J. Dairy Sci. 99:1–6
http://dx.doi.org/10.3168/jds.2016-11348

Prevalence of cytological endometritis and effect on pregnancy outcomes at the time of insemination in nulliparous dairy heifers

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ABSTRACT

The objectives of the present study were to assess the prevalence of cytological endometritis (CYTO) at the time of artificial insemination (AI) and its effect on pregnancy outcomes in nulliparous dairy heifers. In total, 512 endometrial cytology samples were taken during AI from 351 nulliparous Holstein-Friesian heifers using cytotape (a 1.5-cm piece of paper tape rolled on the top of an AI catheter covered with a double guard sheet). After sampling, the top of the AI catheter was gently rolled onto a glass slide, air-dried, and stained using Diff-Quick (Fisher Diagnostics, Newark, DE). For each slide, 300 nucleated cells were counted, and the polymorphonuclear cell ratio (% PMN) was assessed at 400× magnification. We constructed a receiver operating characteristic curve to find the cutoff point at which sensitivity and specificity (% PMN) affected pregnancy outcomes. The receiver operating characteristic curve revealed that the threshold level for diagnosing CYTO in nulliparous dairy heifers was 1% PMN. An insemination was considered successful when pregnancy was confirmed by rectal palpation at least 45 d post-AI. Heifers were considered not pregnant when they received a subsequent insemination or were diagnosed empty by rectal palpation. We built multilevel generalized mixed-effect models to test factors affecting pregnancy outcomes and the occurrence of CYTO at AI. We excluded 16 samples harvested from 12 heifers due to poor sample quality or unavailability of reproductive data. Of the 496 AI samples, the prevalence of CYTO at AI was 7.86% (n = 39). The conception rate was 62.8% (n = 287) in CYTO-negative samples (n = 457) and 38.46% (n = 15) in CYTO-positive samples. Risk factors for non-pregnancy were a previous AI (odds ratio 2.96; 95% confidence interval: 1.21–7.26) and the interaction between CYTO and previous AI. The only risk factor identified as being associated with the occurrence of CYTO was a previous AI (odds ratio 4.7; 95% confidence interval: 2.15–10.34). The performance of unsuccessful inseminations significantly affects reproductive outcomes in subsequent AI and may lead to CYTO in nulliparous dairy heifers.
Key words: cytological endometritis, insemination, reproductive outcome

INTRODUCTION

Ensuring the efficiency of AI involves paying attention to many factors, among them uterine health. It is well known that an adverse uterine environment provokes breakdown of uterine homeostasis, significantly decreasing the reproductive performance of the cow (Gilbert, 1992; Sheldon et al., 2006). Subclinical endometritis is a highly prevalent but asymptomatic uterine disease that can impair a cow's reproductive capacity (Gilbert et al., 2005). In the field, subclinical endometritis is diagnosed primarily by measuring the proportion of inflammatory cells in a cytology sample taken from the uterus and is therefore often referred to as "cytological endometritis" (**CYTO**; Sheldon et al., 2006; Dubuc et al., 2010a). Cytological endometritis can be diagnosed using the cytobrush (Kasimanickam et al., 2004; Pascottini et al., 2016), low-volume lavage (Gilbert et al., 2005; Pascottini et al., 2016), or cytotape (Pascottini et al., 2015). The main advantages of cytotape are its versatility (enabling sampling at AI or during the luteal phase) and its high-quality samples (Pascottini et al., 2015).

Subclinical endometritis is considered a postpartum disease, presumably associated with endometrial recovery after clinical endometritis, trauma, or other nonmicrobial diseases (Sheldon et al., 2009). However, CYTO samples have not, to our knowledge, been collected in nulliparous heifers to diagnose the condition in this group of animals. Although nulliparous heifers have not been exposed to risk factors for CYTO, such as retained placenta, acute metritis, or severe negative energy balance (Kasimanickam et al., 2004; Dubuc et al., 2010b; Cheong et al., 2011), to date there is no evidence of the presence (or absence) of CYTO in these animals. The objectives of the present paper were to

Received April 22, 2016.
Accepted July 27, 2016.
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assess the prevalence of CYTO and its effect on pregnancy outcomes in nulliparous dairy heifers. We also identified risk factors for the occurrence of CYTO and non-pregnancy following AI.

MATERIALS AND METHODS

Experimental Design

A total of 512 AI in Holstein-Friesian nulliparous heifers (n = 351) from 18 commercial dairy herds were included in this prospective observational cohort study, conducted from July 2014 to March 2015 in the Flemish region of Belgium. Participating herds were required to use a computerized record system for herd management. The number of heifers in the herds ranged from 10 to 96. All heifers were housed in freestall barns and had access to pasture during summer time. Heat was detected based on visual observations. Heifers that were showing signs of standing heat and were clinically healthy (no abnormal vaginal discharge) were included in the study at the time of AI. Heifers that required multiple inseminations before pregnancy occurred were sampled more than once.

One experienced inseminator from the Cattle Improvement Co-operative (CRV, Belgium) performed all inseminations. The farmer informed the inseminator when a heifer was detected in estrus, and inseminations were performed using the a.m./p.m. rule (Nebel et al., 1994). Endometrial cytology samples were obtained at the same time as AI using the newly developed cytotape (Pascottini et al., 2015). Briefly, cytotape consists of a 1.5-cm piece of paper tape (Tesa 4322, Hamburg, Germany) rolled on top of a loaded insemination catheter (Agtech, Manhattan, KS), and covered with a 12-inch-long Sani-Shield rod (Agtech). The cytological sampling and AI procedure was as follows. First, the heifer's vulva was cleaned with a paper towel. Then, the AI catheter (with the cytotape on top) was manipulated through the cervix and, once in the uterine lumen, the catheter was released from the Sani-Shield rod. Next, with some gentle pressure of the index finger through the rectum, the top of the catheter was rotated twice on the dorsal wall of the corpus uteri. Finally, after injecting the semen into the lumen of the uterine body, the AI catheter was covered with the Sani-Shield rod and carefully removed from the genital tract. Microscope glass slides (Marienfeld, Lauda-Königshofen, Germany) were prepared on the farm by rolling the top of the AI catheter on the readable area of the glass slide, homogeneously spreading the collected cellular material. Finally, smears were air-dried and housed in a slide box for storage and transportation.

Slide boxes were delivered every 2 wk to the laboratory facilities. All slides were stained with Diff-Quick (Fisher Diagnostics, Newark, DE), and once the slides were dry, Eukitt mounting medium (O. Kindler GmbH, Freiburg, Germany) was used to protect the specimens and hold the coverslips on the slides. Microscopic evaluation was done at 100× and 400× magnifications (Kyowa Optical, Tokyo, Japan) by a single experienced reader. In total, 300 nucleated cells were counted, and the polymorphonuclear cell ratio (**% PMN**) was evaluated (Melcher et al., 2014). The total cellularity and quality of the samples were assessed in 10 high-power fields at 100× (Cocchia et al., 2012; Pascottini et al., 2015). Samples were classified as low cellularity (<50 cells), moderate cellularity (50–100 cells), and high cellularity (>100 cells); and as poor quality (<50% intact cells), good quality (50–75% intact cells), and very good quality (>75% intact cells).

An insemination was considered successful when pregnancy was confirmed by rectal palpation at least 45 d post-AI. Inseminations were considered unsuccessful when they were followed by another insemination or when the animals were diagnosed as not pregnant by rectal palpation at least 45 d post-AI.

Statistical Analyses

Individual heifer data were taken from the data capture forms collected by the inseminator, and from the computerized record system at each farm and exported to Excel (Microsoft Corp., Redmond, WA). Statistical analyses were performed using R version 3.3.0 (R Inc., Boston, MA), considering the AI sample as the unit of interest.

Before the start of the study, a sample-size calculation was conducted to identify the difference in conception rate between diseased and non-diseased animals, with a 95% CI and 80% power (Dohoo et al., 2009). However, because neither the prevalence of CYTO nor the pregnancy risk in affected nulliparous heifers was available, we extrapolated variables that had been described in cows. We used a CYTO prevalence of 30% (Barański et al., 2012) and considered the conception rate in healthy heifers to be 67% (Brickell et al., 2009), versus 40.2% in diseased animals. Cows with CYTO had lower odds [odds ratio (**OR**): 0.6] of becoming pregnant than healthy cows (Cheong et al., 2011).

For the data set assessment, descriptive statistical analyses were conducted using the summary function of the R coding system (package base). A receiver operating characteristic (**ROC**) curve (package pROC; Robin et al., 2011) was constructed to assess the cutoff point where the higher summation of sensitivity and specific-