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Associations between activity of arginase or matrix metalloproteinase-8 (MMP-8) and metritis in periparturient dairy cattle☆,☆☆

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ABSTRACT

Metritis, a uterine disease caused by bacterial infection, is highly prevalent in dairy cattle after parturition. Uterine disease has negative effects on milk production and reproductive efficiency. Finding markers or indicators that can predict cows at greater risk for uterine disease could be beneficial to mitigating these deleterious effects. This study investigates the immune-derived enzymes arginase and matrix metalloproteinase-8 (MMP-8) as potential markers for development of metritis in dairy cows. In a retrospective matched case-control study, 53 lactating Holstein cows diagnosed with metritis were matched and paired to 53 lactating Holstein control cows. In addition to examining cows for diagnosis of metritis on d 4, 7, 10, and 14 after parturition, occurrence of retained fetal membranes, gender of the calf, and the event of a stillbirth were recorded. Blood samples were collected 7 ± 3 d before calving, on the day of calving, and 7 ± 3 d after calving and were assayed for activity of arginase and MMP-8. Associations between metritis and activity of arginase or MMP-8 were determined by conditional logistic regression at each individual sampling time point. An interaction between activity of arginase, before and on the day of parturition, and retained fetal membranes tended ($P \leq 0.13$) to be associated with metritis. After parturition, activity of arginase and the interaction between activity of arginase and retained fetal membranes were not ($P \geq 0.22$) associated with metritis. Activity of MMP-8 was not ($P \geq 0.20$) associated with metritis in the periparturient period. Retained fetal membranes were associated with the odds of developing metritis. Activity of arginase before and at the time of parturition might be a potential marker for occurrence of metritis, especially in cows that develop retained fetal membranes. MMP-8 does not seem to be a potential indicator for metritis.

1. Introduction

Dairy cattle that have uterine disease after parturition have decreased milk production [1,2] and reproductive performance [3]. The two major uterine diseases during the early postpartum period are retained fetal membranes and metritis. Retained fetal membranes is usually defined as the failure to expel fetal membranes by 24 h after parturition [4,5]. Considering that 95% of cows that pass fetal membranes by 24 h had passed membranes by 12 h postpartum, defining the disorder at 12 or 24 h after calving is not relevant [6,7]. Puerperal metritis is defined as a cow having an enlarged uterus with the presence of foul red-brown uterine

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discharge, with decreased milk production, dullness, and fever within 21 d after parturition [8]. Difficult calving or dystocia increases the risk of retained fetal membranes and uterine disease in cows [9]. Factors that can increase the likelihood of cows having dystocia include gender of the calf, stillbirth event (death within 24 h of birth), and twin births [10].

Predicting whether cows will have postpartum uterine disorders may allow implementation of prevention strategies to decrease the negative effects caused by uterine disease on performance. Prepartum dry matter intake [2] and postpartum concentrations of the acute phase protein haptoglobin [11] are associated with metritis risk in dairy cattle. Decreased dry matter intake as early as 2 wk prepartum is associated with an increased risk of metritis [2]. Furthermore, cows with concentration of haptoglobin $\geq$1 g/L 3 d after parturition have 6.7 times greater risk of developing metritis than cows with a concentration <1 g/L [11]. Nevertheless, dry matter intake and concentration of haptoglobin are associated with metritis risk, but poor predictors despite these observed associations.

It is well documented that dairy cows undergo immunosuppression during the periparturient period [12]. Level of immunosuppression could be a factor that influences postpartum health in dairy cattle because neutrophil function is associated with postpartum uterine diseases. In dairy cattle, retained fetal membranes is believed to be a result of decreased neutrophil function and decreased serum concentrations of interleukin-8 [13]. In addition, the cotyledonary placenta of cattle requires release of collagenase from neutrophils to breakdown the microvilli interaction of the cotyledon and caruncle and allow release of the fetal membranes [14]. In dairy cattle, reduced myeloperoxidase activity of neutrophils [15] and increased serum concentrations of tumor necrosis factor-α and interleukin-6 [16] have been reported to be associated with metritis. Arginase and matrix metalloproteinase-8 (MMP-8) are two enzymes produced by polymorphonuclear neutrophils and are released at sites of inflammation [17,18]. Expression of arginase and depletion of L-arginine are signs of immunosuppression [19]. In mice, MMP-8 is essential for a lipopolysaccharide-induced inflammatory response, chemokine production, and is an indicator of neutrophil function [20]. In cattle, gram negative bacteria invade the uterus within 2 wk of calving [21]. Gram negative bacteria can cause metritis via a lipopolysaccharide-induced inflammatory response via the TLR4/CD14/MD-2 receptor complex [21,22]. Because neutrophil function is associated with uterine disorders, activity of enzymes produced by neutrophils, such as arginase and MMP-8, might be potential candidates for indicators or predictors of uterine disease.

The hypothesis of the current study was that activity of arginase and MMP-8 during the periparturient period would be associated with development of metritis in dairy cattle. The objectives of the current study were to (1) quantify activity of arginase and MMP-8 in maternal blood plasma samples during the periparturient period in dairy cattle and (2) determine whether activity of arginase and MMP-8 during the periparturient period are associated with the risk of developing metritis.

2. Materials and methods

2.1. Study design and sampling

This study was a retrospective matched case-control study. Blood samples collected from cows in a previous experiment [23] were used in this study. The experiment [23] was conducted in one commercial dairy herd located in northwestern Wisconsin. Holstein animals housed in naturally ventilated free-stall barns were enrolled in the study at 258.3 $\pm$ 0.2 d of gestation (mean $\pm$ SEM). Nulliparous heifers were housed in separate pens from primiparous and multiparous cows before calving. Animals were fed the same prepartum TMR, except that nulliparous heifers were not supplemented with anionic salts. Animals demonstrating signs of calving (e.g., discomfort, restlessness, tail twitching, and visualization of the allantoic sac through the vulva) were moved to a loose housing pen. After parturition cows were moved to one free-stall pen regardless of parity and were fed the same postpartum TMR. Fifty-three cows diagnosed with metritis were matched and paired to control cows by parity and date of calving. Cows (n = 106) were examined 4, 7, 10, and 14 d after calving for diagnosis of metritis. In addition, cows were examined in the first 24 h for occurrence of retained fetal membranes. Furthermore, gender of the calf and the event of a stillbirth were recorded. Blood samples were collected -7 ± 3 d before parturition, within 24 h of calving (study d 0), and 7 ± 3 d after parturition. Blood samples were collected from a coccygeal vessel into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and placed on ice after collection until centrifugation (1200 x g for 15 min at 4 °C). Blood plasma was separated, aliquoted to microcentrifuge tubes, and stored at −32 °C or −20 °C until analysis. Body condition score was assessed 21 d before and at calving on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) with 0.25 increment [24].

2.2. Arginase and MMP-8 assay

Enzymatic activities of arginase and MMP-8 were quantified using two nanoplatforms that were previously developed by researchers at Kansas State University (Manhattan, KS) [25,26].

Briefly, dopamine-coated Fe/Fe3O4 core/shell nanoparticles with covalently attached fluorescent ligands were synthesized for detecting MMP-8 (consensus sequence: GAGPSG-LRGAG) and arginase I+II (tether: GRRRRRRRG) in aqueous buffer solutions. Each nanoplatform featured a Foerster donor-acceptor pair. The donor was attached to Fe/Fe3O4 via a tether, whereas the acceptor was directly bound to dopamine. This design enabled a subfemtomolar limit of detection of MMP-8, whereas arginase could be detected in the picomolar range. The upper bound for both enzymes was in the micromolar range. Fe/Fe3O4 − based nanoplatforms were dispersed in HEPES buffer solution to form the assay solution (0.3 mg/ml). Next, 5 μL of sample plasma and 125 μL of assay solution were added to a 96-well plate in triplicate. Furthermore, a sample control consisting of 5 μL of sample plasma and 125 μL of HEPES buffer was also added to the 96-well plate for each sample. Plates were analyzed using a Synergy H1 Plate Reader (BioTek Instruments Inc., Winooski, VT). A spectral scan from 600 nm to 700 nm with 2 nm step increment was performed, in accordance with standard procedures. Activities of arginase and MMP-8 were quantified by calculation of the area under the curve from 620 nm to 680 nm for each sample and subtracting the area under the curve of the sample control. All samples were then divided by an assay control to standardize the values across assay. Activities of arginase and MMP-8 are reported in relative florescence units (RFU). Fold-change in activity of arginase and MMP-8 were calculated by dividing the activity before parturition by the activity after parturition, as well as dividing the activity at parturition by the activity after parturition. Fold-change in activity was calculated in order to evaluate differences in activity of arginase and MMP-8 over time.

2.3. Statistical analyses

Descriptive statistics for the activity of arginase and MMP-8 for cows with and without metritis were analyzed by ANOVA for