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Dynamics of postpartum endometrial cytology and bacteriology and their relationship to fertility in dairy cows

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ABSTRACT

Endometrial samples were obtained from 56 consecutively calving dairy cows examined for endometrial cytology and for aerobic and anaerobic bacterial growth. Changes over time, correlations between different cell types and between cell and bacterial populations and with fertility measures were calculated. The proportion of neutrophils in cytologic preparations decreased with time postpartum. Other cell types did not change significantly with time. The proportion of neutrophils early (Day 0 and 7) postpartum was negatively correlated with neutrophil proportion at 5 or 7 weeks postpartum and positively correlated with fertility. Cows with high proportion of neutrophils at 7 days postpartum (>40%) were significantly more likely to become pregnant than those with lower proportions of neutrophils. *Escherichia coli* were the bacteria most frequently isolated at 0 or 7 days postpartum but were uncommon after that. *Trueperella pyogenes* were most prevalent at 3 weeks postpartum and were more likely to infect cows that had previously been infected with *E coli*. The presence of *T pyogenes* at 3 weeks postpartum increased the risk of concomitant or later infection with gram-negative anaerobes. The presence of *T pyogenes* at 3 weeks postpartum significantly reduced the risk of pregnancy at 150 days in milk. The presence of alpha-hemolytic *Streptoccus* spp. at 7 days postpartum was associated with improved reproductive performance. The proportion of neutrophils at 5 and 7 weeks postpartum was related to concomitant bacterial infection. These findings suggest that rapid mobilization of neutrophils to the postpartum uterus is a beneficial response for uterine health in dairy cows.

1. Introduction

Prevalence of endometritis, as diagnosed by endometrial cytology, is high in dairy cows, persisting up to and beyond the end of the traditional postpartum voluntary waiting period. Several studies have confirmed a high-prevalence of endometritis after 40 days postpartum and that the condition has a negative impact on subsequent reproductive performance [1–7]. In contrast, however, most publications maintain that the bovine uterus, although consistently contaminated or infected in the early postpartum period, is essentially sterile by about 28 days postpartum [8–11]. The distinction between proportion of infected cows and cows with subclinical endometritis is well documented by Sheldon et al. [9]. Therefore, one aim of this study was to examine the relationship between bacterial infection and cytologic evidence of endometritis and to determine if endometritis persisted after elimination of bacterial infection.

The relationship between persistent bacterial infection and inflammation is important in devising strategies for prevention and treatment of endometritis. For example, antibacterial treatments are unlikely to be effective if the inflammation occurs in the absence of bacterial infection or

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persists long after the infection is resolved. This study was undertaken to further understand the relationship between uterine infection and evidence of endometrial inflammation (measured by endometrial cytology) and the relationships between uterine infection, endometritis, postpartum endometrial cytology, and fertility in lactating dairy cows. We postulated that uterine bacterial populations would account for observed inflammation and that inflammation (and infection) would steadily decrease with time postpartum.

The relationship between bacterial infection and endometritis, which is often subclinical, could also cast light on the pathogenesis of endometritis. For example, is endometritis largely a reflection of a generalized inflammatory environment attributable to a form of metabolic syndrome (extensive fat mobilization and insulin resistance) that is often recognized in postpartum cows [12], or is it more simply a consequence of local bacterial infection (which itself may follow more generalized immune impairment [13])?

Finally, characterization of the relationship between uterine infection and endometritis could cast light on the mechanism of infertility in endometritis. Do bacteria play a direct role in altering the uterine environment, rendering it hostile to spermatozoa or the developing zygote, or is infertility mediated by more subtle changes brought about by inflammatory mediators at a uterine [14,15] or ovarian [15–17] level?

Although cross-sectional studies of endometrial cytology abound, few have examined cows in a longitudinal fashion and those that have, have concentrated on cows later in lactation, e.g., at 35 and 56 days postpartum [2,18] or at 3, 5, and 7 weeks postpartum [4]. We therefore examined endometrial cytology beginning immediately postpartum and continuing until 49 days postpartum. Knowledge of the progression of endometrial cytology is important for identifying periods in which clinically significant inflammation can be diagnosed and distinguished from the physiological inflammation, which occurs postpartum and is necessary for the extensive uterine tissue remodeling required during uterine involution for return to fertility.

Our goals therefore were to characterize the progression of endometrial cytology in postpartum dairy cows and to evaluate the relationship between uterine bacterial infection and endometrial cytology. The relationship between endometrial cytology or uterine bacterial infection at different postpartum periods and subsequent fertility was also investigated.

2. Materials and methods

2.1. Animals and animal procedures

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee (protocol 2004–0078).

Consecutively, calving cows (n = 56), calving over a 12-month period in 2004 to 2005, in the College of Veterinary Medicine Dairy Herd were enrolled in this experiment. Endometrial samples were obtained by low-volume uterine lavage on the day of calving and 7, 21, 35, and 49 days postpartum as previously described [6]. Briefly, a sterile plastic infusion pipette contained in a chemise was passed through the cervix under transrectal control and 20 mL sterile saline injected into the uterine lumen, gently agitated, and aspirated. Recovered fluid was used for aerobic and anaerobic bacterial culture by thoroughly wetting a Tran-Swab culturette (Fisher Scientific; Pittsburgh, PA, USA) and a Port-a-cul (Becton Dickinson, Franklin Lakes, NJ, USA) swab and submitting them to the Animal Health Diagnostic Laboratory at Cornell University for aerobic and anaerobic bacterial culture, respectively. Remaining fluid was used for cytologic evaluation of the uterus. An aliquot of fluid was transferred to a glass microscope slide using a cytocentrifuge, air dried and stained using a Romanowski staining technique (Diff Quik, VWR, Arlington Heights, IL, USA) [6]. Note that on the day of calving, it was usually not necessary to inject fluid into the uterus before aspirating a sample. A pipette could simply be introduced and uterine content aspirated for further processing.

2.2. Bacterial culture

Bacterial samples were cultured at the Animal Health Diagnostic Laboratory as follows. For aerobic culture, the swabs were inoculated onto the following agar plates: Blood Agar, Chocolate Agar, Eosin Methylene Blue Agar, and Columbia Colistin Nalidixic Acid Agar and struck for isolation of bacteria. The plates were incubated at 35 °C with 6% carbon dioxide overnight before being examined. Individual bacterial colonies were identified using biochemical methods or Trek automated ID or MALDI-TOF biotyper.

The anaerobic cultures were performed in an anaerobic chamber on *Brucella* agar, Phenylethyl alcohol agar, *Bacteroides* bile esculin agar (BBE), and *Brucella*-laked blood agar with kanamycin and vancomycin at 35 °C. Individual bacterial colonies were identified using biochemical methods and/or MALDI-TOF biotyper.

2.3. Endometrial cytology

Endometrial cytology was evaluated by a single examiner blinded to the source of the sample. Nucleated cells (n = 200) were identified as endometrial epithelial cells, polymorphonuclear cells (presumptive neutrophils), large mononuclear cells (presumptive macrophages), and small mononuclear cells (presumptive lymphocytes). Examination was performed independently by both authors using bright field microscopy at × 400 magnification, and the mean result used for analysis [1,4,6,7,19–24]. Progression of cell population with days postpartum was evaluated by linear regression. The relationship of proportion of each cell type to subsequent reproductive performance (pregnancy to first artificial insemination [AI], pregnancy by 150 days postpartum) was evaluated by calculating spearman correlation coefficients because pregnancy is a dichotomous outcome. Where significant correlations were found, predictive values of selected parameters were chosen by Receiver Operating Characteristic curves. The predictor was then dichotomized at the predictive value with the highest