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The responsiveness of subclinical endometritis to a nonsteroidal antiinflammatory drug in pasture-grazed dairy cows

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ABSTRACT

The objective of this study was to determine if the inflammation associated with subclinical endometritis (SCE) is a part of the mechanism by which reproductive performance is reduced in cows with this disease. If it is, reducing inflammation associated with SCE with a nonsteroidal antiinflammatory drug (NSAID) should reduce the severity [as measured by average polymorphonuclear cell (PMN) percentage] of uterine pathology and improve reproductive performance. It was also investigated whether the NSAID treatment reduced metabolic indicators of systemic inflammation previously reported to be altered in cows with SCE. Holstein-Friesian and Friesian-Jersey cross dairy cows (n = 213) were paired by calving date and d-14 uterine PMN percentage and randomly assigned to 3 injections at intervals of 3 d of an NSAID (1.4 mg of carprofen/kg; n = 104) between 21 and 31 d postpartum or left as untreated controls (n = 109). Cows with ≥14% PMN (upper quartile of PMN percentage) in the cytological sample collected at d 14 postpartum were defined as having SCE. The average d-14 PMN percentage was low (9.9%) and a high self-cure rate of SCE (>90%) at d 42 was observed. Treatment with an NSAID reduced plasma concentrations of aspartate aminotransferase and increased pregnancy rate in SCE cows. However, no effect of the NSAID treatment was observed on PMN percentage at d 42, postpartum anovulatory interval, or milk production. Compared with cows without SCE, cows with SCE had lower plasma albumin concentration, albumin:globulin ratio, and body condition score, but higher nonesterified fatty acids on the day of calving. These results indicate that cows with SCE are experiencing a physiological dysfunction, including lower body condition, liver dysfunction, and greater metabolic challenge during the periparturient period. Further research is required to determine the effect of NSAID on SCE and to evaluate the influence of timing of drug application on treatment effectiveness.
Key words: dairy cow, subclinical endometritis, antiinflammatory, Carprofen

INTRODUCTION

Seasonal, pasture-based dairy cows are constrained to a 365-d intercalving interval, leaving 83 d from calving to reestablish pregnancy (Rhodes et al., 2003). A rapid restoration of the uterus to a reproductively capable state after calving is, therefore, critical. Uterine disease, such as subclinical endometritis, impedes uterine recovery. Subclinical endometritis (**SCE**) is a uterine disorder characterized by an increased proportion of polymorphonuclear cells (**PMN**) in the uterus after calving (Barlund et al., 2008; Sheldon et al., 2009; Dubuc et al., 2010). Subclinical endometritis has been reported to be a significant problem in both pasture-based and TMR-based dairy systems, with incidence ranging from 6 to 53% (Gilbert et al., 2005; Green et al., 2009). Negative effects of SCE on reproductive performance include a longer postpartum anovulatory interval (**PPAI**), lower first-service conception rate and overall pregnancy rate, and more services per conception (Gilbert et al., 2005; Barlund et al., 2008; Burke et al., 2010); lower milk production and altered milk composition have also been reported (Green et al., 2009; Burke et al., 2010; McDougall et al., 2011).

The cause of SCE and mechanistic links to reduced fertility are not well understood. Subclinical endometritis may result from bacterial infection, with LPS disruption of the hypothalamic-ovarian axis and uterine secretions reducing reproductive performance (Battaglia et al., 1999; Herath et al., 2007). Recent indications, however, are that PMN percentage-defined

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SCE in pasture-grazed dairy cows is associated with liver dysfunction and systemic inflammation, and that it is the inflammation (as assessed by PMN percentage) associated with endometritis and not necessarily the presence of bacteria in the uterus that causes the negative effect on reproductive performance (Green et al., 2009; Burke et al., 2010; McDougall et al., 2011). A stronger association between poor reproductive performance and inflammation in SCE cows, rather than bacteria, was also reported by Barański et al. (2012) in a TMR-based system. Indicators of liver dysfunction and systemic inflammation reported for SCE cows include a lower albumin:globulin ratio and elevated plasma concentrations of aspartate aminotransferase (**ASAT**) and glutamate dehydrogenase (**GDH**; Bertoni et al., 2008; Burke et al., 2010). This association of SCE with inflammation (both systemic and uterine), rather than uterine bacteria, indicates that treatment of SCE may need to focus on reducing inflammation rather than eliminating uterine bacteria, a case for the use of a nonsteroidal antiinflammatory drug (**NSAID**). This is supported by the lack of a consistent beneficial treatment effect of antibiotics (Kasimanickam et al., 2005; Galvão et al., 2009b) and prostaglandins (Kasimanickam et al., 2005; Galvão et al., 2009a).

To test the role of local (and systemic) inflammation associated with SCE in reducing reproductive performance, an NSAID was used in this study. One pathway through which NSAID reduces inflammation is the cyclooxygenase enzyme-prostaglandin pathway. The NSAID inhibits the action of cyclooxygenase and prevents the secretion of prostaglandin, a proinflammatory molecule (Sordillo et al., 2009; Erdem and Guzeloglu, 2010; Heuwieser et al., 2011). The timing of NSAID treatment is an important consideration, however, because prostaglandin-mediated inflammation is a normal part of the uterine involution process after calving (Barlund et al., 2008). For this reason, NSAID treatment was delayed until 21 d postpartum in the current study.

It was hypothesized that treatment with NSAID between 21 and 31 DIM would reduce the severity (average PMN percentage) of uterine pathology at 42 d postpartum without lengthening the PPAI and would mitigate the negative association of SCE on reproduction and milk production by reducing inflammation and improving liver function. Additionally, the effect of the NSAID treatment on circulating metabolites and minerals previously reported to be altered in cows with SCE was investigated. The objectives of this study were to determine the effect of an NSAID on PMN percentage and associated effects on reproduction, metabolic indicators, and milk production.

MATERIALS AND METHODS

Experimental Design

Multiparous cows (n = 213; 136 Holstein-Friesian and 77 Holstein-Friesian × Jersey) aged 5.4 ± 2.2 yr (±SD) and with a mean BW of 445 ± 56.3 kg were enrolled at Scott Farm (DairyNZ, Hamilton, New Zealand; 37°47′S, 175°19′E) between May and October 2011. Primiparous cows were not included due to a lack of availability at the research farm. The number of cows required for the NSAID and control groups (n = 100) to detect (80% power, 5% significance) a one-third reduction in PMN percentage (e.g., a reduction from 15 to 10% PMN) from d 14 to 42 cytology was calculated using a standard deviation of 0.45 ($\log_{10}$ of PMN percentage; S. Meier, unpublished data) and a detectable difference of 0.18 (difference between the d 14 and 42 $\log_{10}$ PMN percentage counts). Prior approval for animal use was obtained from the Ruakura Animal Ethics Committee (Hamilton, New Zealand; no. 12294).

To ensure that the control and NSAID treatment groups were balanced for PMN percentage, a uterine cytology sample was taken on d 14 to 17 postpartum (d-14 PMN percentage). Cows were blocked on calving date and d-14 PMN percentage before being randomly allocated to either the control (n = 109) or the NSAID (n = 104) treatment group. To allow time for involution to proceed unimpeded, the NSAID treatment was initiated at 21 to 25 d postpartum. The cows in the NSAID treatment group (Carprieve LA; Norbrook New Zealand Ltd., Auckland, New Zealand; 50 mg of carprofen/mL) were given 3 injections (each injection = 1.4 mg of carprofen/kg of BW; 1 mL of carprofen/35 kg of BW) at intervals of 3 d, between 21 and 31 d postpartum. The rationale for this treatment regimen was to ensure an extended period of coverage, as carprofen has a plasma elimination half-life of 45 to 70 h (Ludwig et al., 1989; Norbrook Laboratories, 2011).

All cows were examined using the Metricheck procedure (McDougall et al., 2007) at d 14 and 42. A Metricheck score of ≥3 was determined in 22 cows at d 14 and in 4 cows at d 42. None of these cows received antibiotic treatment and all were included in the analyses. Cows with calving difficulty (n = 1), retained fetal membranes (n = 4), or milk fever (n = 12) were included. Cows (n = 2) were excluded if they received a systemic antibiotic during the experiment.

Uterine Cytology

Uterine endometrial cytology samples were collected on d 14 to 17 (d-14 PMN percentage) and d 42 to 45 (d-42 PMN percentage) postpartum as described by Burke