

# Evaluation of Peripartal Calcium Status and Neutrophil Function of Dairy Cows of Low or High Risk of Developing Uterine Diseases

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## Introduction

Objectives were to establish the relationships among postpartum serum calcium (Ca) status, neutrophil function, and incidence of uterine diseases in dairy cows considered to be of low (LRM) or high risk (HRM) of developing metritis. Our hypothesis was that subclinical hypocalcemia (SCH) impairs *in vitro* neutrophil function and increases the incidence of metritis in early lactation in both LRM and HRM cows.

## Materials and Methods

In this prospective cohort study, 55 Holstein cows considered to be HRM based on having one or more calving problems (dystocia, twins, stillbirth, and/or retained placenta) were matched with 55 LRM (no calving problems) herdmates based on day of calving and parity. Cows were monitored daily for rectal temperature, vaginal discharge, and attitude score for the first 12 days postpartum (dpp). Cows with a fetid, watery discharge, regardless of temperature during the first 12 dpp, were classified as having metritis. Blood was collected and neutrophils analyzed for their phagocytic and oxidative burst activities at 0, 1, and 3 dpp using a dual flow cytometric assay. Blood was also collected at 0, 1, 2, 3, 4, 7, and 12 dpp and analyzed for serum concentrations of Ca. Ovaries were scanned by ultrasonography at 24, 31, and 38 dpp to determine ovulation based on presence of a corpus luteum. Clinical endometritis (CE) was assessed based on vaginal mucus with pus at 31 dpp. Appropriate models for each of the variables of interest were fitted using PROC GLIMMIX in SAS (2001). Receiver operator characteristic curves were used to determine the ideal cut point for serum Ca to define SCH using metritis as the response variable.

## Results

Concentrations of serum Ca reached a nadir at 2 dpp and increased after that. Subclinical hypocalcemia was defined as serum Ca concentration  $\leq 8.59$  mg/dL in at least one sample in the first 3 dpp. This cut point predicted metritis with 88.5% sensitivity and 55.2% specificity (area under the curve = 0.77, 95% CI=0.68-0.84;  $P<0.001$ ). The overall incidence of metritis was 47.3%. Cows with SCH had lower ( $P<0.001$ ) concentration of

neutrophils in blood compared with normocalcemic (NC) cows ( $3.0 \pm 0.2 \times 1,000$  vs  $4.5 \pm 0.3 \times 1,000$  neutrophils/uL), and had a lower ( $P<0.05$ ) percentage of neutrophils with oxidative burst in the first 3 dpp compared with NC cows ( $38.7 \pm 2.0$  vs  $45.4 \pm 2.7\%$ ). Cows with SCH had higher ( $P<0.001$ ) mean rectal temperature during the first 12 dpp compared with NC cows. Furthermore, cows with SCH had greater ( $P<0.01$ ) risk of developing fever (ARR=2.42; 95% CI=1.16-5.03) and metritis (ARR=3.24; 95% CI=1.51-6.95) compared with NC cows. The association between SCH and metritis was observed in LRM and HRM cows. Among cows in the LRM, those with SCH had an increased incidence of metritis compared with NC cows (40.7% (11/27) vs 14.3% (4/28), respectively). Similarly, among HRM cows, those with SCH had a greater incidence of metritis compared with NC cows (77.8% (35/45) vs 20% (2/10), respectively). The odds of developing metritis decreased (AOR=0.38; 95% CI=0.16-0.90) for every 1 mg/dL increase in Ca change (e.g. a drop in 1 mg/dL vs no change in serum Ca), calculated as the difference of Ca concentration from calving to the lowest value in the first 3 dpp. Cows diagnosed with metritis had lower ( $P<0.01$ ) serum Ca concentrations compared with healthy cows in the first 4 dpp ( $8.56 \pm 0.08$  vs  $8.94 \pm 0.08$  mg/dL), which remained lower throughout the 12-day period. Metritis or SCH did not influence resumption of cyclicity at 38 dpp, but metritis predisposed ( $P<0.01$ ) cows to have clinical endometritis. Finally, the population attributable risk for a cow to develop metritis because of SCH, defined as the reduction in the incidence of metritis that would be observed if the population was entirely NC, was 66.6% in the present study.

## Significance

These findings indicate that cows with subclinical hypocalcemia (defined as serum Ca  $\leq 8.59$  mg/dL in at least one day in the first 3 dpp) had lower concentrations of neutrophils in blood, impaired neutrophil function on 1 and 3 dpp, and increased incidence of metritis compared with normocalcemic cows. Based on the calculated population attributable risk, maintaining serum Ca above 8.60 mg/dL in the first 3 dpp, or preventing a reduction in serum Ca concentrations in the first 3 dpp are expected to markedly reduce the incidence of metritis in lactating dairy cows.