J. Dairy Sci. 96:1–7
http://dx.doi.org/10.3168/jds.2012-6269

The relationship between endometrial cytology during estrous cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows

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ABSTRACT

The objectives of this study were to assess the effect of the stage of estrous cycle on the percentage of endometrial polymorphonuclear cells (PMN) obtained by cytobrush to determine cutoff values for the diagnosis of subclinical endometritis under pastoral conditions, to measure the prevalence of subclinical endometritis 21 to 62 d in milk (DIM), and to evaluate the effect of subclinical endometritis on reproductive performance in grazing dairy cows. The first experiment was conducted on a commercial dairy farm in Buenos Aires province (Argentina), where 17 postpartum cyclic dairy cows without clinical endometritis were selected and synchronized by Ovsynch protocol. Endometrial cytology (cytobrush technique) and blood (tail vessels) samples were obtained on d 0, 4, 11, and 18 of the estrous cycle (corresponding to estrus, metestrus, diestrus, and proestrus, respectively) and used for measuring percentage of PMN and P_4 concentration, respectively. The percentage of PMN was determined 3 times by blinded count by 2 operators. Data were analyzed with PROC MIXED, PROC GENMOD, and PROC FREQ from SAS 9.1. The percentage of PMN did not vary with the stage of the estrous cycle. In addition, PMN counts were below any of the reported thresholds in this study (4%) for most of the cows. Therefore, the risk for false positive test results as a consequence of physiological changes in the counts of PMN during estrous cycle is low. The second experiment was conducted on 4 commercial dairy farms in Buenos Aires province (Argentina), where lactating Holstein dairy cows (n = 418) 21 to 62 DIM without clinical endometritis were studied. Samples of endometrial cytology were collected with the cytobrush technique. Data were analyzed with receiver operator characteristic curves with Sigmaplot 10.0, and with PROC GLIMMIX, PROC PHREG, and PROC LIFETEST from SAS 9.1. Cutoff values for the diagnosis of subclinical endometritis in grazing dairy cows are 8% PMN for 21 to 33 DIM, 6% PMN for 34 to 47 DIM, 4% PMN for 48 to 62 DIM, and overall 5% PMN for 21 to 62 DIM; the prevalence of subclinical endometritis 21 to 62 DIM was 17%. Finally, subclinical endometritis diagnosed at 21 to 62 DIM decreases the hazard for pregnancy (hazard ratio = 0.668; 95% confidence interval = 0.492–0.909) and increases the calving to conception interval by d 30 compared with normal cows (median 95% confidence interval = 133 vs. 93, respectively).
Key words: dairy cow, subclinical endometritis, cytobrush, estrous cycle

INTRODUCTION

Endometrial cytology is an accepted practice to evaluate the health status of the uterus (Gilbert et al., 1998) because it is quick, specific, and low cost (Gilbert et al., 2005). Subclinical endometritis is defined based on the proportion of polymorphonuclear cells (**PMN**) in endometrial samples (Sheldon et al., 2006). Cytobrush has been described as the best technique for obtaining uterine cytology in cows due to its reliability and lack of cell distortion (Kasimanickam et al., 2005). Flushing the uterus with low volumes of fluids to collect endometrial cells, however, is also a generally accepted technique (Gilbert et al., 2005; Sheldon et al., 2006). It is well known that cows experience many physiological and structural changes during the estrous cycle depending on the prevailing hormonal profile (Ohtani et al., 1993). One of these changes is an increased infiltration of PMN into the endometrium, especially from proestrus through metestrus (Ohtani et al., 1993). To the best of our knowledge, it is unknown if this physiological change in the percentage of PMN may cause false positive diagnoses of endometritis.

Received October 15, 2012.
Accepted March 5, 2013.
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Conversely, an early and accurate diagnosis of subclinical endometritis would allow us to identify cows for treatment (and cure) and recognize cows with compromised fertility (i.e., mild inflammation) and avoid inseminating them. The main problem regarding the diagnosis of subclinical endometritis is that no general consensus exists among researchers about the cutoff values to differentiate diseased from healthy cows. Some researchers have used receiver operator characteristic (**ROC**) analysis to establish cutoff values (Kasimanickam et al., 2004; Galvão et al., 2009), whereas others used quartiles (McDougall et al., 2011) or arbitrary values (Gilbert et al., 2005; Hammon et al., 2006; Plöntzke et al., 2010). In addition, differences in the timing of sampling and in the diagnostic test used for endometritis have been observed, making comparisons among studies almost unfeasible. The reported prevalence of subclinical endometritis ranges from 12% (Barlund et al., 2008) to more than 50% (Gilbert et al., 2005; Hammon et al., 2006; Galvão et al., 2009). Regarding the effect of subclinical endometritis on reproductive performance, some researchers described a negative effect; specifically, 2 studies (using ROC curve to determine cutoff values) reported an increase of 30 to 60 d in the calving to conception interval in cows with subclinical endometritis (Kasimanickam et al., 2004; Galvão et al., 2009). Another study, carried out in repeat breeding dairy cows (also using cutoff values obtained by ROC curve), found a reduction in the conception rate at the next AI from 47 to 5% in cows with subclinical endometritis (Salasel et al., 2010). Conversely, other studies did not find any negative effect (Kasimanickam et al., 2006; Plöntzke et al., 2010).

Finally, most of the cited studies were carried out in dairy cows reared under confinement systems mainly in Europe and North America. Therefore, a lack of information exists about the effect of the estrous cycle on the likelihood for false positive diagnosis of subclinical endometritis, the cutoff values for its diagnosis, and also on its effect on reproductive performance in grazing dairy cows.

Objectives

The objectives of this study were (1) to assess the effect of the physiological changes in the influx of PMN to the uterus through the estrous cycle on the diagnostic output of subclinical endometritis performed by cytobrush, (2) to determine the cutoff values for the diagnosis of subclinical endometritis under pastoral conditions, (3) to determine the prevalence of subclinical endometritis from 21 to 62 DIM, and (4) to evaluate the effect of subclinical endometritis on reproductive performance in grazing dairy cows. The hypotheses to test were (1) that the greater infiltration of leucocytes (i.e., PMN) to the uterus, observed from proestrus through metestrus, would not induce an increase of false positive diagnoses; (2) that the prevalence of subclinical endometritis is lower under grazing situations from what is reported for more intensively and metabolically stressful production systems; and (3) that subclinical endometritis reduces reproductive performance in grazing dairy cows.

MATERIALS AND METHODS

Experiment 1

Animals and Sampling. This study was performed on a commercial dairy farm located in Buenos Aires province (34°56′S, 58°47′W, Argentina) where lactating Holstein cows (n = 53) 27 to 56 DIM were enrolled. Cows with a BCS <2.5, retention of fetal membranes, or abortion were excluded from the analysis. All cows were examined by gloved-hand vaginal inspection and their vaginal discharge (**VD**) was classified as VD-0 (normal clear discharge), VD-1 (clear discharge with flecks of pus), VD-2 (muco-purulent not fetid discharge), and VD-3 (purulent or brown-colored, and fetid; Sheldon et al., 2002; Williams et al., 2005). Cows also had their ovaries scanned by ultrasound (7.5 MHz; Mindray 6600Vet, Nanshan, China) and categorized as cyclic (with corpora lutea or follicles >8 mm in diameter) or acyclic (no corpora lutea and no follicles >8 mm in diameter). Only cyclic cows with normal VD (without pus) were selected (n = 30) and had estrus synchronized with an Ovsynch protocol (d −9 = 8 μg of Buserelin, GnRH; d −2 = 150 μg of Enzaprost, D-Cloprostenol, and d 0 = 8 μg of Buserelin, GnRH; Biogenesis Bagó, Argentina). Cows that were absent for any of the sampling days or ultrasound scanning (n = 4), failed to complete the Ovsynch protocol (n = 5), did not responding to the Ovsynch (n = 3), or those having clinical mastitis (n = 1) were excluded. Therefore, only cows that completed the protocol (n = 17) were sampled from tail vessels for P_4 measurement and from endometrium for cytological evaluation on d 0, 4, 11, and 18 (representing estrus, metestrus, diestrus, and proestrus, respectively).

Progesterone Measurement. Serum was harvested within 2 h postsampling and stored at −20°C until analyzed by RIA with a commercial kit (Coat-A-Count, Progesterone; Diagnostic Product Corporation, Los Angeles, CA). Intra-assay coefficients of variation were 5.14% for the high pool (6.45 ng/mL) and 10.21% for the low pool (0.8 ng/mL).

Cytological Evaluation. Samples of endometrial cytology were collected using a cytobrush modified for