SHORT COMMUNICATION

Cytological endometritis diagnosed at artificial insemination in repeat breeder dairy cows

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Contents
This study aimed to (i) assess the prevalence of cytological endometritis (CYTO) diagnosed at artificial insemination (AI); (ii) evaluate the effect of CYTO on the pregnancy outcome of the same AI sample; and (iii) determine the risk factors associated with CYTO diagnosed at AI in repeat breeder (RB) dairy cows. We analysed the productive and reproductive performances of 146 RB Holstein-Friesian cows. To obtain a CYTO sample at AI, we used the cytotape technique. Generalized mixed effect models were computed to find the risk factors associated with the pregnancy and CYTO outcome. Based on ≥1% PMN cut-off point, the CYTO prevalence at AI in RB cows was 25.3%. The overall pregnancy at AI was 44.2%. The conception rate in CYTO-positive (n = 37) RB cows was 29.7% versus 49.5% for CYTO-negative (n = 109) cows. A RB cow diagnosed CYTO positive at AI had 0.47 [odds ratio (OR)] odds to become pregnant in comparison with a CYTO-negative cow. Cows that produced more milk than their counterparts in this study had increased odds (OR = 1.01) to be CYTO positive at AI. A novel risk factor positively associated with CYTO diagnosed at AI in RB cows was the level of daily milk urea (OR = 1.11). To conclude, CYTO at the moment of AI had a significantly negative effect on the pregnancy outcome in RB dairy cows. However, as only one of fourth of RB cows is affected with CYTO at AI, it may not be considered a key element associated with the RB syndrome.

1 | INTRODUCTION

Repeat breeder (RB) cows are those with a regular estrous cycle (18–24 days) that fail to conceive after three or more services, in the absence of any detectable clinical abnormality. Reasons for RB are multiple and can be divided into two major categories: intrinsic and extrinsic (Gustafsson & Emanuelson, 2002). Intrinsic factors are linked to alterations in the normal physiology, resulting from an individual abnormality of the cow (Kendall, Flint, & Mann, 2009; Selvaraju et al., 2002). Extrinsic factors refer to managerial and environmental shortcomings, such as inadequate estrous detection, a deficient artificial insemination (AI) technique, poor semen quality and/or the adverse effects of heat stress on fertility (Ferreira et al., 2011; Gustafsson & Emanuelson, 2002; Yusuf et al., 2010). In a previous study, cytological endometritis (CYTO) has been identified as an intrinsic RB factor (Salasel, Mokhtari, & Taktaz, 2010). However, Pothmann et al. (2015) failed to find an association between the CYTO and RB prevalence. In this study, we aimed to diagnose the CYTO prevalence at AI in RB cows. Moreover, we studied both the effect of CYTO on the subsequent pregnancy outcome as well as the risk factors associated with CYTO diagnosed at AI in RB cows.

2 | MATERIALS AND METHODS

This retrospective cross-sectional cohort study was conducted from July 2014 to March 2015. We selected RB animals from a data set in which 1,625 AIs were performed in 873 Holstein-Friesian cows at 18 dairy farms. Repeat breeder cows were identified as cows that had not conceived after three or more consecutive AIs, without any clinically visible abnormality. Herd size ranged from 24 to 176 lactating cows, all housed in free stall barns and fed a TMR. Cows were milked twice.
daily and bred exclusively via AI. Endometrial cytology samples were harvested during AI using the cytotype (CT) technique (Pascottini, Dini, Hostens, Ducatelle, & Opsomer, 2015). As the AI sampling was performed based on commercial settings by one experienced veterinarian from the company Cattle Improvement Co-operative (CRV-Belgium), an ethical approval was not required for this experiment. The CYTO cut-off point at AI was set at ≥1% PMN (Pascottini, Hostens, Sys, Vercauteren, & Opsomer, 2017a).

Statistical analyses were performed via R Studio version 3.3.0 (R Inc., Boston, USA). Generalized mixed effects models (package lme4) were computed to assess the risk factors associated with both the pregnancy outcome and CYTO at AI. The fixed effects tested were as follows: ease of calving at previous parturition (eutocia, dystocia), BCS (lean [<1.5], normal [2–3.5] and fat [>3.5]) at AI, month of the AI, parity (primiparous, multiparous), CYTO (positive, negative) and milk production measurements; daily milk yield (kg), protein (g/L), fat (g/L), urea (mg/L) and somatic cell count (SCC) in milk ×1,000/ml. Unfortunately, farmers did not provide credible records of postpartum uterine disease (metritis and purulent vaginal discharge); therefore, we did not include them as fixed effects. The cow was set as the unit of interest. Farm was included as random effect. Univariable models were constructed, and variables with a p < .2 were identified and offered to the multivariable models. The multivariable models were built by manual stepwise backward elimination, and fixed effects and first degree interactions (p ≥ .05) were retained.

3 | RESULTS

Cows with both adequate cytologic and reproductive data were 146 in total. The mean AI number was 4.3 ± 0.7, and cows were at 208.6 ± 96.2 days in milk at AI sampling. Of all included cows, 34% (n = 50) were primiparous and 66% (n = 98) multiparous. The average milk yield closest to the AI-sample was 28 ± 6 kg. The mean (±SD) protein, fat, lactose, urea and SCC in milk were 36.9 ± 3.7 g/L, 43.5 ± 6.9 g/L, 45.5 ± 1.9 g/L, 233 ± 64.2 mg/L and 189.1 ± 405.8 ×1,000/ml, respectively. The overall conception rate of the 146 AIs was 44.2%. The CYTO prevalence at AI was 25.3% (n = 37). The conception rate was 49.5% for CYTO-negative and 29.7% for CYTO-positive RB cows. The results of the multivariable analyses for both the risk factors associated with fertility as well as those associated with CYTO are depicted in Table 1a,b.

4 | DISCUSSION

In a previous study in which we harvested endometrial cytology samples at AI, we found that ≥1% PMN was already detrimentally affecting fertility (Pascottini et al., 2017). Therefore, we decided to use the same cut-off point to assess the CYTO prevalence during AI in RB cows. The 25.3% CYTO rate in RB cows is equivalent to the overall prevalence of CYTO at AI in dairy cows (27.8%) (Pascottini et al., 2017). Therefore, results of the present study suggest that CYTO may not be the key reason for RB. On the other hand, Salasel et al. (2010) found a CYTO prevalence of 52.7% in RB dairy cows, indicating a close association between RB and CYTO. In agreement with our results, Pothmann et al. (2015) found a CYTO prevalence in RB cows of only 12.7%, suggesting only a minor association between CYTO and RB. These contradictory results may be related to the technique implemented to acquire samples and the CYTO cut-off point. Salasel et al. (2010) used the low-volume lavage technique (LVL) while Pothmann et al. (2015) used the cytobrush. The LVL collects PMNs already segregated in the uterine lumen, but also PMNs at the superficial endometrial epithelia (Pascottini et al., 2016). By cytobrush sampling, PMNs from only a very limited area of the superficial endometrium are harvested. These two different approaches may have had an influence on the reported prevalence of CYTO in RB cows. The CT resembles more the cytobrush technique, which may explain why our results are closer to those obtained by Pothmann et al. (2015). Moreover, Salasel et al. (2010) used 3% PMN threshold level, and Pothmann et al. (2015) set the cut-off point at 5% PMN. High-milk yield was already found to have counterproductive effect on fertility and also associated with higher risk of CYTO. However, the very novel finding in this study was the positive association between the milk urea level and CYTO prevalence in RB cows. In this context, we refer to the well-described correlation between high urea nitrogen concentration in plasma and milk and altered uterine secretions, and a concomitantly lower pregnancy rate (Butler, Calaman, & Beam, 1996; Rhoads, Rhoads, Gilbert, Toole, & Butler, 2006).

Our main conclusion is that CYTO may be not a key factor contributing to the RB syndrome. Moreover, we opened the possibility of a "new" cause of CYTO in dairy cows, an elevated urea level in milk.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.
AUTHOR CONTRIBUTIONS

OBP involved in study design, samples evaluation, statistical analysis and writing of the manuscript. MH involved in study design and statistical analysis. GO involved in study design and critical analysis of the manuscript.

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REFERENCES


