Theriogenology 86 (2016) 1550–1556

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Comparison between cytology and histopathology to evaluate subclinical endometritis in dairy cows

Osvaldo Bogado Pascottini [a,*], Miel Hostens [a], Pouya Dini [a], Jan Vandepitte [a], Richard Ducatelle [b], Geert Opsomer [a]

[a] Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
[b] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 17 May 2016
Accepted 17 May 2016

Keywords:
Dairy cow
Subclinical endometritis
Endometrial histopathology
Endometrial cytology

ABSTRACT

The aim of the present study was to compare endometrial cytology with histopathology to diagnose subclinical endometritis (SCE) in dairy cows. Endometrial cytology samples were collected from Holstein-Friesian cows (n = 32) just before slaughtering. Half of them were obtained by *in vivo* cytobrush (IV-CB), whereas the other half by *in vivo* low-volume lavage (IV-LVL). After slaughtering, reproductive tracts were collected, and the endometrium was sampled at eight locations. At each location, both a *ex vivo* cytobrush sample (EV-CB) and a tissue sample for histopathologic examination were taken. In the histopathology slides, polymorphonuclear (PMN) cell counts were differentiated as PMN cells in direct contact with the epithelial cells of the endometrium (PMN-EP), and PMN cells present in the deeper stratum compactum (PMN-SC). Summation of both countings was referred to as PMN-total. Pearson's correlation and Cohen's kappa coefficient were used to assess the correlation and agreement between both sampling methods (*in vivo* cytology [IV-CB and IV-LVL] with EV-CB and PMN-total). A Poisson mixed effect model was used to analyze the PMN cells' distribution. The prevalence of SCE was 18.75% (n = 6/32) for *in vivo* cytology. The SCE prevalence based on EV-CB analyses and on the assessment of PMN-total was determined both at the sample (n = 256) as well as at the cow level (n = 32): EV-CB 25% (n = 64/256) and 35.5% (n = 12/32), and PMN-total 37.11% (n = 95/256) and 59.38% (n = 19/32). Correlation and agreement between IV-CB and EV-CB were r = 0.81 and k = 0.97, whereas between IV-CB and PMN-total r = 0.15 and k = 0.23, respectively. *In vivo* low-volume lavage correlation and agreement were r = 0.52 and k = 0.66 with EV-CB, and r = 0.45 and k = 0.44 with PMN-total. Moreover, correlation and agreement between EV-CB and PMN-total were r = 0.60 and k = 0.50, respectively. More PMN cells ($P < 0.05$) were detected in PMN-SC when compared to PMN-EP and EV-CB. A higher SCE prevalence was found using histopathology, rendering the latter as a more sensitive method to diagnose SCE in comparison to *in vivo* and *ex vivo* cytology. Although cytology had low and/or moderate sensitivity to diagnose SCE when compared with histopathology, its specificity is 100%, implying that all cows that were indicated to suffer from SCE using *in vivo* cytology were confirmed to do so by histopathologic examination. There is an uneven distribution of PMN cells throughout the endometrium, generally more PMN cells being found in the deeper stratum compactum than in contact with the superficial layers of the endometrium.

1. Introduction

Uterine inflammatory processes in dairy cows may persist for enduring time periods, triggering a detrimental

* Corresponding author. Tel.: +32 497 177 718; fax: +32 9 264 75 46.
E-mail address: osvaldo.bogado@ugent.be (O. Bogado Pascottini).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.05.014

effect on further reproductive capacity [1–3]. Histologically, endometritis is defined as the presence of inflammatory cells in the uterine endometrium, with disruption (or not) of the epithelial layer [4]. When endometritis occurs without the presence of clinical signs, it is designated as "Subclinical Endometritis" (SCE). Subclinical endometritis refers to cows showing no clinical signs of endometritis but having an increased percentage of polymorphonuclear (PMN) cells in endometrial cytology being associated with reduced reproductive performance [5]. As SCE cannot be detected by simple visual inspection, complementary examinations are necessary for its diagnosis.

Cytology is the preferred technique to diagnose SCE in both field and research setups, mainly for reasons of simplicity and low cost [6,7]. Measuring the proportion of PMN cells in endometrial cytology is the hallmark of SCE diagnosis, which is therefore often also referred to as "cytological endometritis" [1,5,8]. The controversy, however, lies in which cytologic technique is most reliable, cytobrush (CB) or low-volume lavage (LVL) [9]. Advantages and disadvantages have been described for both techniques [10]. Sampling with CB is easier, yields an *in situ* sample with less distorted cells [8], and provides results faster in comparison to LVL [8]. However, CB evaluates only a very small portion of the endometrium [7,11,12], whereas LVL is considered to provide a more representative sample of the entire uterus [13–15], yielding a higher chance to harvest PMN cells from a larger endometrial surface [16,17].

Although no diagnostic test can be considered 100% accurate [18,19], histopathology is considered the gold standard to diagnose endometrial alterations, mainly because it allows to directly visualize both acute and chronic alterations in the epithelium and stratum compactum of the endometrium [14,20]. Unfortunately, at least in cows, biopsy sampling for histopathology is technically complicated and may be detrimental to subsequent fertility [19,21–24]. Because biopsy sampling itself may affect fertility [21,23,24], it is hard to objectively interpret the reproductive performance of sampled animals and identify which alterations should be considered as critically interfering with fertility. To the best of our knowledge, there currently are no peer reviewed articles available that demonstrate a significant association between the results of a histopathologic examination of the bovine uterus and the reproductive capacity of the sampled animal. Consequently, endometrial cytology has become more common in the last 10 years [25], among other reasons, because of its possibility to predict the cows' further reproductive capacity even in cows that have calved for longer periods [3].

Thus, the main objective of the present study was to assess the accuracy and efficacy of endometrial *in vivo* cytology to diagnose SCE using *ex vivo* cytology and histopathology as the gold standard, and to find out which *in vivo* sampling technique (CB vs. LVL) renders more reliable results. Moreover, we aimed to assess the representativeness of *ex vivo* endometrial cytology (CB sampling) versus histopathology by comparing multiple samples taken in close proximity of each other evenly spread over the whole uterus.

2. Materials and methods

All experiments described in the present article were carried out with permission of the Ethical Committee of the Faculty of Veterinary Medicine of the Ghent University (EC 2013/174).

2.1. Animals and procedures

For the present study, 35 Holstein-Friesian cows from one single dairy farm were initially enrolled. The day before cows were planned to be slaughtered, the first author was informed about this decision by the herd manager. Main reasons for culling were reproductive failure and high somatic cell count. Additional reasons were lameness and aging. Just before slaughter, a complete reproductive examination was performed by an experienced veterinarian. This included vaginal examination by the gloved hand method [26] and transrectal reproductive ultrasonography (Tringa, Esaote-Pie Medical, Maastricht, The Netherlands). Cows presenting any type of purulent vaginal discharge were excluded from the study. Uterus and ovaries were evaluated by ultrasound for presence (>0.5 cm) of fluid in the uterine lumen [1] or presence of follicular or luteal cysts (fluid-filled anechoic structure > 2.5 cm in diameter) on either of the ovaries [27]. Presence of fluid (>0.5 cm) in the uterine lumen, an ovarian cyst or severe lameness, were reasons to discard cows from the study.

2.2. In vivo sampling

Endometrial cytology samples were taken based on the ear tag of the cow. *In vivo* CB (IV-CB) was performed in even-, whereas *in vivo* LVL (IV-LVL) in odd-numbered cows. Before sampling, the perineum of the cows was cleaned with fresh water and dried with a paper towel. For the IV-CB technique, a Cytobrush Plus GT (Cooper Surgical, Berlin, Germany) was adapted to a stainless steel stylette of an universal insemination gun (Agtech, Manhattan, KS, USA), by heating the tip of the stylette with a lighter and fitting it to the base of the handle of the CB. After this, the CB was introduced in a sterile 22″ long equine infusion pipette (Agtech). Then, to protect the infusion pipette from contamination by vaginal and cervical cells, it was covered with a 21″ long sanitary sheath (IMV, L'Aigle, France). Next, under rectal guidance of a gloved hand, the pipette was introduced into the vagina and manipulated through the cervix. Once in the entrance of the uterine lumen, the sanitary sheath was punctured with the tip of the pipette so that the CB was released. Then, it was rolled twice with some gentle pressure of the index finger through the rectum, sampling in this way the dorsal part of the uterine body. In the end, the CB was reintroduced in the pipette, and the device was carefully removed from the genital tract. Immediately after the IV-CB sampling, the bristles of the brush were gently rolled on a microscope slide (Marienfeld, Lauda-Königshofen, Germany), which was then air-dried and subsequently housed in a slide box.

For the IV-LVL, a sterile 22″-long equine infusion pipette (Agtech) was introduced into the reproductive tract of the cow as described for the IV-CB sampling. Once in the