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Risk factors associated with cytological endometritis diagnosed at artificial insemination in dairy cows

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ABSTRACT

In this study, we aimed to determine risk factors associated with cytological endometritis (CYTO) diagnosed at artificial insemination (AI) in dairy cows. The CYTO risk factors were evaluated based on 1.625 AI-CYTO samples obtained from 873 Holstein-Friesian cows from in total 18 dairy herds in Flanders (Belgium). The endometrial cytology samples were obtained using the cytotape technique, which consisted of adapting a 1.5 cm piece of paper tape to a standardly loaded AI catheter, covered with a double guard sheet. The polymorphonuclear cells' (PMNs) cut-off point for CYTO at AI was set at $\geq$ 1%. We constructed multilevel generalized mixed effect models in order to identify the risk factors associated with the presence of CYTO at AI. The CYTO prevalence at AI was 27.8% at the animal level, while the within-herd level prevalence ranged from 10.7 to 39.7%, with an average of 28.1%. Risk factors associated with the occurrence of CYTO were parity $\geq$2 [odds ratio (OR) = 1.8], days in milk (DIM) at AI $\geq$ 124 (OR = 0.4), and warm months of the year [July (OR = 2.9), August (OR = 2.3), and September (OR = 1.4)]. In conclusion, the present study supports that multiparous cows and cows that are inseminated in the summer months have a higher risk to suffer from CYTO at insemination, while the risk for CYTO is lower when the insemination is taking place at $\geq$ 124 DIM.

1. Introduction

Dairy cows farmed in intensive systems commonly need to deal with a high microbial contamination during and immediately after parturition [1]. If this bacterial contamination evolves to uterine infection, a proportion of the postpartum cows will eventually develop uterine disease [2]. Within the postpartum uterine disease complex, cytological endometritis (CYTO) plays an extraordinary role, being: highly prevalent, asymptomatic, and with a negative effect on subsequent fertility [3,4]. Although endometrial cytology (cytobrush [5] or low volume lavage [6]) are well-accepted techniques to diagnose CYTO during the voluntary waiting period (VWP) [7], it may not reflect the uterine health status at the time of fertilization and subsequent implantation. Consequently, an innovative technique (cytotape [CT]) was recently developed to take endometrial samples during artificial insemination (AI) in order to evaluate the prevalence and effect of endometrial inflammation at this time [8–10].

Prevalence of CYTO widely ranges among farms (9–76%) [6,11], suggesting that herd as well as individual risk factors are highly associated with the occurrence of this disease [11]. Risk factors for CYTO occurring during the VWP have been identified as retained placenta, negative energy balance, and acute metritis [5,11,12]. However, to the best of our knowledge, no study exists about the risk factors that are significantly associated with CYTO at AI. A high CYTO prevalence causes considerable economic losses to dairy farmers due to inadequate fertility results. Consequently, identifying risk factors associated with CYTO at AI, may allow to reduce the prevalence of this disease and the concomitant costs by relatively simple management interventions. Thus, in the present study, we aimed to determine the risk factors associated with CYTO diagnosed at AI in dairy cows.

2. Materials and methods

A convenience sampling protocol of dairy farms in the Flemish region of Belgium based on the availability of a computerized record system and the farmers' willingness to collaborate, was implemented for this prospective cross-sectional cohort study.

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First, a sample size calculation was done to assess the minimal number of inseminations to be included in order to perform a study with 80% power and a 95% confidence interval (CI) [13]. This sample size was designed based on an expected CYTO prevalence of 25% and an anticipated difference of 10% in the conception rate between affected (30%) versus unaffected (40%) cows. Based on this calculation, from July 2014 to March 2015, a total of 1.625 AI's in 873 Holstein-Frisian cows from 18 herds were performed in this study. Herd size ranged from 24 to 176 with an average of 76 ± 38 cows per herd, all cows being housed in free-stall barns with cubicles. Cows were milked twice daily and fed a TMR according to their milk production level. All included farms participated in the official milk recording system in which milk samples were analyzed every four to six weeks in order to assess milk yield as well as the fat, protein, lactose, urea and somatic cell count (SCC) level in milk. Cows presenting standing estrus (visual observation), and that were identified by the farmer to be inseminated, were enrolled in the trial. Cows showing signs of abnormal vaginal discharge were not included. Cows that not conceived at the first AI-sample were eventually sampled more than once. The body condition score (BCS; 1-5) [14] was assessed at AI.

All herds implemented exclusively AI for breeding the cows. All inseminations were done after the VWP [which was set at 60 days in milk (DIM)] by one experienced veterinarian from the company Cattle Improvement Co-operative (CRV-Belgium), generally after spontaneous heat expression (based on the a.m./p.m. rule [15]). Endometrial cytology samples were taken during AI using the CT technique. Briefly, the AI sheath (IMV, L'Aigle, France) was prepared in advance by rolling an 1,5 cm piece of paper tape (Tesa 4322, Hamburg, Germany) on its tip [8]. At the farm, after conventionally loading the universal insemination gun (Agtech, Manhattan, KS, USA) with a frozen-thawed semen straw (0.5 or 0.25 ml), it was mounted with the CT-AI sheath and covered with a 12-inch-long Sani-Shield Rod® (Agtech, Manhattan, KS, USA). A schematic illustration showing how the CT was assembled and adapted to the AI gun is depicted in Fig. 1. Once ready, the CT-AI gun was carefully introduced in the genital tract of the cow and manipulated through the cervix under rectal guidance. In the uterine lumen, the tip of the CT-AI gun was released from the Sani-Shield Rod®. Then, with some gentle pressure of the index finger through the rectum, the cytology sample was taken by rotating the top of the AI catheter on the dorsal wall of the *corpus uteri* [16]. Next, after injection of the seminal material, the catheter was covered with the Sani-Shield Rod® and carefully removed from the genital tract of the cow. Finally, the cellular material on the tip of the paper tape was uniformly spread (rolled) on a clean microscope slide (Marienfeld, Lauda-Königshofen, Germany), air-dried, and housed in slide boxes. At the laboratory facilities, slides were stained with Diff-Quick (Fisher Diagnostics, Newark, DE, USA), and once dry mounted with Eukitt® mounting media (O.Kindler GmbH, Freiburg, Germany). The cytologic evaluation was made by light microscopy (Kyowa Optical, Tokyo, Japan) at 400 X. The polymorphonuclear cells proportion (PMN%) was assessed by counting 300 cells (epithelial and PMNs) [17]. The threshold level for CYTO was set at ≥1% PMN [10].

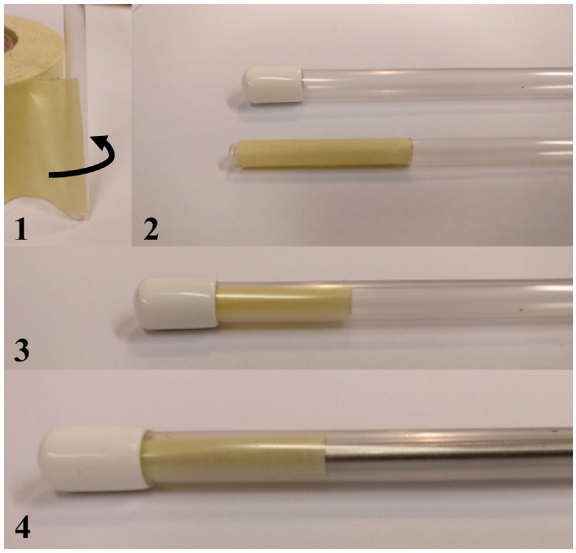

Fig. 1. Schematic illustration showing how the cytotape (CT) is assembled and adapted to the AI gun. 1) An 1,5 cm of paper tape is rolled on the tip of an AI sheath, 2 and 3) the CT-AI sheath is covered with a 12-inch-long Sani-Shield Rod®, and finally 4) the CT-AI sheath covered with the 12-inch-long Sani-Shield Rod® is adapted to the universal AI gun.

All statistical analyses were performed using R version 3.3.0, 2016 (R Inc., Boston, USA). Individual cow data such as CYTO (positive, negative), previous calving ease [eutocia, dystocia (traction, caesarean section, twins)], performance of a previous AI (no, yes), BCS at AI (1-5) [14], DIM at AI, month in which the AI was performed, parity (primiparous, multiparous) and milk yield measurements that were obtained during the official milk recording closest to the AI [milk yield (Kg), protein (g/l), fat (g/l), lactose (g/l), urea (mg/l) and SCC in milk (x 1000/ml)], were exported from the data record-keeping software of the farm into a Microsoft Excel spreadsheet file (Microsoft Corporation, Seattle, USA), and the R working environment. First, descriptive statistics were computed using the package Base of the R code system. Then, in order to determine the risk factors being significantly associated with CYTO (positive, negative), we constructed multilevel generalized mixed effects models (function glmer, package lme4 [18]). The CT sample number nested within cow, nested within herd (3 level model) were included as a random effect. The binomial CYTO condition (positive, negative) was set as the responsive variable. All information collected at individual cow level (previously mentioned above) was tested as fixed effects (explanatory variables). The BCS was categorized as lean (<1.5), normal (2−3.5) and fat (>3.5). The DIM at AI was categorized as follows: 61 to 81, 82 to 102, 103 to 123, 124 to 144, and ≥145 DIM [19,20]. The SCC in milk was expressed as SCC-log in order to normalize the data distribution. Moreover, the proportional 305 d milk yield was calculated and offered to the models. The proportional 305 d milk yield consisted in the 305 d milk yield of each cow blocked by parity and by farm [19]. All fixed factors showing $P < 0.2$ in the univariable models, and which were not highly inter-correlated ($r < 55$), were offered to the multivariable analysis by backward stepwise elimination. The final model of the multivariable analysis consisted of fixed effects (and first degree interactions) with P-value < 0.05. Results are expressed as odds ratios with their respective 95% confidence interval [13].

3. Results

Initially, a total 1.625 AI-samples from 873 cows in 18 dairy farms were included. However, 27 cows (47 AI-samples) were excluded due to the unavailability of their fertility outcome (culled or sold; n = 40) or bad quality of cytologic samples (null or scant cells; n = 7). The PMN average in CYTO positive samples was 5.3 ± 8.3% (median, 3%), ranging from 1 to 70%. Finally, 1.578 AI-samples from 846 cows had satisfactory cytology and reproductive follow-up data. Of the 1.578 AI-samples, 537 (34%) were harvested in primiparous heifers and 1.041 (66%) in multiparous cows.