

# Validation of two diagnostic methods for postpartum endometritis in dairy cows

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## Introduction

Postpartum uterine diseases can impair reproductive performance of dairy cows. Diagnostic tools have been developed to identify cows at risk of poor subsequent reproductive performance. Most of these tools focus on identification of inflammation in the reproductive tract during the postpartum period. Endometrial cytology was proposed as a good tool to identify cows with cytological endometritis; however, cytology is not an easy technique to implement for an on-farm uterine health surveillance program in dairy herds because it requires the use of a microscope. Therefore, a more convenient on-farm method needs to be identified for determination of uterine status of dairy cows. A leucocyte esterase (LE) colorimetric test is commercially available (Multistix®, Bayer Corporation, Elkhart, IN) and could be used to detect endometrial inflammation. The objectives of this study were to determine diagnostic criteria for endometritis in dairy cows by use of endometrial cytology and LE testing, to quantify the agreement between results of these two methods, and to quantify their impact on subsequent reproductive performance.

## Materials and Methods

A cohort study was conducted on 25 commercial dairy herds from October 2011 to November 2012. Herds were visited biweekly and cows were examined at 34 ( $\pm 7$ ) days in milk (DIM) for endometritis by means of a standard endometrial cytobrush technique. After collection of the endometrial specimen, the cytobrush was rolled on a microscope slide for cytological evaluation with a microscope (laboratory testing), and was dipped into 1 mL of sterile water for LE colorimetric testing (Multistix®; on-farm testing). Cytologic results were recorded as the proportion of polymorphonuclear cells present in the endometrial specimen, and LE results were classified into 1 of 5 categories (negative, trace, small [+], moderate [++], and large [+++]). The voluntary waiting period for breeding was 50 days. Subsequent reproductive events were recorded up to 250 DIM. Diagnostic criteria for endometritis were determined on the basis of the maximal sum of sensitivity and specificity

for predicting the risk of pregnancy within 120 DIM. Statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC). The extent of agreement beyond chance between the results from the two tests was calculated by use of a kappa ( $\kappa$ ) coefficient (PROC FREQ). The impact of these diagnostic criteria on subsequent reproductive performance (pregnancy risk at first service and proportional hazard of pregnancy) were quantified by use of a generalized linear mixed model (PROC GLIMMIX) and a Cox proportional hazard model (PROC PHREG), both of which included herd as a random effect to adjust for clustering of cows within herd.

## Results

A total of 566 Holstein cows were enrolled in the study. Cytologic endometritis (CYTO) was defined as cytologic results with  $\geq 6\%$  polymorphonuclear cells, and LE endometritis was defined as a score  $\geq ++$ . On the basis of those thresholds, sensitivity and specificity to predict pregnancy with 120 DIM were 39.9% and 72.1%, respectively, for endometrial cytology, and 35.4% and 75.2%, respectively, for LE testing. Prevalence of CYTO and LE endometritis were 34% and 30%, respectively, and agreement beyond chance between CYTO and LE endometritis results was moderate ( $\kappa = 0.54$ ;  $P < 0.01$ ). Both CYTO and LE endometritis diagnostic criteria were associated with a detrimental impact on first-service pregnancy risk (CYTO, risk ratio = 2.05,  $P < 0.01$ ; LE, risk ratio = 1.71,  $P < 0.01$ ), and on median time to pregnancy (CYTO, hazard ratio = 1.47,  $P < 0.01$ ; LE, hazard ratio = 1.32,  $P = 0.01$ ).

## Significance

Endometritis, defined as cytologic results with  $\geq 6\%$  polymorphonuclear cells or a LE score  $\geq ++$  (moderate), had a significant detrimental impact on subsequent reproductive performance of dairy cows. Results of this study indicated that CYTO and LE techniques can be used to identify cows at risk of subsequent reduced reproductive performance. Discrepancies between CYTO and LE results need further investigation.