J. Dairy Sci. 97:195–201
http://dx.doi.org/10.3168/jds.2013-6836

Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows

L. V. Madoz,*† M. J. Giuliodori,‡ A. L. Migliorisi,* M. Jaureguiberry,* and R. L. de la Sota*†[1]
*Laboratorio y Servicio de Reproducción Animal, Facultad de Ciencias Veterinarias-Universidad Nacional de La Plata (FCV-UNLP), La Plata, B1900AVW, Argentina
†Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, C1033AAJ, Argentina
‡Cátedra de Fisiología, FCV-UNLP, La Plata, B1900AVW, Argentina

ABSTRACT

The objectives of this study were to assess the agreement between endometrial cytology and uterine biopsy for the diagnosis of subclinical endometritis (SEND) in grazing dairy cows, the interobserver agreement of the biopsy's readings, and the bacterial population isolated from the uterus of cows having SEND. In experiment 1, lactating Holstein cows (n = 44) 31 to 59 d in milk (DIM) at sampling were enrolled. Clinical endometritis was diagnosed by direct evaluation of vaginal discharge and SEND by endometrial cytology evaluation. Two hundred cells per smear were counted to determine the percentage of polymorphonuclear neutrophilic leukocytes (PMNL). Cut-off values used were ≥8% PMNL at ≤33 DIM, ≥6% PMNL at 34 to 47 DIM, and ≥4% PMNL at ≥48 DIM. Biopsies were assessed blindly by 2 observers who categorized them into 4 groups according to their inflammatory changes: none, minimal, moderate, and severe inflammatory changes. Data were analyzed using the kappa coefficient and logistic regression. In experiment 2, lactating Holstein cows (n = 60) 21 to 62 DIM were enrolled. Clinical endometritis and SEND were diagnosed as previously described. Samples were cultured for aerobic and anaerobic bacteria by routine methods of bacteriological testing. Data were analyzed with logistic regression. In experiment 1, little agreement was observed between cytology and biopsy outputs (kappa = 0.151), and strong agreement between the 2 operators (kappa = 0.854). The likelihood of having a normal biopsy (no inflammatory change) was greater for healthy cows than for those having SEND (odds ratio = 13.145). The probability for getting normal uterine tissue decreased 2.1% for every increasing percentage point in PMNL. In experiment 2, no bacteria were isolated from cows with SEND, coagulase-negative staphylococci were commonly isolated from healthy cows, and *Trueperella pyogenes* was frequently isolated from cows with clinical endometritis. The likelihood of isolating *T. pyogenes* from uterine samples increased with the percentage of PMNL (odds ratio = 1.100). In conclusion, biopsy showed low agreement with cytology for the diagnosis of SEND. Nevertheless, fertility trials using uterine biopsies to predict pregnancy outcomes are needed to determine its diagnostic usefulness. Finally, bacteriology would not be recommended as a diagnostic tool because no bacteria were isolated from cows with SEND.

Key words: dairy cow, subclinical endometritis, uterine biopsy, bacteriology

INTRODUCTION

Subclinical endometritis (**SEND**) was first described as cytological endometritis considering the presence of PMNL in the endometrial lumen (Gilbert et al., 1998), and then standardized by Kasimanickam et al. (2004) based on its negative effects on reproductive performance. Thus, those authors stated a threshold of percentage of PMNL, above which animals are diagnosed as having SEND and below which as not having it. Since then, some studies have shown that SEND is a common disease in postpartum dairy cows highly associated with poor reproductive performance (Kasimanickam et al., 2004; Gilbert et al., 2005). No gold standard exists for the diagnosis of SEND, which turns the task into a challenging one. Nevertheless, uterine cytological evaluation is the most used tool for SEND diagnosis (Kasimanickam et al., 2005). Cytobrushing is considered the best technique for obtaining endometrial cytological samples because it is easy and quick to perform (Barlund et al., 2008; Kasimanickam et al., 2005), and it is also safe and effective (Oral et al., 2009).

Other tools used for the diagnosis of uterine diseases in large animals are biopsy and bacteriology (Studer and Morrow, 1978; Dubuc et al., 2010). Uterine biopsies were used initially for the study of infertility in mares (Chapwanya et al., 2010) to predict the ability of the mare to conceive and carry out a new pregnancy (Kenney, 1978). Biopsy provides detailed information about

Received March 20, 2013.
Accepted September 5, 2013.
[1]Corresponding author: dairydoc82@gmail.com

uterine health status, and a 4-point scale has been developed for use in cows (Chapwanya et al., 2009) but, as far as we know, studies are still lacking relating biopsy scores with future fertility of the cow. In addition, only 1 work exists evaluating its use as a diagnostic tool for SEND (Meira et al., 2012).

On the other hand, several bacteriological studies have shown that metritis and clinical endometritis in cows (Messier et al., 1984; Williams et al., 2005; Santos et al., 2010; Westermann et al., 2010) are related to nonspecific mixed infections involving environmental bacteria (Rutter et al., 1999; Sheldon et al., 2002; Petit et al., 2009) that invade the uterus at parturition and immediately after it. Among bacterial species causing endometrial diseases, *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*) and *Escherichia coli* are the most prevalent, but *Prevotella melaninogenica*, *Proteus* spp., and *Fusobacterium necrophorum* have also been reported (Griffin et al., 1974; Williams et al., 2005). So, it is logical to expect the same bacteria to be involved in the pathogenesis of SEND. Whereas several bacteriological studies were carried out in the past to investigate the pathogenesis of puerperal metritis and clinical endometritis (Silva et al., 2009; Bicalho et al., 2010, 2012; Sheldon et al., 2010), to our knowledge, only a few bacteriological studies have been carried out in this regard on SEND, with no conclusive results (McDougall et al., 2011; Barański et al., 2012).

In summary, information is lacking about the usefulness of uterine biopsy for SEND diagnosis and about bacterial population involved in SEND cases in postpartum dairy cows. Therefore, the main hypotheses to test were that a correlation exists between diagnostic outputs obtained by both cytology and biopsy, that high repeatability and agreement exist between biopsy readings, and that the bacterial population involved in subclinical cases is similar to that found in clinical endometritis. So, the objectives of this study were to assess (1) the agreement between endometrial cytology (obtained by cytobrush) and uterine biopsy for the diagnosis of SEND in grazing dairy cows, (2) the interobserver agreement of the biopsy readings, and (3) the bacterial populations isolated from the uterus of cows having SEND.

MATERIALS AND METHODS

Experiment 1

Animals and Evaluation of Vaginal Discharge. The study was performed in the experimental dairy farm of the National University of La Plata (Lomas de Zamora, province of Buenos Aires, Argentina; 34°75′S, 58°46′W) where 44 lactating Holstein cows 31 to 59 DIM at sampling were enrolled. Cows with BCS <2.5 (5-point scale with 0.25 increments; Ferguson et al. (1994), retention of fetal membranes, abortion, or with intrauterine or systemic treatments were excluded from the study. Manual examination of the vagina and withdrawal of the mucus by a gloved hand for direct inspection was performed in all cows. Vaginal discharge (**VD**) was classified as normal clear discharge (**VD-0**); clear discharge with flecks of pus (**VD-1**); mucopurulent, not fetid discharge (**VD-2**); or purulent or brown-colored, and fetid (**VD-3**; Williams et al., 2005). Clinical endometritis was declared in cows having VD-1 through -3.

Cytological Evaluation. Samples of endometrial cytology were collected using a cytobrush modified for use in cattle (Madoz et al., 2013). Briefly, a stainless steel device was attached with a sterile brush (Medibrush XL; Medical Engineering Co. SA, Buenos Aires, Argentina). The device was covered for protection from vaginal contamination with a bovine split universal sheath (IMV Technologies, Paris, France). Once the cervix was passed, the cytobrush was exposed and rolled into the endometrium and then covered again with the protective sheath. Outside the vagina, the cytobrush was removed from the pistol grip and rotated on a microscopic slide. Smears were fixed with spray (Roby; Argencos SA, San Martín, Argentina) to preserve cellular morphology and stained (Tinción 15; Biopur S.r.l., Rosario, Argentina). Evaluations were performed under a microscope at 400× magnification, where 200 cells were counted to determine the percentages of PMNL. Cut-off values for the diagnosis of SEND were ≥8% PMNL at 21 to 33 DIM, ≥6% PMNL at 34 to 47 DIM, and ≥4% at 48 to 62 DIM (Madoz et al., 2013).

Uterine Biopsy. Samples of endometrial tissue were collected using a stainless steel biopsy instrument 53 cm in length, having a jaw with cutting edges of 0.6 × 0.4 cm (Chapwanya et al., 2010). Once the cervix was passed, the device's jaw was opened into the uterine horn and then the jaw was closed and rotated 90 degrees to obtain an endometrial tissue sample. Then, the device was removed from the cow and the sample was placed in a 1.5-mL tube containing a10% formaldehyde buffered solution; then, formalin-fixed tissues were dehydrated through a graded series of ethanol solutions, cleared in acetone, paraffin-embedded, sectioned at 5- to 6-μm thickness, and stained with hematoxylin and eosin. Later, they were evaluated under a microscope at 400× magnification and scored, independently of clinical findings, by the grades of endometrial inflammatory infiltrates. All biopsies were assessed by 2 observers blinded to the cows' identification and categorized with a simplified scale described by Chapwanya et al. (2009), using the following categories: 0 = uterus with no inflammatory infiltrate (**UB-0**), 1 = minimal inflamma-