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 (ULSOD₆₂₀). Incidences of clinical endometritis, metritis, and retained placenta were 10, 15.2, and 5.6%, respectively. The ULSOD₆₂₀ 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; ULSOD₆₂₀ was selected for further analysis because it presented the best ROC curve to detect clinical endometritis. The recommended threshold for ULSOD₆₂₀ 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 ULSOD₆₂₀. The recommended threshold was 0.059, where the sensitivity and specificity were 100 and 82.2%, respectively. Cows with ULSOD₆₂₀ ≤ 0.058 were 1.21 times more likely to conceive than cows with ULSOD₆₂₀ > 0.058 ; moreover, the median calving-to-conception interval for cows that had ULSOD₆₂₀ ≤ 0.058 was 122 d compared with 148 d for cows that had ULSOD₆₂₀ > 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 ULSOD₆₂₀ > 0.058 , respectively. Cows with ULSOD₆₂₀ > 0.058 had a higher percentage of neutrophils in the uterine lavage samples than cows with ULSOD₆₂₀ ≤ 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*, *kpsMIII*, *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 (Glaser, 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

the time of each uterine lavage using a 5-point scale (1 = emaciated, 5 = obese, scored in 0.25-point intervals) as described by Edmonson et al. (1989). Uterine lavage samples were kept in ice until they were processed in the laboratory and cultured for *A. pyogenes* on Mueller-Hinton agar plates (BBL TM 7, Becton, Dickinson and Co., Sparks, MD) supplemented with 5% defibrinated sheep blood for 48 h aerobically in 7% CO₂ at 38°C. Colonies of *A. pyogenes* were distinguished by colony morphology, hemolysis, and Gram stain. After culturing, the samples were frozen at -80°C. After all the uterine lavage samples were collected, they were thawed and processed for optical density measurement as follows: hypertonic saline solution (10%) was added to the uterine lavage sample in a 1:1 proportion, samples were then incubated at room temperature for 30 min and subsequently centrifuged for 30 min at $3,000 \times g$ at 4°C. The supernatant was collected and an aliquot of 0.2 mL was added to a 96-well microplate (Greiner Bio-One, Frickenhausen, Germany). The optical density was assessed using a Synergy HT microplate reader (BioTek Instruments, Winooski, VT) and was measured at the following wavelengths for a subset of the study cows (554 cows): 200, 352, 620, 790, 860, and 960 nm. Uterine lavage sample optical density measured at 620 nm (**ULSOD₆₂₀**) was assessed for all 1,742 cows enrolled in the study. Information regarding parity, calving, presence of retained placenta, and metritis was obtained through the farms' management software.

Additionally, a subgroup (n = 85) of the studied cows was examined for cytological evidence of inflammation with the use of endometrial cytology (Gilbert et al., 2005). Briefly, the uterine lavage samples were mixed by vortex to resuspend cellular material, and an aliquot was transferred to a cytocentrifuge chamber. Samples were centrifuged onto glass slides at $105 \times g$ for 3 min. The slides were then air-dried, and stained with modified Wright-Giemsa stain (Diff-Quik 1, Dade Diagnostics, West Monroe, LA). Coverslips were applied to dried, stained slides using toluene (Permount, Fisher Scientific, Pittsburgh, PA). Each slide was examined by the same examiner under bright-field microscopy at 250 $\times$ and 400 $\times$. The examiner counted 200 cells from each slide and the percentage of neutrophils among all other cells was estimated. Based on reports from previous studies (Kasimanickam et al., 2004), a threshold of 18% was used, above which cows were characterized as suffering from endometritis. The 18% threshold was used because our samples were taken around 35 DIM. Gilbert et al. (2005) suggested a 5% threshold using samples obtained 40 to 60 DIM.

Statistical Analysis

To facilitate data analysis and interpretation, the variables BCS group (**BCSG**), lactation group (**LACGP**), calf information (**CALF**), calving ease information (**EASE**), presence of clinical endometritis at 35 ± 3 DIM (**ENDOM**, yes = 1 and no = 0), presence of metritis during second week of lactation (**MET**, yes = 1 and no = 0), presence of retained placenta during first week of lactation (**RP**, yes = 1 and no = 0), and the outcome of the uterine lavage sample culture for *A. pyogenes* at 35 ± 3 DIM (**APYO**, yes = 1 and no = 0) were created. Body condition score group was categorized as follows: BCSG = 1 if BCS <3, BCSG = 2 if BCS = 3, and BCSG = 3 if BCS >3. Lactation group was categorized as follows: LACGP = 1 if lactation = 1, LACGP = 2 if lactation = 2, and LACGP = 3 if lactation >2. Calf information was categorized as CALF = 1 if the cow had a live female calf, CALF = 2 if the cow had a live male calf, CALF = 3 if the cow had a stillbirth calf, and CALF = 4 if the cow had twins. A 5-point scale was used for EASE: EASE 1 was defined as calvings that occurred easily without assistance; EASE 2 was defined as unassisted, but more difficult than EASE 1, calvings; EASE 3 was defined as calvings requiring easy assistance from a person; EASE 4 was defined as vaginally delivered calvings requiring the calf position to be corrected or hard traction to be applied to deliver the calf; and EASE 5 was defined as calvings requiring fetotomy or caesarian section.

Descriptive statistics analysis was undertaken in SAS using the FREQ procedure (SAS Institute Inc., Cary, NC). An ANOVA model was fitted in SAS using the MIXED procedure to assess the effect of several independent variables on ULSOD₆₂₀. The independent variables BCSG, LACGP, APYO, pregnancy at 100 DIM, pregnancy at 120 DIM, death or culling, CALF, EASE, abortion, RP, and MET were offered to the model. Variables were manually and stepwise removed from the model when the *P*-value >0.05. The variable ENDOM was not offered to the model because of its multicollinearity with ULSOD₆₂₀. All possible 2-way interactions between the independent variables were added to the model; none was significant. To compare the accuracy of OD measured at several different wavelengths (200, 352, 620, 790, 860, and 960 nm) to predict the dichotomized endometritis score of the uterine lavage sample (ENDOM = 0 if mucus score ≤ 1 , ENDOM = 1 if mucus score >1), a receiver operating characteristic (**ROC**) curve analysis was performed using MedCalc version 11.5.1.0 software.

(MedCalc Software, Mariakerke, Belgium). The area under the curve (**AUC**) was used to select the most accurate wavelength of OD; 620 nm had the greatest AUC and was selected for further analysis. To estimate a threshold of ULSOD₆₂₀, a second ROC analysis was performed and a threshold of 0.058 was indicated. In addition to the AUC, the following measures were estimated using MedCalc: sensitivity, specificity, positive predictive value, and negative predictive value. Because it was used for the previous analysis, the gold standard was the dichotomous endometritis diagnosis (endometritis score >1). A similar ROC analysis was performed with data regarding ULSOD₆₂₀ and the percentage of neutrophils in uterine lavage samples. In this case, the gold standard was the dichotomous endometritis diagnosis based on information regarding percentage of neutrophils in uterine lavage samples. Cows having a percentage of neutrophils >18% were characterized as suffering from endometritis.

The effect of the dichotomized ULSOD₆₂₀ on reproduction was analyzed by Cox's proportional hazard using MedCalc. For this analysis of reproduction, cows were right censored if not diagnosed as being pregnant before culling, death, or the end of the data collection period. Variables offered to the models included dichotomized ULSOD₆₂₀ and LACGP.

A logistic regression was fitted in SAS using the Logistic procedure to examine in detail the association of the independent variables BCSG, LACGP, APYO, pregnancy at 100 DIM, pregnancy at 120 DIM, death

or culling, CALF, EASE, abortion, RP and MET with the odds of endometritis as defined by the dichotomized ULSOD₆₂₀.

Finally, association between clinical endometritis (as defined by visual uterine lavage scores) and dichotomized ULSOD₆₂₀ scores with the cytological evidence of inflammation (measured as the percentage of neutrophils among other cells in uterine lavage samples) were estimated with the use of 1-way ANOVA in JMP 9 (SAS Institute Inc., Cary, NC).

RESULTS

Descriptive Statistics

In total, 1,742 cows from 3 farms were enrolled in the study; descriptive statistics concerning the incidence of metritis, retained placenta, and clinical endometritis by lactation, farm and BCSG are in Table 1. Additionally, 29 cows had missing BCS data, and only 554 cows had OD data for all evaluated wavelengths (200, 352, 620, 790, 860, and 960 nm). All cows enrolled had data for OD measured at 620 nm.

Effect of Clinical Endometritis, Metritis, *A. pyogenes* Infection, and Lactation on Measured ULSOD₆₂₀

Uterine lavage sample OD₆₂₀ was associated with clinical endometritis ($P < 0.0001$). The mean ULSOD₆₂₀ was 0.18 compared with 0.06 for cows diagnosed with

Table 1. Descriptive statistics of 1,742 cows at study enrollment by lactation, BCS group (BCSG), and farm, including the number of cows per group and the incidence of clinical endometritis, metritis, and retained placenta

Item	Cows, no.	Incidence, %		
		Clinical endometritis (35 ± 3 DIM)	Metritis (wk 2 of lactation)	Retained placenta (wk 1 of lactation)
Lactation				
1	565	11.4	27.1	6.4
2	650	8.2	9.4	3.4
≥3	5,527	10.6	9.7	7.6
<i>P</i> -value		0.14	<0.01	<0.01
Total	1,742	10.0	15.2	5.6
BCSG				
1	294	11.2	16.0	6.1
2	1,118	10.8	14.9	6.1
3	301	6.0	15.3	3.6
<i>P</i> -value		0.02	0.77	0.22
Total	1,713	10.04	15.29	5.66
Farm				
1	303	7.6	5.3	4.6
2	248	6.2	8.1	8.5
3	1,191	11.4	19.2	5.3
<i>P</i> -value		0.01	<0.01	0.12
Total	1,742	10.0	15.2	5.6

Figure 1. Mean optical density (OD) measured at 620 nm of uterine lavage samples for cows diagnosed with clinical endometritis (1) and cows diagnosed without clinical endometritis (0). Cows diagnosed with clinical endometritis had mean OD of 0.18 (95% CI: 0.17–0.20) and cows diagnosed without clinical endometritis had mean OD of 0.06 (95% CI: 0.05–0.07). Error bars represent the 95% CI for the means ($P < 0.0001$).

and without clinical endometritis, respectively (Figure 1). Clinical endometritis was not subjected to ANOVA.

Uterine lavage sample optical density measured at 620 nm differed significantly based on lactation, diagnosis of metritis, and presence of intrauterine *A. pyogenes* infection. Cows in lactation 1, 2, and ≥ 3 had ULSOD₆₂₀ averages of 0.12 (95% CI: 0.11–0.13), 0.10 (95% CI: 0.09–0.11), and 0.11 (95% CI: 0.10–0.12), respectively ($P = 0.02$). In addition, the average ULSOD₆₂₀ for cows diagnosed with or without metritis was 0.13 (95% CI: 0.11–0.14) and 0.09 (95% CI: 0.08–0.10), respectively ($P < 0.001$). Furthermore, cows positive for *A. pyogenes* had a ULSOD₆₂₀ average of 0.14 (95% CI: 0.12–0.16), which was significantly higher than the

ULSOD₆₂₀ of 0.08 (95% CI: 0.07–0.09) for cows negative for *A. pyogenes* ($P < 0.001$).

ROC Curve Analysis

A ROC analysis was performed to evaluate the accuracy of optical density measured at several different wavelengths (200, 352, 620, 790, 860, and 960 nm). The AUC was 0.84, 0.845, 0.848, 0.830, 0.815, and 0.754 for optical density measured at wavelengths 200, 352, 620, 790, 860, and 960 nm, respectively. The ULSOD₆₂₀ was selected for further analysis because it presented the greatest AUC between the wavelengths evaluated, and therefore, the best ROC curve to detect clinical endometritis. A second ROC analysis was conducted for ULSOD₆₂₀ using the data from all cows enrolled, and the AUC for this new ROC curve was 0.85. The recommended threshold for this ROC curve was 0.058, where the sensitivity was 76.3% and the specificity 78.3% (Table 2). A ROC analysis was conducted for ULSOD₆₂₀ using percentage of neutrophils in uterine lavage samples as the gold standard; cows with $>18\%$ neutrophils were considered endometritis positive and cows with $\leq 18\%$ neutrophils were considered endometritis negative. The AUC for this curve was 0.862. The recommended threshold for this ROC curve was 0.059, where the sensitivity was 100% and the specificity 82.2%.

Effect of Dichotomized ULSOD₆₂₀ on Reproduction

A multivariable Cox proportional hazard model was performed, and the only variables retained in this model were LACGP and the dichotomized ULSOD₆₂₀; cows with ULSOD₆₂₀ ≤ 0.058 were 1.21 times more likely to conceive compared with cows with ULSOD₆₂₀ >0.058 (hazard ratio = 1.21, $P < 0.01$). Additionally, the median calving-to-conception interval for cows that

Table 2. Outcome of the receiver operating characteristic (ROC) curve analysis performed to evaluate the optimum uterine lavage sample optical density measured at 620 nm threshold for the detection of clinical endometritis¹

Probability threshold	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, %	Negative predictive value, %	Overall accuracy, %	Cows above threshold, %
>0.054	80.9 (74.3–86.5)	72.29 (70.0–74.5)	24.5	97.2	73.1	33.0
>0.055	80.3 (73.6–86.0)	73.6 (71.3–75.7)	25.2	97.1	74.2	31.8
>0.056	78.6 (71.7–84.5)	75.82 (73.6–77.9)	26.5	97.0	76.1	29.6
>0.057	76.3 (69.3–82.4)	77.5 (75.3–79.5)	27.3	96.7	77.4	27.9
$>0.058^2$	76.3 (69.3–82.4)	78.3 (76.1–80.3)	28.0	96.7	78.1	27.2
>0.059	74.0 (66.8–80.4)	79.28 (77.2–81.3)	28.4	96.5	78.7	26.0
>0.060	72.8 (65.6–79.3)	80.3 (78.2–82.3)	29.1	96.4	79.6	25.0
>0.061	72.2 (64.9–78.8)	81.0 (79.0–82.9)	29.7	96.3	80.1	24.3
>0.062	71.7 (64.3–78.3)	82.0 (80.0–83.9)	30.6	96.3	80.9	23.4

¹Sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy, and percentage of cows above the suggested probability threshold were estimated for each hypothetical probability threshold.

²Probability threshold suggested by the ROC curve analysis.

Figure 2. Cox proportional hazards survival analysis of the calving-to-conception interval for cows that presented optical density (OD) measured at 620 nm of uterine lavage sample >0.058 . Cows below this threshold (solid line, $n = 1,072$) had a median calving-to-conception interval of 122 d compared with 148 d for cows that had uterine sample lavage optical density measured at 620 nm above 0.058 (dashed line, $n = 400$). Variables offered to the models included dichotomized uterine sample lavage OD measured at 620 nm and lactation group ($P < 0.001$).

had $ULSOD_{620} \leq 0.058$ was 122 d compared with 148 d for cows with $ULSOD_{620} > 0.058$ (Log rank test, $P < 0.001$, Figure 2). Moreover, by 250 DIM, the percentage of cows pregnant was significantly lower for cows with $ULSOD_{620}$ above the threshold. By 250 DIM, 68% of cows with $ULSOD_{620} > 0.058$ were pregnant, whereas 75% of cows with $ULSOD_{620} \leq 0.058$ had conceived (Figure 2).

Association of Metritis, Retained Placenta, and *A. pyogenes* with the Odds of Endometritis as Defined by the Dichotomized $ULSOD_{620}$

The use of a multivariable logistic regression model revealed that the variables APYO, MET, and RP were significantly associated with the dichotomized $ULSOD_{620}$. Cows that were positive for APYO had 4.0 times higher odds of having $ULSOD_{620} > 0.058$ ($P < 0.001$); 59.6% of the APYO positive cows ($n = 151$) had $ULSOD_{620} > 0.058$, and only 24% ($n = 1,577$) of the APYO negative cows had $ULSOD_{620} > 0.058$. Additionally, cows diagnosed with MET were at a 1.4 increased odds of having $ULSOD_{620} > 0.058$ ($P = 0.035$); 39.6 and 24.9% of the cows that were diagnosed with and with-

out MET ($n = 265$ and 1,477) had $ULSOD_{620} > 0.058$, respectively. Moreover, cows with RP had 1.7 times higher odds of having $ULSOD_{620}$ above the probability threshold suggested by the ROC curve analysis (26.1%, $n = 1,644$; $P = 0.04$); 44.9% of the cows with RP ($n = 98$) had $ULSOD_{620} > 0.058$.

Association of Clinical Endometritis and Dichotomized $ULSOD_{620}$ Scores with Cytological Evidence of Inflammation

Results regarding the association of clinical endometritis (as defined by visual uterine lavage scores) and dichotomized $ULSOD_{620}$ scores with the percentage of neutrophils among other cells in uterine lavage samples are in Table 3. Different visual scores or dichotomized $ULSOD_{620}$ scores were accompanied by statistically significant differences in cytological evidence of inflammation. However, the differences were clearer and of greater significance when dichotomized $ULSOD_{620}$ scores were used.

DISCUSSION

Optical density, also known as absorbance, refers to the mathematical quantity of the physical process of

Table 3. Association of clinical endometritis and dichotomized uterine lavage sample optical density measured at 620 nm (ULSOD₆₂₀) with mean percentages of neutrophils in uterine lavage samples

Item	Mean $\pm$ SE	P-value
Clinical endometritis		
No	4.4 $\pm$ 1.1	0.013
Yes	19.5 $\pm$ 5.9	
ULSOD ₆₂₀		
≤ 0.058	2.7 $\pm$ 1.2	0.0001
> 0.058	12.6 $\pm$ 2.1	

absorbing light (Gold and International Union of Pure and Applied Chemistry, 1987). It is largely used to measure the concentration of proteins or cells in suspension in a liquid sample (Glaser, 1995; Metris et al., 2006). In the present study, ULSOD₆₂₀ was analyzed by using ROC and visual scoring of clinical endometritis as the gold standard, from which it was determined that a threshold of ULSOD₆₂₀ > 0.058 should be applied to define diseased cows, achieving an overall accuracy of 78%. It is important to highlight that, currently, no perfect diagnostic test (gold standard) exists for clinical endometritis and, therefore, our ROC analysis was carried out with that limitation.

The incidence of clinical endometritis was 10%, considered low compared with the 15 to 42% found in other studies (LeBlanc et al., 2002; LeBlanc, 2008; Plontzke et al., 2010). The diagnostic method used in this study may be the main reason for this low incidence of disease. With the low-volume uterine lavage technique, only the presence of a mucopurulent secretion inside the uterus was assessed, eliminating false-positive diagnoses that are more common with other methods of diagnosis such as rectal palpation and visual inspection of vaginal discharge (LeBlanc, 2008; Plontzke et al., 2010; Potter et al., 2010).

Uterine lavage sample optical density measured at 620 nm was highly associated with the diagnosis of clinical endometritis; the risk factors for high ULSOD₆₂₀ found in this study supported those associated with clinical endometritis found in other studies: presence of *A. pyogenes* in the uterine lumen (Bondurant, 1999), metritis (Dubuc et al., 2010b), and parity (Potter et al., 2010). In contrast, other risk factors described in previous studies were not important for the ULSOD₆₂₀, such as dystocia, stillbirth, and offspring (Dubuc et al., 2010b; Potter et al., 2010). Retained placenta is a risk factor for clinical endometritis (Potter et al., 2010). In the present study it was not an important risk factor for a high ULSOD₆₂₀ value; it was only a risk factor for the dichotomized ULSOD₆₂₀.

The incidence of retained placenta was 5.6%, which can be considered low when compared with other studies, which reported incidence of 8.6 and 11.8% (Kelton

et al., 1998; Dubuc et al., 2010b). This low incidence could explain why retained placenta was not a risk factor for a high ULSOD₆₂₀ value. The ULSOD₆₂₀ mean for cows with retained placenta was greater than that for cows without retained placenta, but because of the low incidence of retained placenta, the statistical power was also low. Nevertheless, retained placenta was significantly important for the dichotomized ULSOD₆₂₀.

Metritis was one of the most important factors associated with ULSOD₆₂₀. Clinical endometritis and metritis appear to be linked uterine diseases; however, metritis is not necessary for the development of endometritis (Bondurant, 1999; Dubuc et al., 2010b). *Arcanobacterium pyogenes* can be recovered from the uterus of cows with either normal puerperium or metritis (Silva et al., 2008; Santos et al., 2010), and this infection can persist inside the uterine lumen as endometritis (Bondurant, 1999; Miller et al., 2007). Additionally, clinical endometritis is associated with factors that caused lesions in the uterus, such as dystocia, twin parturition, and retained placenta (Dubuc et al., 2010b; Potter et al., 2010). Therefore, clinical endometritis could be linked to metritis because of the uterine damage caused by metritis (Bondurant, 1999).

The presence of *A. pyogenes* in the uterine lumen has been associated with chronic uterine inflammation (Bondurant, 1999), and thus bacterial culture was used as a means to detect false-positive diagnosis of clinical endometritis when the diagnosis is based on the presence of a mucopurulent discharge in the vagina (Westermann et al., 2010). In the present study, the presence of *A. pyogenes* in the uterine lavage sample was associated with a high mean ULSOD₆₂₀. It was reported that *A. pyogenes* causes severe endometrial lesions (Miller et al., 2007), leading to an antiinflammatory response (Westermann et al., 2010). Therefore, a high ULSOD₆₂₀ could be not only a result of the presence of bacterial cells in the uterine lavage sample, but also the outcome of the endothelial inflammatory process caused by this pathogen. This assumption is supported by the results regarding ULSOD₆₂₀ and the percentage of neutrophils among other cells in uterine lavage samples. Cows with high ULSOD₆₂₀ (> 0.058) had a significantly higher percentage of neutrophils (Table 3).

Endometrial cytology has been used for the diagnosis of endometritis in postpartum Holstein cows. Kasimanickam et al. (2004) defined subclinical endometritis as the presence of $> 18\%$ neutrophils in samples obtained from the uterine lumen of clinically healthy cows at 20 to 33 DIM. The data presented suggested that we could use the dichotomized ULSOD₆₂₀ to accurately (100% sensitivity, 82.3% specificity) identify cows with endometritis (defined as the presence of $> 18\%$ neutrophils in samples obtained from the uterine lumen of all

cows with endometrial cytology information available). Endometrial cytology is considered an objective technique for the diagnosis of endometritis (Gilbert et al., 2005; Westermann et al., 2010). Therefore, ULSOD₆₂₀ measurements could be used as an alternative to endometrial cytology diagnostic techniques, and it should be noted that obtaining ULSOD₆₂₀ measurements is much less laborious than obtaining endometrial cytology information. The results observed in this study must be replicated by other researchers using a distinct population of animals. It should be acknowledged that the 18% threshold is under discussion because different thresholds have been suggested by other researchers.

Cows diagnosed with endometritis have impaired reproductive performance (Gilbert et al., 2005; Barlund et al., 2008; Plontzke et al., 2010). The extended calving interval and increased culling caused by inefficient reproductive performance cause severe economic loss in the dairy industry (Lee and Kim, 2007). To evaluate the effect of dichotomized ULSOD₆₂₀ on reproductive performance, a survival analysis was performed (Cox proportional hazard), controlling for parity. Cows with ULSOD₆₂₀ >0.058 were less likely to conceive and had a greater median calving-to-conception interval than cows with ULSOD₆₂₀ ≤0.058. This finding shows that elevated ULSOD₆₂₀ had a negative effect on reproductive performance and consequently a potential negative economic impact on the dairy herd.

The ULSOD₆₂₀ value could be a useful tool for assessment of uterine health in research. For instance, it could be used as a continuous variable for comparison of different treatment groups. The technique of measuring ULSOD is simple and fast and does not require special training. Moreover, it can prevent diagnostic bias because it provides a numerical value that cannot be influenced by the researcher. It is important that sample collection and processing be standardized to avoid excessive variation between research groups.

In conclusion, we suggest that ULSOD₆₂₀ could be used for the evaluation of postpartum uterine health. It is a technique that provides objective information of similar quality and accuracy to that obtained by endometrial cytology.

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