Diagnosis of endometritis in postpartum dairy cows
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Abstract
The prevalence of endometritis in postpartum dairy cows is high and the condition impairs subsequent reproductive performance once the voluntary waiting period (VWP) has passed. In addition to the direct financial losses, there are also important indirect losses due to infertility and its subsequent loss of a potential profitability from genetic selection for milk production. To reduce the negative impact of endometritis on the dairy industry, an accurate diagnostic test is needed. Since there is no gold-standard to test for the condition, most diagnostic tests can only differentiate partially between animals with and without the disease. To further complicate the situation, the cause of endometritis is often multi-factorial, with several predisposing factors exhibiting different levels of impact such that the distinction between the physiological process of the involution of the uterus and a pathological process establishing disease becomes difficult.

The present article reviews the different methods used to diagnose endometritis in postpartum dairy cows. The diagnostic accuracy, the limitations and advantages of trans-rectal palpation, visual assessment of the vaginal discharge, endometrial cytology, uterine bacteriology, chemical testing, ultrasonography, and the measurement of the optical density of a uterine lavage are discussed. A better knowledge of the diagnostic methods of endometritis will allow veterinarians to be proactive in monitoring uterine health in postpartum dairy cows which is the key to restore reproductive performance in postpartum animals and to establish a strong base for an effective herd health management.

Introduction
Reproductive performance in dairy cows and farm economic efficiency are related to uterine health status at the end of the voluntary waiting period.1-4 Uterine diseases affect about half of all dairy cows in the postpartum period,5,6 causing infertility by disrupting uterine and ovarian function.7 The resulting reduction in fertility leads to an estimated 50% loss of the potential profitability from genetic selection for milk production.8 Postpartum uterine infections in dairy cows cost more than 1.4 billion, 650 million and 300 million to the European, American, and Canadian industries, respectively.9,10 Yet, despite the significant economic impact of postpartum uterine infections, controversies still exist around the diagnosis.11

Postpartum uterine diseases in dairy cows include puerperal and postpartum metritis, pyometra, clinical endometritis (CE) or purulent vaginal discharge, and subclinical endometritis (SE) or cytological endometritis. Compared to puerperal metritis and pyometra, where clinical signs are more obvious, endometritis is a challenging condition for veterinarians to diagnose. Historically, endometritis has been histologically defined: a superficial inflammation limited to the endometrium and extending no deeper than the stratum spongiosum. It is characterized by disruption of the surface epithelium with infiltration of inflammatory cells, vascular congestion and stromal edema.12,13 Diagnosis of the condition, therefore, requires endometrial tissue sampling (uterine biopsy) and histological analysis. In the last decade, a more practical definition based on clinical context and reproductive performance has been proposed.1 This new approach allows the veterinarian to make a more straightforward, medically rational and economically based decision. The CE is defined as an infection of the endometrium with uterine exudate, varying from clear mucus with flecks of pus to purulent discharge in the vagina, 21 days or more postpartum. The SE is defined as inflammation of the endometrium based on the presence of a certain percentage of polymorphonuclear neutrophils (PMN) in the absence of purulent vaginal discharge. Because both conditions have different risk factors and that their negative effect on reproductive performance are additive, they are assumed to be two different uterine pathological entities.14,15 Based on these definitions, it is estimated that about 20% of postpartum dairy cows have CE and 30% have SE.9,16 To efficiently treat
CE and SE, and improve reproductive performance, the veterinarian needs accurate diagnostic methods. What are the currently available diagnostic methods, and how accurate and useful are they?

The accuracy of diagnostic tests

In veterinary medicine, decisions around animal health are based on a number of factors, including medical knowledge, professional experience, clinical intuition and clinical diagnostic testing. Diagnostic testing is a relatively objective method that reduces uncertainty about factors during diagnosis. Of primary importance is diagnostic accuracy, or the ability of a test to discriminate between the pathologic and healthy status. The discriminative ability of a test can be quantified by a variety of different measures: sensitivity (Se), specificity (Sp), negative and positive predictive values (NPV and PPV, respectively), likelihood ratio (LR), area under the ROC curve (AUC), odds ratio (OR), and kappa (K) test (Tables 1-5). Of course, there are a number of other criteria that determine the most appropriate test – criteria that are not directly linked to the medical performance of the test, but rather more to its feasibility and practicality in a given situation, such as cost, time required to perform the test, availability, and time to final results (the issue of cow-side vs laboratory tests). Moreover, measures of test performance are not absolute indicators of test quality and performance because they are sensitive to factors like population characteristics, disease prevalence and disease spectrum. For veterinarians, it is of utmost importance to know how to interpret a test, and when and under what conditions to use a test.

There is no perfect test because most diagnostic test results can make only a partial distinction between subjects with and without a disease. In bovine theriogenology, the first Koch’s postulate for establishing the causative relationship between a microbe and a disease are not always applicable. The first postulate of Koch says that the microorganism must be found in abundance in all animals suffering from the disease, but should not be found in healthy organisms. The cause of uterine conditions is often multi-factorial, with several predisposing factors exhibiting different levels of impact. The concept of HOST-PATHOGEN-ENVIRONMENT is closer to the reality in the field situation. This concept captures well the complex interplay of factors that result in disease at the individual and population levels. In short, a pathogen is a necessary but not sufficient cause of disease. Asymptomatic or subclinical infection carriers are known to be a common feature of many diseases as in the present case.

The result of such complexity is that there is no real gold standard for evaluating a diagnostic test. Rather, we have to evaluate a diagnostic test by examining its level of agreement with a near-gold standard or a more accepted diagnostic method. We can also assess outcomes in terms of economic, reproductive or management impacts. The kappa test measures the level of agreement between two tests beyond what would be obtained by chance alone. Note that the kappa result gives no indication of test quality per se because a good agreement may mean only that both tests are equally good or equally bad.

The overall accuracy of diagnostic tests for endometritis

In general, veterinary reproductive interventions are only beneficial if they result in pregnancy in a timely fashion. The value of reproductive examinations before the breeding period depends on the ability of veterinarians to identify cows at a higher risk of pregnancy failure and then, through a diagnostic process, identify those that could benefit from treatment for endometritis. Veterinarians need to perform diagnostic tests for a number of reasons:

- Confirm the diagnosis in symptomatic animals
- Screen for disease in asymptomatic animals (systematic postpartum examination)
- Provide prognostic information in cows with the disease
- Monitor the benefits and/or side effects of treatment (follow-up)
- Confirm disease-free status

The most important postpartum uterine disease in dairy cows is endometritis, which is prognostic for impaired reproductive performance. In bovine reproduction, Se, Sp, PPV and NPV are the most important measurements for validating diagnosis methods (see above). In the case of the postpartum endometritis in dairy cows, it is very difficult to estimate the real sensitivity and specificity of diagnostic methods because there is no gold standard. The diagnosis of endometritis in cows has also long been
hampered by the lack of a well-accepted definition of the condition and a simple, effective diagnostic technique. Added to these difficulties is the fact that all postpartum cows experience some degree of endometrial inflammation for a certain period of time is associated with normal uterine involution. Establishing the best time window of opportunity for testing uterine health status along the timeline of uterine involution is needed. As diagnostic tests are often interpreted using a dichotomous outcome (normal/abnormal or diseased/healthy), it is often easier to interpret results if the test itself is dichotomous, such as presence or absence of a pathogen. Interpretation is more difficult when the outcome is continuous or categorical, such as neutrophil counts or vaginal discharge categories. Variation in the cut-off point used to separate positive (disease) and negative (non-disease) results introduces a level of uncertainty (false positives/false negatives). This in turn affects estimates of prevalence. For endometritis in dairy cows, estimates of prevalence range from 0 to 68%.24-28 This large variation is due not only to the above-mentioned differences in test cut-offs, but also to a variety of factors:

- Inconsistencies in the timing of examination during the postpartum period
- Differences in diagnostic methods
- Definition of the pathology
- True difference in prevalence between populations
- Variation between individuals
- Host-pathogen-environment interactions
- Complex interactions between etiological pathogens

Diagnostic test: transrectal palpation

In practice, transrectal palpation is always part of a complete physical examination and still the predominant method used by veterinarians to diagnose uterine diseases. However, several studies have demonstrated that this method results in a large number of false-positive diagnoses.29 The diagnosis of endometritis by transrectal uterine palpation in the postpartum period relies on the location of the uterus (in the pelvic cavity, over the pelvic brim, or over the pelvic brim but retractable or not completely retractable), size of the uterus (enlarged or asymmetrical uterine horns), thickness of the uterine wall, presence of the uterine lumen or fluid within the lumen of the uterus,30 and size of the cervix.20,31 Transrectal palpation of the uterus lacks diagnostic accuracy, as a large uterus may be the result of physical damage or variation associated with breed, age or nutrition.1,20,32 It is a subjective measure and is not reliably associated with reproductive performance.33,34

A cervical diameter of > 7.5 cm as diagnosed by transrectal palpation has been demonstrated to be a more reliable predictor of poor reproductive performance. However, large cervix represents less than 3% at 27-33 days in milk (DIM).30 Cows with a large cervix have been shown to have a significantly decreased likelihood of pregnancy at first insemination and significantly increased mean days open. The greatest difference in cervical diameter between healthy cows and those with abnormal discharge is only 10 mm at 21 DIM.31 The likelihood of returning to cyclicity decreases for each 1-cm increase in cervical diameter as measured by ultrasound between 15 and 21 d postpartum.35 Dubuc et al found a sensitivity of 40.9% and 50.8% at 35 DIM based on thresholds of > 7.5 cm and > 5.0 cm, respectively, using predicted pregnancy status at 120 DIM as the reference.14 The overall sensitivity and specificity of rectal palpation of the cervix for all investigators, using ultrasound as the reference standard, was 37.5% and 96.2% based on thresholds of > 7.5 cm and > 5.0 cm respectively.36 This low sensitivity may be explained in part by the considerable time lag between diagnosis and pregnancy confirmation, and by the multiple factors that influence pregnancy status.24,37 Likewise, the repeatability of the estimate of the cervical diameter obtained through transrectal palpation is moderate, and decreases with increasing size diameter.38 A cervical diameter of > 6.0 cm seems to confuse investigators and lead to more false-positives. By 25 DIM, both uterine horns and the cervix are expected to reach a diameter of less than 5 cm in normal cows.39 Therefore, the lack of accuracy of the transrectal palpation of the uterine tract in identifying cows with endometritis is not surprising. A similar lack of accuracy has also been demonstrated for ovarian
examination, with significant inter-clinician variability in assessing corpus luteum function in dairy cows.40

Given the low inter-investigator agreement for the manual assessment of cervical and uterine horn diameter, and the lack of association between this measure and reproductive performance, the usefulness of widespread manual transrectal examination of the reproductive tract during the postpartum period in dairy cows remains controversial. The time required to perform a complete and meticulous examination is considered too long and too costly by veterinary practitioners and farmers. The main objective of characterizing the uterus and the cervix in postpartum cows is to identify those at increased risk of failure to become pregnant in a timely fashion. Transrectal palpation of the reproductive tract of dairy cows performed before 21 days postpartum may more likely overestimate the real prevalence of endometritis because of incomplete uterine involution and the difficulty in manually palpating a large uterus. However, even though the published data do not support transrectal palpation of the uterus as a useful diagnostic method for endometritis during the postpartum period, the exam is still an important part of a complete genital examination that allows the veterinarian to rapidly rule out obvious abnormalities or abnormalities that would not otherwise be noticed, such as pyometra, tumors, abscesses and lacerations.

Diagnostic test: visual examination of the vaginal discharge

There are several methods for diagnosing CE in postpartum dairy cows based on the visualization and characterization of vaginal discharge (Figure 1). They are: 1) gloved hand method,41 2) the Metricheck device (Metricheck, Simcro, New Zealand),18 and 3) vaginoscopy or speculum method.20,42 Purulent vaginal discharge resulting from bacterial infection is characterized by a mixture of neutrophils, necrotic tissue and fluid, and amount of these materials, or pus, is correlated with the growth density of pathogenic bacteria. By contrast, the growth density of bacteria categorized as opportunist contaminants of the cow reproductive tract is not associated with purulent vaginal discharge.7 William et al proposed a 4-point scoring system for grading vaginal discharge: 0=clear mucus, 1=mucus containing flecks of pus, 2=discharge containing fewer than 50% pus, and 3=discharge containing more than 50% pus.7 Although perceived as an inconvenient method by veterinarians, the visual assessment of vaginal discharge via vaginoscopy examination is a simple tool that can rapidly distinguish healthy from diseased cows.1,14,22,43 In fact, vaginal examination with a speculum has been shown to be more accurate than transrectal palpation in detecting abnormal discharge.44

It would appear, then, that transrectal palpation is of little value in terms of monitoring uterine health in postpartum period when vaginoscopy is performed.19,20 Yet, transrectal palpation remains an important part of a complete genital examination. Note that the stimulation of uterine contractions caused by previous transrectal palpation of the reproductive tract does not, as previously thought, increase the detection of vaginal discharge.18,45

The relative risk analysis does not reveal a significant effect of transrectal palpation of the uterus on the prevalence of CE as diagnosed by visual examination.38 The prevalence of CE ranges from 10% to 20%,20,46 however more recent studies reported prevalence > 40%.38,45,38,47 This large variation between studies could be explained by different diagnostic criteria and methods, definitions of the disease (cutoff points), or the population and farm effect. The interobserver (k=0.47 to 0.55) and intraobserver (k=0.61-0.82) agreement for visual examination is high. The visualization of vaginal secretions by vaginoscopy does enable the detection of a fine variation in colors of purulent discharge. The repeatability of vaginoscopic examination for different investigators and different times is acceptable.48 Furthermore, the visual assessment of the vaginal vault enables the differentiation of CE from vaginitis, cervicitis and vaginal trauma/lesions. While primary postpartum vaginitis in dairy cows is rare, the prevalence of cervicitis is estimated at 60.8%, with 29.1% of cows presenting prolapsed and hyperemic cervical mucosa.49 This is more than the prevalence of CE. While vaginoscopy is a rapid and simple technique, it may underestimate the prevalence of endometritis because of its low sensitivity. Vaginal discharge assessed by vaginoscopy between 27 and 33 DIM had 21% sensitivity and 89% specificity when referenced to 150-day pregnancy status.20 Barlund et al showed that vaginoscopy lacks sensitivity when compared to cytobrush endometrial cytology (Se=54%, cut-off=8%) and to 150-day pregnancy status.
between 28 and 41 DIM (Se=7.1%), with moderate agreement (kappa=0.52). In both studies, Sp was higher than 87% and all eligible cows were included. If the vaginoscopy is performed before 27 days postpartum, a larger number of normal cows may have mucopurulent discharge, thereby overestimating the real prevalence of CE. However, the false positive rate is probably not important because of the consistent and significant negative effect of vaginal discharge on reproductive performance, and the clear positive effect of treatment in restoring normal fertility of cows.

It should be noted that care is required when performing vaginal examinations to monitor uterine health in postpartum dairy cows. Performing vaginal examinations on several cows with the same instrument increases the risk of spreading transmissible diseases throughout the herd. Instruments need to be cleaned after each vaginal examination or disposable instruments used. Such examinations should always be done in a consistent and thorough manner.

Other diagnostic methods, such as Metricheck (Figure 2) or gloved hand, have been described as accurate vaginal examination techniques. With vaginoscopy as the reference diagnostic method, the Metricheck device and gloved hand have been shown to produce comparable results, although prevalence estimates tend to be higher with Metricheck. This is to be expected because the device collects a considerable amount of cellular debris in the vaginal mucosa that cannot be visualized. There is a higher rate of false positives (for CE) with Metricheck, the consequence of which would be the unnecessary or inadequate treatment of cows during the postpartum period and the resulting financial losses.

The gloved hand and Metricheck methods are also unable to distinguish certain categories of vaginal discharge, such as the cloudy category of vaginal discharge without macroscopic purulent flecks, which has been associated with decreased FSCR. This increases the risk of a misclassification. Cloudy mucus on vaginoscopic examination has also been associated with endometritis.

It is especially important to have a good scoring system for vaginal discharge because treatment efficacy varies among discharge categories over the threshold point. It is therefore vital to have a standardized diagnostic method and to know the limitations of the diagnostic test being used. As with transrectal palpation, vaginal examination is an indirect measure of uterine inflammation. False-positive findings, such as in the case of vaginitis or cervicitis, or false-negative findings where endometritis is not diagnosed because of a closed cervix, may occur. Madoz et al showed that hysteroscopy can be used as a direct measurement of uterus health status and to test the real prevalence of false-positive and false-negative diagnosis for endometritis. The Se, Sp, PPV, PNV and accuracy of the transrectal palpation and the vaginoscopy were 75%, 85%, 43%, 96% and 83%, and 100%, 85%, 50%, 100% and 86%, respectively, using hysteroscopy as the reference method.

All visual examination methods for categorizing vaginal discharge are recommended for diagnosing CE in postpartum dairy cows. They are useful routine diagnostic tools for veterinarians whose aim is to improve herd health management and maintain reproductive performance. The three visual vaginal examination methods show similar efficacy when it comes to characterizing vaginal discharge, and they have no negative effects on reproductive performance. Vaginoscopy does have the advantage of enabling the veterinary practitioner to rule out other abnormalities of the reproductive tract (Figure 3).

**Diagnostic test: endometrial cytology**

Subclinical endometritis is characterized by the absence of purulent vaginal discharge but inflammation of the endometrium as determined by cytology. Bacterial pathogens that contaminate the uterus during the postpartum period are recognized by the innate immune system, which then attracts inflammatory cells. While lymphocytes, macrophages, eosinophil leukocytes and neutrophils are all involved in eliminating uterine infections, the dominant type of professional cell, because of their ability to phagocyte bacteria, is the polymorphonuclear neutrophil (PMN, Figure#3). These neutrophils are recruited from the blood stream into the uterine lumen in order to fight bacterial pathogens. After the elimination of these pathogens, inflammation subsides and the neutrophils become limited to the fluid in the uterine lumen, later to be expelled by uterine contractions (Figure 4). Thus the proportion of neutrophils relative to endometrial cells is an indicator of the inflammatory process and an important characteristic of cytological endometritis. Gilbert and Santos showed that the proportion of
neutrophils on endometrial cytology (uterine lavage) in the first three weeks postpartum was high and likely reflecting the physiological inflammatory process of a normal uterine involution and remodeling occurring during that period and, of course, the uterine infection with the large amount of bacteria. All sampling in the study were from a single dairy farm representing a limitation of the study. The criterion for inflammation of the endometrium is percentage of PMN (#PMN/#PMN+endometrial cells) in a cytological sample from the uterus. As cytology of the endometrium is a direct evaluation of intrauterine cavity inflammation, and as there is no effect of estrus cycle stage on the percentage of uterine PMN in cows, PMN% is the near-gold standard for diagnosing postpartum endometritis in dairy cows and the reference for evaluating surrogate tests. The presence of a large amount of neutrophils on endometrial cytology after 21 days postpartum is most likely associated with the bacterial uterine infection. Barlund et al showed that cows with more than 8% PMN had a 1.9 greater risk of not being pregnant at 150 DIM, with a Se and Sp of 13% and 90%, respectively (n=189). In a study of over a thousand cows, based on a threshold of more than 6% PMN, cytobrush cytology showed a Se, Sp, PPV, and a NPV of 42%, 80%, 85%, and 34%, respectively. Cytological examination of the uterus is the most widely accepted definitive diagnosis for endometritis, although it cannot rule out other abnormalities of the caudal reproductive tract (eg. vaginitis or cervicitis, etc.).

Cytology analysis requires staining of the specimen and then examination under a microscope. The problem arises in the quantification step: there is little standardization in the number and type of cells counted, the magnification used, the number of high-power fields assessed, and the field selection on the slide. Melcher et al showed that six different slide assessments (count of 100, 300 and 500 cells at magnifications of 10x and 40x high-power fields) showed strong compliance (r=0.77-0.90), with the highest correlation coefficients found for counts of 100 and 300 cells. Agreement between methods (k=0.30 to 0.85) and observers (k=0.79) was good. Similarly, DeGuillaume showed a strong inter- and intra-readers agreement (CI95%, 0.89-0.94 and CI95%, 0.80-0.97, respectively). Another possible methodology for taking differential counts for neutrophils, lymphocytes, macrophages and epithelial cells is counting a maximum of 500 cells along five predetermined reading paths on the slide (Figure 5). However, counts of only neutrophils from 10 randomly selected fields at 40x magnification showed good agreement when compared to the previous method (R.C. LeFebvre, unpublished data). Different methods with different standards in different studies were designed to evaluate endometrial cytology (neutrophil counts). However, all studies reported a good reader agreement suggesting that the reading and scoring techniques used in endometrial cytology are solid.

Methods to collect endometrial material to assess inflammation include uterine lavage and cytobrush, both of which are well accepted diagnostic techniques. However, the cytobrush method (Figure 6) seems to be more reliable, rapid and consistent because it results in a better cell sample with less distortion (Figure 7). With uterine lavage, up to 17% of attempts do not result in the recovery of uterine fluid in a study. However, the cytobrush method is far from perfect. Like the biopsy technique, pressure on the cytobrush may influence the PMN/epithelial cell ratio. Furthermore, the small area sampled by the cytobrush may not be representative of the entire endometrium. In the mare, the technique appears to have low within-horse repeatability. Sampling of the cervix, the uterine body, the right and left uterine horns showed that inflammation is not equally present through the whole reproductive tract. However, SE in postpartum dairy cows diagnosed by endometrial cytobrush or uterine lavage shows similar negative effects in reproductive performance.

It is clear that more studies are needed to standardize endometrial cytology and thereby improve its accuracy. In general, the slide is prepared by rolling the cytobrush or using a centrifuge (fluid collected by uterine lavage) on a predetermined surface area of a clean glass microscope slide. The staining method of the endometrial material may also affect the analysis. Normally, the material is air-dried and the slide stained using a modified Wright-Giemsa stain (Diff-Quick™, Dabe Diagnostic, West Monroe, LA) or naphthol-AS-D-chloroacetate-esterase (Sigma, Montreal, Canada), then evaluated under the microscope. Naphthol-AS-D-chloroacetate-esterase is the more effective stain for identifying and counting neutrophils. However, its preparation is very labor-intensive compared to the Diff-Quick™ stain, making it impractical and not rapid enough for the field situation.
Overall, endometrial cytology with cytobrush is accepted as the best diagnostic test and the most practical in the field for postpartum endometritis. However, based on its low-to-moderate accuracy, its overall accuracy still needs to be improved.

**Diagnostic test: uterine bacteriology**

The uterine lumen in early postpartum dairy cows is contaminated with a variety of bacteria, but most cows will eliminate any bacterial infection by three weeks after calving. Based on the time of sampling after calving, the proportion of positive uterine bacteriology varies between 80% and 100% in the first 21 days postpartum.46 Essentially, the uterine cavity is assumed sterile by about 28 days postpartum.46 For cows that do not have a sterile endometrial cavity, the persisting infection will lead to endometritis.64,65 Recognized uterine pathogens associated with uterine lesions are *Trueperella pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum* and *Prevotella melaninoginica*.7 The presence of these bacteria in the uterus causes inflammation, delays uterine involution, and causes histologic lesions in the endometrium.66,67 Analyses of the bacteriological data of the uterine microbiome indicate that *E. coli* is the most prevalent bacteria in the first three days postpartum, with equally importance with *Streptococcus uberis* and *T. pyogenes* at nine days postpartum and where *T. pyogenes* is the most prevalent after 15 days postpartum.47 As with *E. coli*, *Clostrium perfringens* was the most prevalent anaerobic bacteria in the first seven days postpartum.56

Uterine bacterial infections perturb uterine and ovarian function, and fertility.58,69 There is a strong association between *Trueperella pyogenes* in the uterus of dairy cows during the postpartum period and CE and uterine pus.33,70,71 Cows with a positive culture for *Trueperella pyogenes* are more at risk of clinical endometritis.53 Although impaired fertility is more closely associated with certain pathogens, cows with intrauterine bacterial growth of any species at 21 DIM exhibit decreased fertility.72 Uterine bacteriology has been used to investigate false-positive cases of CE as determined by vaginoscopy. At 21-28 DIM, *Trueperella pyogenes* was found in 33.5% of samples and was correlated with vaginal discharge. In total, 17.3% and 28.5% of cows with endometritis as diagnosed with vaginal discharge showed negative bacteriology and endometrial cytology, respectively. Galvao et al., reported that 41% of samples collected in cows with purulent vaginal discharge were *Trueperella pyogenes*-culture positive.52 For sampling the uterine cavity, cytobrush and cotton swab are both acceptable techniques for generating reliable bacteriological results on uterine tract infection.74 The Se, Sp, PPV, and NPV of the presence of *Trueperella pyogenes* were 90.7%, 13.6%, 69.6%, and 22.1% for predicting nonpregnant status at 300 DIM.71 These accuracy measurements were similar to vaginal discharge, visual characteristic of uterine discharge and the presence of neutrophils. However, several factors can potentially affect the pregnancy status between the moment the test is performed (35±3 DIM) and 300 DIM. The uterine microbiome is very dynamic if certain bacteria are recognized as pathogens other may reduce the risk of uterine infection. For instance, Streptococcus has been reported as having a negative correlation on uterine health 56,67 although other reported a protective effect of it presence in early postpartum.47 Several factors can explain the different results and the question is still debated.

While bacteriology would seem to be a valuable alternative diagnostic tool, the test is very expensive, not practical to perform in the field, and incapable of yielding quick results. Uterine bacteriology is more appropriate for further clinical investigation of individual infertility cases and for research.

**Diagnostic test: leukocyte esterase, a new chemical test for endometritis**

The cytobrush technique is simpler and more rapid than uterine lavage,75 and more precise than ultrasonography of the uterus.76 It is considered the reference method for the cytological diagnosis of endometritis because it generates quality samples and exhibits the highest repeatability. However, it is not a cow-side test. Although neither complex nor expensive, endometrial cytology requires special instrumentation, technical expertise and time. From the standpoint of practicality and efficiency, a diagnostic test for endometritis must be rapid and provide results at the farm. This would enable the veterinary practitioner to make a treatment decision and execute this decision at the time of the diagnosis.
An alternative method to assessing inflammatory cells in the lumen of the uterus is the leukocyte esterase (LE) test. Leukocyte esterase is released from neutrophil cells and reacts with indoxil carbonic acid ester. The esterase releases indoxil, which reacts with diazonium salt and is oxidized, yielding a violet azo dye. The intensity of color in this dye is correlated with leukocyte count. This method has been used for the rapid diagnosis of inflammation in many body fluids, including urine, pleural fluid, peritoneal fluid and cerebrospinal fluid. It could also potentially be used as an indirect method for detecting neutrophils in the cow uterus. Santos et al., evaluated the efficacy of this method in diagnosing endometrial diagnosis in dairy cows. They used a commercial urine test strip containing leukocyte esterase (Multistix 10 SG, Bayer Corporation) in uterine fluid derived through saline lavage. They reported a high correlation between endometrial cytology and leukocyte esterase activity, with 96% Se and 98% Sp. However, with a larger number of cows (n=563), Cheong et al showed that at the optimal cut-off point, the esterase test had Se, Sp, PPV, and NPV of only 77%, 52%, 38% and 85%, respectively. Using a similar test, Denis-Robichaud et Dubuc showed Se, Sp, PPV, and NPV of 52%, 60%, 79% and 32%, respectively. There was moderate agreement between PMN and LE (k=0.43), compared to the good agreement found by Santos et al. (k=0.6). The LE was strongly correlated with proportion of neutrophils found in the uterus of cows with SE (Figure 8), and was also associated with reproductive performance.

In sum, leukocyte esterase shows promise as a cow-side test in the diagnosis of endometritis in dairy cows. However, more research is needed to further characterize and standardize the test, as well as to improve its accuracy.

**Diagnostic test: uterine lavage optical density, a new test for endometritis**

Several diagnostic techniques based on the visual scoring of vaginal discharge have been proposed for the diagnosis of CE. However, these techniques are indirect diagnostic methods and are subject to observer bias. A new method, uterine lavage optical density, represents a more objective, numerical measurement of vaginal discharge. The technique involves using a spectrophotometer to measure the concentration of substances in a suspension. As visible light passes through the suspension, it is scattered by particles present in the suspension. Greater scatter indicates more material in the suspension. In the case of the uterine lavage in postpartum dairy cows, optical density measures the amount of material in the uterine suspension (e.g. bacteria, exudates, cellular debris, inflammatory cells). Uterine lavage optical density (620 nm) was found to have Se, Sp, PPV, PNV and overall accuracy of 76.3%, 78.3%, 28, 96.7%, and 78.1%, respectively, when ROC and visual scoring of clinical endometritis were used as the gold standard. The presence of *Trueperella pyogenes* in uterine lavage was associated with high mean uterine lavage optical density, and cows with high optical density showed a higher percentage of neutrophils. Although uterine lavage optical density is a simple and rapid test that does not require special training, it is not a cow-side test. In addition to equipment cost considerations, there are a number of other issues to be resolved. The choice of optimal wavelength changes with the type and color of the uterine suspension. Furthermore, real light absorption may not be representative of the actual number of bacterial cells because many bacteria are colorless. This technique has not been compared yet to other diagnostic techniques.

**Overall perspective on diagnostic tests for endometritis**

Even though endometritis in postpartum has significant consequences on reproductive performance of dairy cows and on the economic sustainability of the dairy industry, diagnostic methods for the condition are still actively investigated. Veterinarians should be able to identify cows at a higher risk to remain open and to have a diagnostic test that identifies cows that would benefit from treatment. Diagnostic tests reviewed in this article have low-to-moderate Se and PPV, and a moderate overall accuracy. As the characterization and standardization of the diagnostic tests improve, tests will become more accurate and therefore more useful to veterinarians and dairy farmers. As the number of cows per farm increases and the number of people actually working on farms decreases, less time is available to
monitor general animal health and even more so the postpartum uterine health. Therefore, a reliable, simple and rapid cow-side test is desperately needed.

Very little is known about the overall diversity and dynamics of the uterine microbiota between the calving and the end of the voluntary period. Transversal studies where sampling is performed at one point of time during postpartum period and usually more often in the second half of the VWP (between 30 to 50 DIM) have not shed sufficient light on the dynamics of the process of uterine involution. Longitudinal studies where several, subsequent samples are taken during the whole periparturient period may help researchers to understand if the postpartum uterine pathologies are associated with other body functions of the entire animal such as the general metabolic status and the immune system of high producing dairy cows. As uterine remodeling and its associated physiological inflammation are normal during uterine involution, longitudinal studies would help to draw the line between physiological and pathological processes and therefore increasing the overall accuracy of the diagnostic tests when performed at the right time. Longitudinal studies may further help to understand the carryover effect of infertility in cows with endometritis at the end of the VWP.

There is growing evidence that the female reproductive tract infection is associated with a complex immune system. It is becoming clear that several features of innate immunity play a major role in uterine defense and the normal return to fertility in dairy cows at the end of the voluntary period (Lefebvre et al., 2016 companion article). As the same mediators play a role in both immunity and female physiological reproductive functions during the postpartum uterine repair, tests on an immunological basis may become better predictive tests for endometritis. In addition, longitudinal studies will allow researchers to understand the relationship between uterine disease and infertility.

Take-home messages
1. Diagnosing endometritis in postpartum dairy cows remains challenging for veterinarians.
2. The accuracy of a diagnostic test can be quantified using a number of different measures: sensitivity, specificity, negative and positive predictive values, likelihood ratio, area under the ROC curve, and odds ratio.
3. The concept of HOST-PATHOGEN-ENVIRONMENT interactions is a useful model for describing the complex interplay of factors resulting in uterine diseases in postpartum period.
4. The most important postpartum uterine disease in dairy cows is endometritis, which is prognostic for impaired reproductive performance.
5. The large variation in reports of the prevalence of endometritis is due to a number of factors: 1) inconsistency in the timing of examination during the postpartum period, 2) difference in diagnostic methods, 3) difference in how the pathology is defined, 4) true differences in prevalence between populations, 5) variation between individuals, 6) host-pathogen-environment interactions, and 7) complexity in the interaction between etiological pathogens.
6. All visual examination methods used to characterize vaginal discharge – vaginoscopy, gloved hand and Metricheck – are recommended for the diagnosis of CE in postpartum dairy cows. They are effective routine diagnostic tools that veterinarians can use to improve herd health management and maintain reproductive performance. Risk of contamination between cows with the use of Metricheck and visual recognition of other abnormalities with the vaginoscopy (cervicitis, vaginitis, lacerations, etc.) have to be taken in account.
7. Endometrial cytology with cytobrush or uterine lavage are both accepted methods for establishing a diagnosis of SE, although the cytobrush technique results in a better sampling, but both are not cow-sided methods.
8. Even though uterine pathogens have been isolated from the uterus of cows without CE/SE, the prevalence of *Trueperella pyogenes* is higher in categories 2 and 3 of vaginal discharge and is correlated to higher numbers of PMN on endometrial cytology. When establishing a diagnosis of CE, one should expect a rate of false positives of 17-28%, depending on the threshold used.
Practical implications

In general, most currently available diagnostic tests for monitoring uterine health status do not exhibit a very high Se, PPV or overall accuracy and are not always practical for the veterinarians to perform in a routine postpartum uterine health monitoring in dairy cows. However, whatever their weakness, most of the tests described in this paper are able to predict a significant negative effect on reproductive performance. With a more accurate and practical diagnostic test, veterinarians will be more confident in monitoring uterine health in postpartum dairy cows, restoring reproductive performance in animals with endometritis, applying the appropriate treatment and therefore ensuring effective herd health management.

Issues yet to be resolved

1. The best time window during the postpartum period in which to test uterine health status.
2. The value of combining different tests for diagnosing endometritis.
3. The real sequence of events during the whole periparturient period in dairy cow which are associated with postpartum uterine infection.
4. The dynamic behavior of the whole microbiota of the reproductive tract and its relationship with each pathologic conditions affecting the high producing dairy cows.
5. With more evidence of the importance of immunity in the etiology of postpartum uterine diseases, the value of immunological tests, alone or in combination with other tests, for predicting or diagnosing endometritis will become more and more important.

Conclusion

Bovine veterinarians need a simple and reliable cow-side diagnostic test to assess herd uterine health and estimate the risk of reproductive outcomes in postpartum dairy cows. The diagnostic tests proposed in the past fifteen years all have weaknesses, and it is clear that more research is needed to standardize their use and improve their accuracy. However, the lack of consensus on what constitutes the best predictor of a negative reproductive outcome should not stop veterinarians from being proactive in monitoring uterine health, nor from taking action when it comes to the diagnosis and treatment of endometritis in postpartum dairy cows.

Acknowledgement

The authors thank Susan Lempriere for reviewing an earlier version of this manuscript.

References


Table 1. Measures of diagnostic accuracy: definitions

<table>
<thead>
<tr>
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<th>LR+</th>
<th>LR-</th>
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<tr>
<td>Excellent</td>
<td>10.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Very good</td>
<td>6.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Fair</td>
<td>2.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Useless</td>
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Table 2. Classifying diagnostic accuracy based on AUC

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<tr>
<td>0.9-1.0</td>
<td>Excellent</td>
</tr>
<tr>
<td>0.8-0.9</td>
<td>Very good</td>
</tr>
<tr>
<td>0.7-0.8</td>
<td>Good</td>
</tr>
<tr>
<td>0.6-0.7</td>
<td>Sufficient</td>
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<tr>
<td>0.5-0.6</td>
<td>Bad</td>
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<tr>
<td>&lt;0.5</td>
<td>Useless</td>
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Table 4. Arbitrary benchmarks for kappa values (Landis and Koch, 1977)

<table>
<thead>
<tr>
<th>Kappa Value</th>
<th>Agreement</th>
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<tr>
<td>&gt;0.81</td>
<td>Almost perfect agreement</td>
</tr>
<tr>
<td>0.61-0.8</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>0.41-0.60</td>
<td>Moderate agreement</td>
</tr>
<tr>
<td>0.21-0.40</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>0.01-0.20</td>
<td>Slight agreement</td>
</tr>
<tr>
<td>0.0-0.01</td>
<td>Poor agreement</td>
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</table>

Table 5. Criterion standard test for diagnostic accuracy

<table>
<thead>
<tr>
<th></th>
<th>Disease+</th>
<th>Disease-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test+</td>
<td>TP</td>
<td>FP</td>
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<tr>
<td>Test-</td>
<td>FN</td>
<td>TN</td>
</tr>
</tbody>
</table>

Figure 1. Vaginoscopic examination on a postpartum dairy cow (R.C. LeFebvre).
Figure 2. Postpartum genital examination using the Metricheck device (Metricure Clinic Talker, Merck 2011). Troubled vaginal mucus with less than 50% pus, cathegory #2.

Figure 3. Vaginal visualization of the cervix and a lymphoma mass (right side of the cervix) spreading to vaginal wall and to the cervix during the vaginoscopy of the postpartum genital examination at 35 DIM.
Figure 4. Correlation between endometrial cytology percentage of neutrophils and DIM. Data represent least-square means ± SEM (Couto et al., 2011)

Figure 5. Methodology of RM slide evaluation. Slides were placed on a squared paper (0.5cm) and small dots were made at the bottom of the slide following the dots represented on the sketch previously made on the square paper. Following a virtual straight line bottom to top starting on 1 to 5, every 3rd field was assessed and the cells present were counted until a total of 500 cells were counted or until the top end of the 5th line was reached.
Figure 6. Uterine sample is collected using a cytobrush (VWR Canlab, Mississauga, ON, Canada). Briefly, the cytobrush is screwed onto a stainless steel rod and placed in a 65 cm-long stainless steel tube for passage through the cervix. The instrument is inserted into a guarded pipette, (Continental Plastic Corp.) for protection from vaginal contamination (R.C. LeFebvre).

Figure 7. Endometrial cytology made with a cytobrush (400X). A large number of neutrophils are present on the slide with endometrial cells and mucus and cytoplasm.
Figure 8. Relationship between endometrial cytology (EC % neutrophils) and leukocyte esterase activity scores in the uterus (n=218) and cervix (n=204). Data represent least-square means ± SEM. Bars with different superscript differ (P<0.05; Couto et al., 2011)

(Editor’s note: Photographs in this manuscript are available in color in the online edition of Clinical Theriogenology.)