

## Peripartum blood concentrations of calcium, phosphorus and magnesium in dairy cows with normal puerperium or puerperal endometritis

### Concentrações sanguíneas de cálcio, fósforo e magnésio no periparto de vacas leiteiras com puerpério normal ou com endometrite puerperal

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**Summary:** In Experiment 1, plasma concentrations of Ca, Ph and Mg were measured twice weekly from parturition until the sixth week postpartum in cows with normal puerperium (n = 7), with mild puerperal endometritis (n = 11) or with severe puerperal endometritis (n = 8). Plasma Ca concentrations were significantly lower in cows with severe endometritis than in cows with mild endometritis (P < 0.01) and in normal puerperium cows (P < 0.00001). Cows with mild endometritis had significantly lower plasma Ca concentrations than normal puerperium cows (P < 0.01). Overall the sampling period, plasma Ca concentrations < 8.0 mg/dL were present in 0, 4 (36 %) and 6 (75 %) cows with normal puerperium, mild endometritis or severe endometritis, respectively. No significant effects were observed for the plasma Ph and Mg concentrations and for the relation Ca:Ph.

In Experiment 2, serum Ca and Ph concentrations were measured twice a week from a minimum of two weeks before parturition until the sixth week postpartum in dairy cows with mild puerperal endometritis (n = 6) and heavy puerperal endometritis (n = 8) that spontaneously recovered from the infection. Only the effect of sampling day for the Ca concentrations was significant (P < 0.00001). Serum Ca concentrations were significantly higher before parturition than after parturition. During the sampling period, 13 (93 %) cows presented serum Ca concentrations < 8.0 mg/dL.

In dairy cows, the establishment of puerperal endometritis is associated to a subclinical hypocalcemia and the severity of the infection is related to the blood total Ca levels.

**Resumo:** No Ensaio 1, foram mensuradas as concentrações plasmáticas de Ca, Ph e Mg, duas vezes por semana, desde a parição até à sexta semana pós-parto, em vacas com puerpério normal (n = 7), com endometrite puerperal ligeira (n = 11) ou com endometrite puerperal severa (n = 8). A concentração de Ca foi maior nas vacas com puerpério normal do que nas vacas com endometrite ligeira (P < 0,01) ou com endometrite severa (P < 0,00001). As vacas com endometrite severa apresentaram concentrações de Ca menores do que as vacas com endometrite ligeira (P < 0,01). Durante o período de amostragem foram observadas concentrações de Ca < 8,0 mg/dL em 0, 4 (36 %) e 6 (75 %) das vacas com puerpério normal, endometrite ligeira ou endometrite severa, respectivamente.

No ensaio 2, as concentrações séricas de Ca e Ph foram mensuradas, duas vezes por semana, desde duas semanas antes da parição até à sexta semana pós-parto, em vacas leiteiras com endometrite puerperal ligeira (n = 6) e endometrite puerperal se-

vera (n = 8), que recuperaram espontaneamente da infecção. O efeito dia de amostragem sobre a concentração de Ca foi significativo (P < 0,00001). As concentrações de Ca foram significativamente maiores antes do que depois da parição. Durante o período de amostragem 13 vacas (93 %) apresentaram concentrações de Ca < 8,0 mg/dL. Em ambos os ensaios não foram observados efeitos significativos sobre as concentrações de Ph, Mg e a relação Ca:Ph.

Na vaca leiteira, existe uma associação entre o estabelecimento da endometrite puerperal e a hipocalcemia subclínica. A severidade da endometrite puerperal poderá estar relacionada com as concentrações sanguíneas de Ca.

### Introduction

The postpartum uterine involution is the result of the reduction in uterine size and of the uterine depuration from the tissue debris (uterine and placental) and from bacteria that gained access to the uterus. Myometrial contraction plays an important role in uterine involution (Arthur *et al.*, 1996). This contraction of the smooth muscular layer of the uterus is mediated by several agents, including estrogens, prostaglandins, oxytocin and ions like Ca (Risco *et al.*, 1994; Arthur *et al.*, 1996).

In dairy cows, the blood concentrations of Ca significantly decrease at the start of milking (Hove, 1986). The range of physiologic blood total Ca concentrations is 8-10 mg/dL, with values below this threshold representing a sub-clinical hypocalcemia and normally attaining a clinical hypocalcemia below 5 mg/dL (Houe *et al.*, 2001). Risk assessment studies showed that metabolic disorders of the peripartum period are associated with several pathological conditions including dystocia, uterine prolapse, retention of fetal membranes, metritis, ovarian cysts and abnormal ovarian function (Curtis *et al.*, 1983; Erb *et al.*, 1985; Opsomer *et al.*, 2000). However, these associations can be either direct or act indirectly. Subclinical hypocalcemia was related to the severity of the postpartum vaginal discharge (Titterton and Weaver, 1999), to poor uterine involution (Risco *et al.*, 1984, 1994; Jonsson, 1999), metritis and

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ovarian dysfunction (Risco *et al.*, 1994; Houe *et al.*, 2001).

The objective of these experiments was to evaluate the relationship between the blood concentrations of Ca, Ph and Mg in the peripartum period of dairy cows and the establishment and the severity of the puerperal uterine infection.

### Materials and methods

Two experiments were conducted in a dairy herd with 250 Holstein-Friesian cows, with an average milk yield of 7,500 Kg per 305 day corrected lactation. The cows were kept in free stalls and milked twice a day. They were fed a total mixed ration, according to milk yield, consisting of corn silage, ryegrass silage, alfalfa hay and a commercial concentrate in the lactation phase and consisting of corn silage, straw and a commercial concentrate in the dry phase. This ration was calculated to cover the daily maintenance and production requirements of nutrients, according to the National Research Council (NRC, 1989) guidelines. At about three weeks before the estimated parturition date, dry cows were moved to a closed barn and observed for impending signs of parturition at which time they were moved to a calving box where they remained until 12 to 24 hours after parturition. All cows calved normally and no clinical signs other than those related to puerperal endometritis were noted.

**Table 1** - Results (P value) of multivariate analysis of variance with repeated measures for total Ca, Ph and Mg concentrations in plasma (Experiment 1) and for total Ca and Ph concentrations in serum (Experiment 2).

Parameter	Effect		
	Between group <sup>1</sup>	Within group <sup>2</sup>	Inter action <sup>3</sup>
Experiment 1			
Plasma total Ca concentrations	0.0001 <sup>a</sup>	0.12	0.17
Plasma total Ph concentrations	0.50	0.82	0.96
Plasma total Mg concentrations	0.32	0.89	0.78
Relation Ca:Ph	0.44	0.67	0.64
Experiment 2			
Serum total Ca concentrations	0.34	0.00001	0.73
Serum total Ph concentrations	0.47	0.93	0.96
Relation Ca:Ph	0.84	0.80	0.64

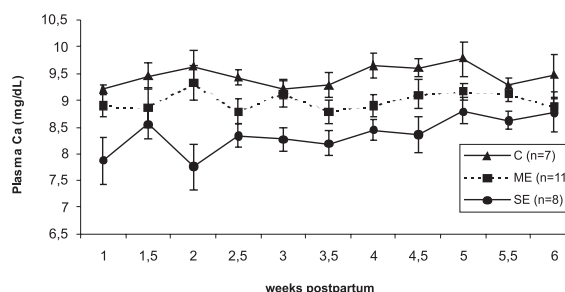
<sup>1</sup> Groups control versus mild endometritis versus severe endometritis (Experiment 1) and mild endometritis versus heavy endometritis (Experiment 2);

<sup>2</sup> repeated measures factor = sampling days;

<sup>3</sup> interaction between-within effect.

<sup>a</sup> Group control versus group mild endometritis, P < 0.01; group mild endometritis versus group severe endometritis, P < 0.01; group control versus group severe endometritis, P < 0.00001 (least significant differences).

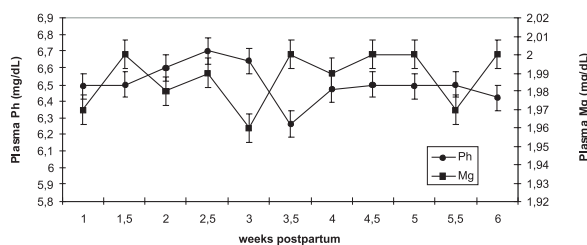
Experiments 1 and 2 were conducted from October 1997 through December 1998 and from April to September 2000, respectively. In both experiments, in each session, an average of 5 cows with estimated close parturition date was allocated to the study. Cows that were allocated to the different groups of the experiments were present in each session.



**Figure1** - Plasma total Ca concentrations in the postpartum period of dairy cows with normal puerperium, with mild puerperal endometritis or with severe puerperal endometritis. C = group control (normal puerperium); ME = group mild endometritis; SE = group severe endometritis. The effect of group was significant (P < 0.0001, MANOVA with repeated measures). The effect of sampling day tended to be significant (P < 0.12).

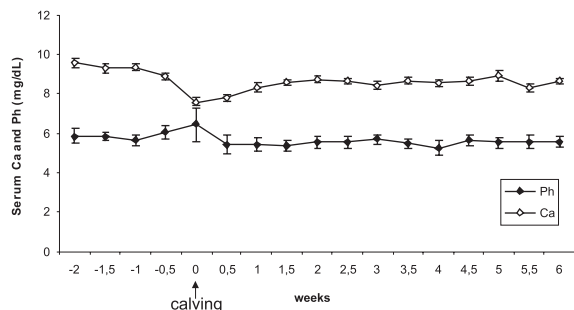
In Experiment 1, cows (n = 26) were allocated to three groups: group control (n = 7), including cows with normal puerperium; group mild endometritis (n = 11) and group severe endometritis (n = 8), including cows with mild and severe clinical signs of puerperal endometritis, respectively. In experiment 2, fourteen cows that developed puerperal endometritis and that spontaneously recovered from the infection until 35 days postpartum were used and allocated to two groups: group mild endometritis (n = 6), including cows with mucopurulent to purulent lochia and group heavy endometritis (n = 8), including cows with fetid sanguine-purulent lochia (no systemic symptoms were noted). The diagnosis and the evaluation of endometritis severity were based on the clinical signs (lochia type, cervical and uterine findings evaluated by palpation per rectum and ultrasound) and the bacteriological findings (isolation of uterine pathogenic bacterial species). Except for two cows from group severe endometritis of Experiment 1, that developed serious systemic illness and were treated with systemic and local antibiotics, no other cows were treated. The clinical and bacteriological methods used in this study are described in a previously reported study (Mateus *et al.*, 2002).

In both experiments, blood samples were collected twice a week from the jugular vein from parturition until



**Figure2** - Plasma total Ph and Mg concentrations in the postpartum period of dairy cows (n = 26). No differences were observed among sampling days (P > 0.05, MANOVA with repeated measures).

the sixth week postpartum (Experiment 1) or from a minimum of two weeks before parturition until the sixth week postpartum (Experiment 2). In Experiment 1, blood samples were collected into heparinized tubes (NH4-heparin, Monovette, Sarstedt, Nümbrecht, Germany), transported at 4 °C to the laboratory within three hours



**Figure 3** – Serum total Ca and Ph concentrations in the peripartum period of dairy cows ( $n = 14$ ) that spontaneously recovered from puerperal endometritis. The effect of sampling day was significant ( $P < 0.00001$ , MANOVA with repeated measures) for the serum total Ca concentrations. No significant effects were observed for the serum total Ph concentrations ( $P > 0.05$ ).

and centrifuged at 3,000 rpm during 10 minutes at 4 °C. In experiment 2, blood samples were collected into serum tubes (Serum Z, Monovette, Sarstedt, Nümbrecht, Germany), transported at 4 °C to the laboratory within three hours and allowed to form a blood clot. The tubes were then centrifuged at 3,000 rpm during 10 minutes at 4 °C. The plasma and serum samples were aliquoted into 1.5 mL micro tubes (Sarstedt, Nümbrecht, Germany) and stored at –20 °C until assay.

In Experiment 1, plasma total Ca, Ph and Mg concentrations were measured and in Experiment 2, serum total Ca and Ph concentrations were measured. The plasma or serum Ca:Ph relation was calculated by dividing the Ca value by the corresponding Ph value. The Ca, Ph and Mg concentrations were measured by a colorimetric test, using commercial kits (Spinreact, Reactivos Spinreact, SA, Girona, Spain), according to the manufacturers instructions. The sample optical density was measured in a spectrophotometer (Spectronic 20 Genesis, Spectronic Instruments, USA) at a wavelength of 570 nm, 710 nm and 520 nm for the Ca, Ph and Mg determinations, respectively. The results were expressed in mg/dL units. All samples were assayed in duplicate. The intra-assay coefficients of variation for the Ca, Ph and Mg assays were 4.9 %, 2.3 % and 2.4 %, respectively. The inter-assay coefficients of variation for the Ca and Ph assays were 13.0 % and 1.2 %, respectively. This latter coefficient of variation was determined in sessions ( $n = 13$ ) where control solutions with known Ca and Ph concentrations, available commercially (Unitrol, Biomérieux, Marcy-L'Étoile, France), were run. The Mg concentrations were determined in a single session.

Data were computed by a statistical software package (Statistica 5.0, Statsoft) and analysed by general linear models, multivariate analysis of variance (MANOVA) with repeated measures, considering as fixed effects, a between-group (control versus mild endometritis versus severe endometritis in Experiment 1 and mild endometritis versus heavy endometritis in Experiment 2) effect and a within-group (sampling day) effect. The levels of the repeated measures effect were  $n = 11$  and  $n = 17$  for Experiments 1 and 2, respectively. These levels

represent the minimum number of sampling days that all cows experienced. Significant effects were further analysed by least significant differences. The assumptions of the analysis of variance were tested by the software functions (Bartlett test, normal distribution, correlation between means and standard deviations). In Experiment 1, the number of cows with Ca concentrations  $< 8.0$  mg/dL was compared among groups by Chi-square tests in contingency tables.

## Results

Table 1 shows the results of the multivariate analysis of variance with repeated measures of Experiments 1 and 2. In Experiment 1, for the plasma Ca concentrations, the between-group effect was significant ( $P < 0.0001$ ). For the plasma Ph and Mg concentrations and for the relation Ca:Ph there were no significant effects. Figure 1 shows the profiles of plasma Ca concentrations for groups of Experiment 1. Overall the sampling period, plasma Ca concentrations  $< 8.0$  mg/dL were present in 0, 4 (36 %) and 6 (75 %) cows with normal puerperium, mild endometritis or severe endometritis, respectively. The difference between the groups control and severe endometritis tended to be significant ( $P < 0.06$ ). As no significant group effect was observed on the Ph and Mg concentrations, the respective values of the three groups were combined and the profiles are shown in Figure 2. For the groups control, mild endometritis and severe endometritis, the range of the measured values, in mg/dL units, were 8.0-11.3, 7.2-11.6 and 6.7-10.1 for Ca values, 4.7-8.7, 3.7-9.4 and 4.0-9.5 for Ph values and 1.8-2.5, 1.7-2.3 and 1.8-2.4 for Mg values, respectively. The range of the calculated Ca:Ph relation values was 1.04-1.96, 0.93-2.79 and 0.82-2.24 for the groups control, mild endometritis and severe endometritis, respectively.

In Experiment 2, only the within-effect (sampling day) of the serum Ca concentrations was significant ( $P < 0.00001$ ). Figure 3 shows the profile of serum Ca and Ph concentrations of the 14 cows (the group-effect was not significant, so the values of both groups were combined). Serum Ca concentrations were significantly higher before parturition than after parturition. The range of the Ca and Ph concentrations in mg/dL units and of the Ca:Ph relation values were 6.1-11.7, 3.1-8.8 and 1.0-2.6, respectively. During the sampling period, 13 (93 %) cows presented serum Ca concentrations  $< 8.0$  mg/dL.

## Discussion

The cows used in Experiment 1 correspond to those used in a previously reported study (Mateus *et al.*, 2002). In that study we found that the puerperal endometritis significantly retarded the uterine involution and induced abnormal patterns of postpartum ovarian activity. Here we present the Ca, Ph and Mg profiles of those animals. We observed that the plasma Ca concentrations were significantly lower in cows with severe endometritis than

in cows with mild endometritis and than in control normal puerperium cows. Also, cows with endometritis presented plasma Ca concentrations indicative of subclinical hypocalcemia throughout the sampling period, which was not observed in normal puerperium cows.

These findings show a relationship between blood Ca concentrations and the establishment and severity of the puerperal endometritis. Titterton and Weaver (1999) reported a significant relationship between serum Ca concentrations and the severity of the postpartum vaginal discharge. In cows with milk fever the uterine involution between 15-32 days postpartum was retarded, compared to control cows (Risco *et al.*, 1994). Also, Kamgarpour *et al.* (1999) observed that the interval from parturition until the complete involution of the uterus was negatively and significantly correlated with the mean plasma Ca concentrations. Altogether, these observations suggest that blood Ca concentrations below the physiologic range affect the normal uterine involution and predispose the uterus to infection. This might be explained by the relevant role of Ca in myometrial contraction in the postpartum period, which promotes the uterine clearance of the lochia.

The blood Ph and Mg concentrations did not show significant changes in the postpartum period and were not associated to the presence of endometritis. We could not find any reference relating the blood concentrations of Ph with the puerperal uterine infection. Erb *et al.* (1985) reported that blood Mg concentrations were related to uterine involution. However, Titterton and Weaver (1999) did not find a significant association between serum Mg concentrations and the vaginal discharge and the uterine tone, which supports our results.

Borsberry and Dobson (1989) and Risco *et al.* (1994) reported that cows with milk fever had extended calving to conception intervals. Kamgarpour *et al.* (1999) observed that the mean size of the first ovulated follicle was significantly smaller and the mean number of ovulatory-size follicles was significantly lower in cows showing episodes of subclinical hypocalcemia than in normocalcemic cows. The cows with severe endometritis of Experiment 1 showed a higher incidence of abnormal ovarian activity patterns (delayed anestrus, prolonged luteal phases and ovarian cysts) than the control normal puerperium cows (Mateus *et al.*, 2002). Whether the effect of the subclinical hypocalcemia acts directly on the ovarian function or indirectly through the uterine infection cannot be depicted from this study.

The results here reported suggest that, in dairy cows, the establishment of puerperal endometritis is associated with a subclinical hypocalcemia and that the severity of the infection is related to the blood total Ca levels.

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