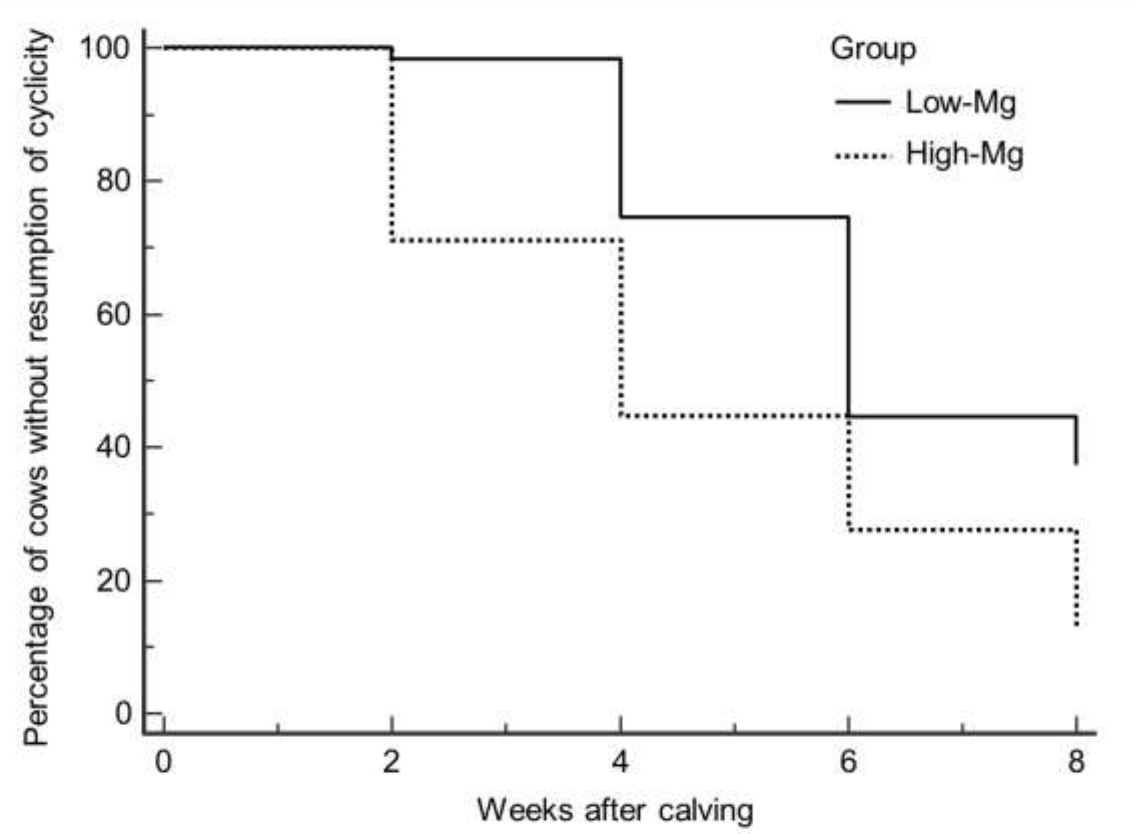

Percentage of cows without resumption of cyclicity
100
80
60
40
20
0
0
2
4
6
8
Weeks after calving
Group
Low-Mg
High-Mg

Fig 1.

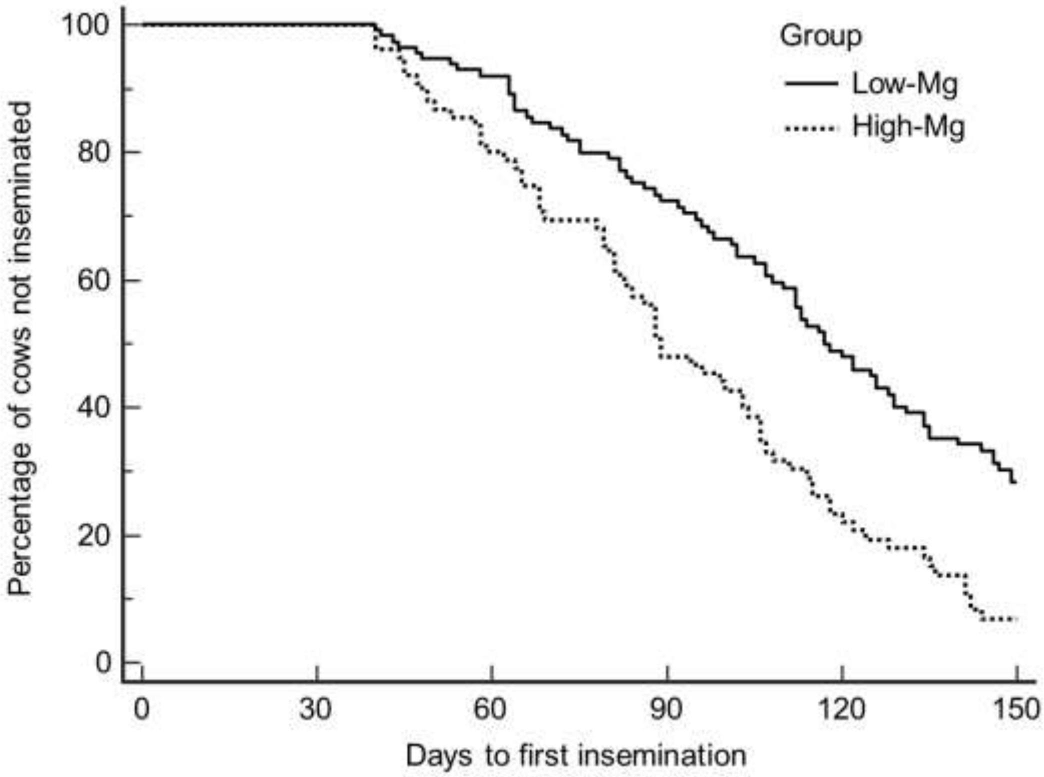

Percentage of cows not inseminated
100
80
60
40
20
0
0
30
60
90
120
150
Days to first insemination
Group
Low-Mg
High-Mg

Fig 2.

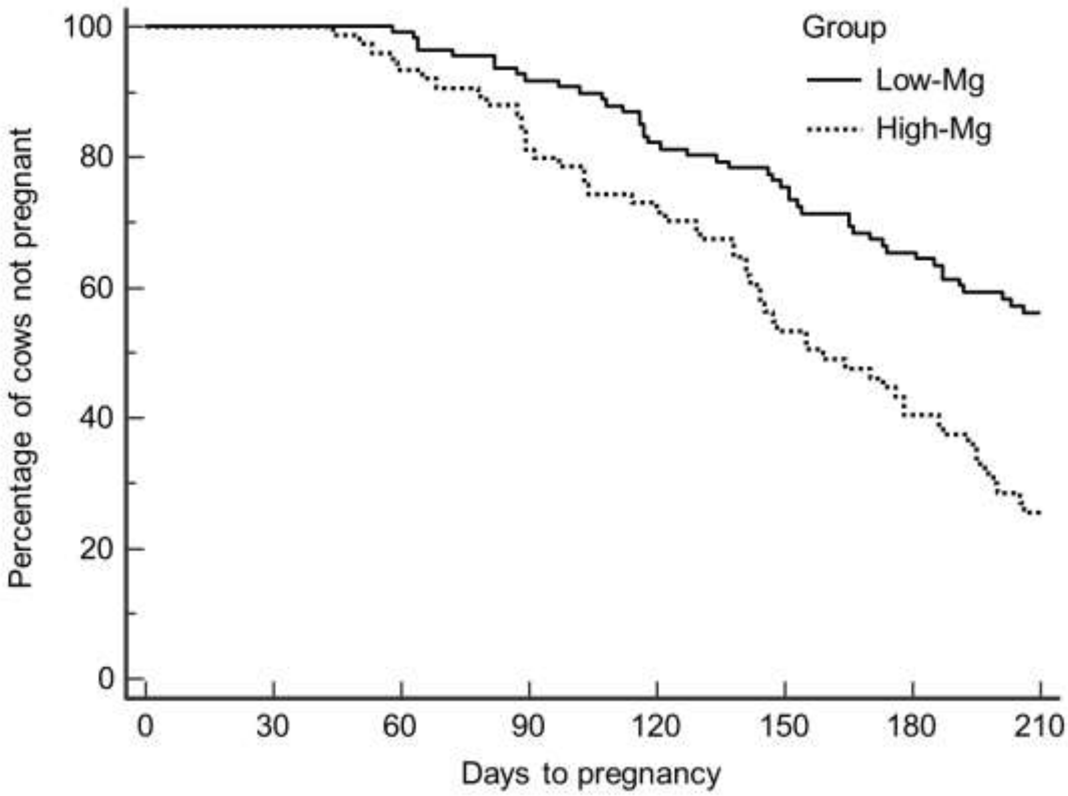

Group
Low-Mg
High-Mg
Percentage of cows not pregnant
100
80
60
40
20
0
0
30
60
90
120
150
180
210
Days to pregnancy

Fig 3.

Table 1

Comparison of the incidences of peri- and postpartum disorders and removal from herds according to mean serum magnesium concentrations 4 weeks prepartum and just after calving.

Group[1]	Peri- and postpartum disorders % (n)								Removal[3] % (n)
	Dystocia	Retained placenta	Metritis	Endometritis	Metabolic disorders[2]	Digestive disease	Mastitis	Lameness	
Low-Mg	29.5 (31/105)[c]	38.1 (40/105)[a]	39.6 (40/101)	39.6 (40/101)[c]	22.3 (23/103)	5.9 (6/101)	11.9 (12/101)	15.8 (16/101)[c]	3.8 (4/105)
High-Mg	12.9 (12/93)[d]	24.7 (23/93)[b]	36.3 (33/91)	20.0 (18/90)[d]	22.6 (21/93)	4.4 (4/91)	8.9 (8/90)	3.3 (3/90)[d]	3.2 (3/93)

[1] Low-Mg: 1.45–2.65 mg/dL; High-Mg: 2.70–5.40 mg/dL.

[2] Metabolic disorders included ketosis, milk fever, and abomasal displacement.

[3] Removal included cows that died or were culled.

[a,b] Means with different superscripts differ with $P < 0.05$ between groups.

[c,d] Means with different superscripts differ with $P < 0.01$ between groups.

Table 2

Factors affecting the probability of resumption of cyclicity by 8 weeks postpartum, analyzed using the PHREG procedure.

Variable	Hazard ratio	95% CI[1]	*P*-value
Group[2]			
Low-Mg	Reference		
High-Mg	1.70	1.172–2.464	< 0.01
Calving season			> 0.05
Cow parity			> 0.05

[1] Confidence interval.

[2] Low-Mg: 1.80–2.59 mg/dL (n = 116); High-Mg: 2.60–4.17 mg/dL (n = 82).

Table 3

Factors affecting the probability of first insemination by 150 days postpartum, analyzed using the PHREG procedure.

Variable	Hazard ratio	95% CI[1]	*P*-value
Group[2]			
Low-Mg	Reference		
High-Mg	2.16	1.465–3.192	0.0001
Calving season			> 0.05
Cow parity			> 0.05

[1] Confidence interval.

[2] Low-Mg: 1.80–2.59 mg/dL (n = 116); High-Mg: 2.60–4.17 mg/dL (n = 82).

Table 4

Factors affecting the probability of pregnancy by 210 days postpartum, analyzed using the PHREG procedure.

Variable	Hazard ratio	95% CI[1]	*P*-value
Group[2]			
Low-Mg	Reference		
High-Mg	2.36	1.547–3.595	< 0.0001
Calving season			> 0.05
Cow parity			> 0.05

[1] Confidence interval.

[2] Low-Mg: 1.80–2.59 mg/dL (n = 116); High-Mg: 2.60–4.17 mg/dL (n = 82).