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A novel diagnostic technique to determine uterine health of Holstein cows at 35 days postpartum

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ABSTRACT

The objectives were (1) to evaluate the association of uterine lavage sample optical density (ULSOD) with uterine health, and (2) to estimate and evaluate a threshold value that will maximize the accuracy of ULSOD as a diagnostic tool for clinical endometritis. The study enrolled 1,742 cows from 3 dairy farms located near Ithaca, New York. The samples were collected at 35 ± 3 d in milk (DIM) by using low-volume uterine lavage. Cows with a purulent or mucopurulent secretion in the sample were diagnosed with clinical endometritis, whereas a subgroup of all studied cows was examined for cytological evidence of inflammation by endometrial cytology. Data for ULSOD measured at different wavelengths (200, 352, 620, 790, 860, and 960 nm) were available for 554 cows; all 1,742 cows had data for ULSOD measured at 620 nm ($ULSOD_{620}$). Incidences of clinical endometritis, metritis, and retained placenta were 10, 15.2, and 5.6%, respectively. The $ULSOD_{620}$ was associated with clinical endometritis. Receiver operating characteristic (ROC) analysis of the accuracy of optical density in the detection of clinical endometritis was conducted for ULSOD measured at different wavelengths; $ULSOD_{620}$ was selected for further analysis because it presented the best ROC curve to detect clinical endometritis. The recommended threshold for $ULSOD_{620}$ ROC was 0.058, where the sensitivity and specificity were 76.3 and 78.3%, respectively. The ROC analysis of the accuracy of optical density in the detection of endometritis defined as a percentage of neutrophils in the uterine lavage samples higher than 18% was conducted for $ULSOD_{620}$. The recommended threshold was 0.059, where the sensitivity and specificity were 100 and 82.2%, respectively. Cows with $ULSOD_{620}$ ≤0.058 were 1.21 times more likely to conceive than cows with $ULSOD_{620}$ >0.058; moreover, the median calving-to-conception interval for cows that had $ULSOD_{620}$ ≤0.058 was 122 d compared with 148 d for cows that had $ULSOD_{620}$ >0.058. Cows that were positive for *Arcanobacterium pyogenes*, diagnosed with metritis, or had retained placenta had 4.0, 1.4, and 1.7 times higher odds of having $ULSOD_{620}$ >0.058, respectively. Cows with $ULSOD_{620}$ >0.058 had a higher percentage of neutrophils in the uterine lavage samples than cows with $ULSOD_{620}$ ≤0.058. Uterine lavage sample optical density measured at 620 nm can be used as an objective indicator of uterine health in dairy cows, principally for clinical endometritis.

Key words: dairy cow, clinical endometritis, uterine disease, metritis

INTRODUCTION

Reproductive efficiency is undoubtedly a trait of great importance for the dairy industry and significantly affects the overall economic outcome of a dairy enterprise. A healthy reproductive tract is a prerequisite for satisfactory reproductive performance. After parturition, the uterine lumen is usually contaminated by bacteria (Foldi et al., 2006). The complexity of the bacterial community in the postpartum uterus of dairy cows differs between healthy cows and cows with metritis (Santos et al., 2011). *Escherichia coli*, *Arcanobacterium pyogenes*, and *Fusobacterium necrophorum* are considered important etiological agents of uterine diseases (Miller et al., 2007; Bicalho et al., 2010; Santos et al., 2011). *Escherichia coli* virulence factors, such as *fimH*, *hlyA*, *cdt*, *kpsMII*, *ibeA*, and *astA*, are associated with uterine diseases and impaired reproductive performance (Bicalho et al., 2010). Additionally, *F. necrophorum* is the most prevalent bacterium in the intrauterine environment of metritic cows, while being completely absent in the uterus of healthy cows (Santos et al., 2011). Furthermore, the presence of *A. pyogenes* in the uterus is associated with impaired reproduction performance (Williams et al., 2005).

Clinical endometritis is defined as the presence of purulent or mucopurulent uterine exudates in the vagina, after 21 d postpartum, not accompanied by systemic signs (Sheldon et al., 2006). Although this definition is largely accepted and used by clinicians and researchers, a recent study challenged assumptions of this method of diagnosis, showing that cows with purulent vaginal discharge did not always present endometrial inflam-

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mation (Dubuc et al., 2010a). Clinical endometritis is a disease that affects 15 to 42% of cows (Plontzke et al., 2010; Potter et al., 2010; Westermann et al., 2010); parity, dystocia, stillbirth, offspring, and metritis are risk factors for the disease (Dubuc et al., 2010b; Potter et al., 2010). The harmful effect of clinical endometritis on reproductive performance (Barlund et al., 2008; Plontzke et al., 2010) and the resulting negative economic impact on the dairy industry (Lee and Kim, 2007) necessitate means for the accurate diagnosis and better investigation and handling of the disease.

Several diagnostic techniques for clinical endometritis are used by researchers, including vaginoscopy, ultrasonographic assessment of uterine fluid volume and endometrial thickness, hysteroscopy, and the use of an intravaginal device (McDougall et al., 2007; Barlund et al., 2008; Madoz et al., 2010). Vaginoscopy and the use of a vaginal mucus collection device (Metricheck, Simcro, Hamilton, New Zealand) are used to evaluate vaginal mucus and diagnose uterine inflammation; however, vaginal discharge can be erroneously associated with uterine inflammation when the discharge is a result of vaginitis or cervicitis. Uterine bacteriology and cytology were used to determine false-positive findings of clinical endometritis by vaginoscopy (Westermann et al., 2010). In addition, ultrasonographic assessment of uterine fluid volume and endometrial thickness was not a good predictor of reproductive performance (Barlund et al., 2008), and hysteroscopy is not a practical technique in the field. Some of these techniques are prone to observer bias and variation between observers, and lack of training could lead to an erroneous diagnosis of clinical endometritis when scoring vaginal discharge by vaginoscopy.

Optical density measurement provides a numerical and objective value of the absorbed light in a sample, and it has been used to measure concentration of cells or proteins in samples (Glasel, 1995; Metris et al., 2006). Uterine lavage sample optical density (**ULSOD**) might be influenced by the exudates and cellular debris accumulated because of endothelium inflammation (Bondurant, 1999), and by the presence of bacteria inside the uterine lumen of cows affected with endometritis (Miller et al., 2007). Therefore, the objectives were (1) to evaluate the association of ULSOD with uterine health and reproductive performance, and (2) to estimate and evaluate a threshold value that would maximize the accuracy of ULSOD as a diagnostic tool for clinical endometritis.

MATERIALS AND METHODS

Farms and Management

The study enrolled 1,742 cows from 3 dairy farms located near Ithaca, New York, from May 4, 2010, until January 17, 2011. The farms were selected because of their long working relationship with the Ambulatory and Production Medicine Clinic at Cornell University. Farm A milked 3,000 cows, farm B milked 1,600 cows, and farm C milked 2,800 cows. The cows were housed in freestall barns with concrete stalls covered with mattresses and bedded with waste paper pulp. Cows were milked 3 times daily in milking parlors. All cows were offered a TMR consisting of approximately 55% forage (corn silage, haylage, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cottonseed, and citrus pulp) on a DM basis of the diet. The diet was formulated to meet or exceed the National Research Council nutrient requirements (NRC, 2001) for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM. The reproductive management utilized a combination of Presynch (Moreira et al., 2001), Ovsynch (Pursley et al., 1995), Resynch (Fricke et al., 2003), and detection of estrus, with 25 to 30% of cows bred via timed AI and the remainder bred after detection of estrus solely by activity monitors (Alpro, DeLaval, Kansas City, MO).

Case Definition, Sample Collection, and OD Measurement

Clinical endometritis was previously diagnosed primarily by presence of a mucopurulent vaginal discharge (LeBlanc et al., 2002; Sheldon et al., 2006). More recently, it has been established that many cows with purulent or mucopurulent vaginal discharge are free of endometrial inflammation (Dubuc et al., 2010a,b). Clinical endometritis was evaluated at 34 to 36 DIM by visual inspection of a uterine lavage sample obtained as described previously (Gilbert et al., 2005). In this way, visible signs of inflammation (purulent or mucopurulent exudate) emanating from the uterus, rather than from another site, would be assured. Briefly, the cows were restrained, the perineum area was cleansed and disinfected with 70% ethanol, and a plastic infusion pipette was introduced into the cranial vagina and manipulated through the cervix into the uterus. A total of 20 mL of sterile saline solution was infused into the uterus and agitated gently, and a sample of the fluid was aspirated. The volume of recovered fluid ranged from 5 to 15 mL. All of the samples were visually scored by one investigator, who assessed the presence of a purulent or mucopurulent secretion in the uterine lavage sample. The score ranged from 0 to 2, with 0 indicating absence of a purulent or mucopurulent secretion in the lavage sample, 1 indicating a bloody but not purulent sample, and 2 the presence of pus in the lavage sample. Cows with a score of 2 were considered with clinical endometritis. Body condition scores were recorded at