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Cytological endometritis at artificial insemination in dairy cows: Prevalence and effect on pregnancy outcome

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ABSTRACT

The aims of our field study in dairy cows were (1) to consolidate cytotape (CT) as a valid technique to diagnose cytological endometritis (CYTO) during artificial insemination (AI); (2) to establish a cutoff point concerning the polymorphonuclear cells (PMN) proportion to diagnose CYTO at AI; (3) to assess the prevalence of CYTO at AI; and (4) to evaluate the effect of CYTO on the pregnancy outcome of that AI. The investigation was performed using 1,625 AI-CT samples harvested from 873 Holstein-Friesian cows from 18 dairy farms in the Flemish region of Belgium. The CT device consisted of adapting a 1.5-cm piece of paper tape on the top of a conventional AI catheter covered with a double guard sheet, allowing an endometrial cytology sample to be taken when performing an AI. A receiving operator characteristic curve was built to assess the threshold level above which the PMN proportion significantly affected the AI success. Multilevel generalized mixed-effect models were built to identify factors affecting the pregnancy outcome of the AI under investigation. Only 7 samples (0.4%) harvested in 5 cows were discarded because of low-quality parameters. The cutoff point for CYTO at AI was set at ≥1% PMN (sensitivity = 33.8%, specificity = 88.6%). Prevalence of CYTO at AI was 27.8%. The conception rate for CYTO-positive samples was 32.7%, whereas it was 47% for CYTO-negative samples. A CYTO-negative AI had 1.8 [odds ratio (OR)] more chances to become pregnant than a CYTO-positive one. Other factors identified as detrimental for the pregnancy outcome were body condition score ≤1.5 (OR = 0.6), relative 305-d milk yield (OR = 0.9), dystocia (OR = 0.3), parity ≥2 (OR = 0.7), and warm months of the year. In conclusion, CT is a consolidated technique to diagnose CYTO at AI, PMN 1% is the threshold level to diagnose CYTO at AI, around one-quarter of inseminated uteri suffer from CYTO, and affected uteri having a significantly lower chance to become pregnant from that insemination.
Key words: dairy cow, artificial insemination, subclinical endometritis, cytotape

INTRODUCTION

High reproductive performance is a decisive factor for production and, hence, profitability in modern dairy herds (Plaizier et al., 1997; Krpálková et al., 2014). To assess an adequate reproductive efficiency, the first insemination conception rate must be as high as possible. However, multiple factors, such as semen quality, insemination technique, heat detection efficiency, delayed ovulation, and uterine diseases such as purulent vaginal discharge or cytological endometritis (**CYTO**), are known to significantly affect the success of AI (LeBlanc, 2008; Dubuc et al., 2010).

It is well accepted that the most convenient method to diagnose CYTO is by measuring the polymorphonuclear (**PMN**)-to-epithelial cell ratio in endometrial cytology samples (Sheldon et al., 2006); however, standardization of endometrial cytology diagnosis in dairy cows has not been fully established yet (Pascottini et al., 2015). In different publications, the time relative to calving the samples were taken varied from 21 to 64 DIM (Kasimanickam et al., 2004; Gilbert et al., 2005; Hammon et al., 2006, Barlund et al., 2008). Concomitantly, PMN threshold levels to diagnose CYTO ranged from 3 to 18% (Salasel et al., 2010; de Boer et al., 2014), resulting in a wide variation (9 to 76%) in reported CYTO prevalence (Barański et al., 2012). Consequently, comparing results between studies is almost unfeasible. However, a novel technique, cytotape (**CT**), was developed to take endometrial samples at the moment of AI (Pascottini et al., 2015). This simple technique achieved high cytology standards when compared with the cytobrush, its main advantage being the possibility to sample cows during AI by using ordinary material. Sampling during AI may have 3 significant benefits: (1) standardization of the moment of sampling and assessment of the uterine health status at the most critical point, the moment of insemination; (2) allowing

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the use of a universal PMN cutoff point, as the moment of sampling is standardized; and (3) no extra manipulation of the animal is required, as CYTO sampling and AI are performed simultaneously.

The timing of CYTO examination should allow consideration of the proper process of uterine involution, which is based on an inflammatory reaction (Sheldon et al., 2006). However, an inflammatory status at inappropriate stages of the reproductive cycle inflicts damage on gametes and zygotes, impairing the pregnancy outcome (Gilbert, 2011). Polymorphonuclear cells represent the first defense line and the principal cell type recruited during uterine inflammation (Rogan and Strauss, 2005; Herath et al., 2006). The presence of PMN in the uterine lumen is the result of an inflammatory cascade begun by the activation of immune receptors, leading to a proinflammatory state characterized by the secretion of inflammatory cytokines and chemokines (Gilbert, 2011; Ghasemi et al., 2012; Hailemariam et al., 2014; Kasimanickam et al., 2014; Sheldon et al., 2014). This proinflammatory milieu interferes with fertility by creating suboptimal conditions for sperm cell transportation and storage, oocyte maturation and ovulation, fertilization, zygote development, implantation, and embryonic and fetal growth (Gilbert, 2011), increasing the amount of subfertile animals in dairy farms. Thus, the aims of the present study were to (1) consolidate CT as a valid technique to diagnose CYTO during AI, (2) define the PMN proportion cutoff point above which the conception result of the AI is significantly decreased, (3) establish the prevalence of CYTO at AI in dairy cows, and (4) evaluate the effect of CYTO at AI on the conception rate.

MATERIALS AND METHODS

Study Design

This prospective observational cohort study was conducted from July 2014 to March 2015. To achieve a study with 80% power and a 95% confidence interval, the sample size was calculated to identify a difference of 10% in conception rate between diseased (30%) and nondiseased (40%) cows, with an expected CYTO prevalence of 25%. Based on this calculation, a total of 1,625 AI were performed in 873 Holstein-Friesian cows from 18 dairy farms. All participating dairy herds were located in the Flemish region of Belgium and were using freestalls for housing. Herd size ranged from 24 to 176, with an average of 76 ± 38 cows per herd. Cows were fed a TMR according to their production level and were milked twice daily. Farm-level inclusion criteria were the willingness of the farmer to cooperate and the availability of a data record-keeping software for fertility and milk production parameters. All included herds participated in the official milk-recording system in which cows are sampled every 4 to 6 wk to assess daily milk yield, fat, protein, lactose, urea, and SCC level. Cow-level inclusion criteria were healthy Holstein-Friesian cows presenting estrus and offered for insemination. Cows were sampled more than once if they did not conceive at the first AI sample and were offered for a next AI. Body condition score (1–5) was evaluated just before the AI sample in all cows (Ferguson et al., 1994).

Sampling Procedure

Cows were inseminated based on the a.m.-p.m. rule (Nebel et al., 1994), generally after spontaneous heat expression. All inseminations were done after the voluntary waiting period, which was set at 60 DIM. One experienced veterinarian from the company Cattle Improvement Co-operative (CRV-Belgium) performed all AI and simultaneously acquired the endometrial cytology samples using the CT. Cytotape consisted of a 1.5-cm piece of paper tape (Tesa 4322, Hamburg, Germany) rolled on the top of a standard AI catheter, covered with a double guard sheet (Sani-Shield Rod, Agtech, Manhattan, KS; Pascottini et al., 2015). All the CT sheets were prepared in advance to be ready for use at the farm. Briefly, the AI gun was introduced into the vagina and under rectal guidance manipulated through the cervix. Once in the uterine lumen (corpus uteri), the tip of the catheter was released from the double guard sheet, and then it was rolled twice on the dorsal wall of the uterine body (Bogado Pascottini et al., 2016) with a gentle pressure of the index finger through the rectum. At the end, before the removal of the AI gun from the genital tract, the AI was performed, and the catheter was covered with the double guard sheet and removed from the cow's genital tract.

Preparation, Staining of the Slides, and Microscopic Evaluation

Slides were prepared at the farm immediately following the AI sampling. The tip of the CT was gently rolled on a clean microscope slide (Marienfeld, Lauda-Königshofen, Germany), spreading the cellular material homogeneously over the entire slide. Next, after proper identification, slides were air-dried and housed in a slide box. Approximately every 2 wk boxes were delivered to the laboratory facilities for staining and evaluation. Stainings were done using Diff-Quick (Fisher Diagnostics, Newark, DE) according to the instructions of the manufacturer. Mounting media (Eukitt, O.Kindler GmbH, Freiburg, Germany) was applied on