Theriogenology xxx (2014) 1–7

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Degree of variation and reproducibility of different methods for the diagnosis of subclinical endometritis

Y. Melcher [1], I. Prunner, M. Drillich*

Vetmeduni Vienna, Department for Farm Animals and Veterinary Public Health, University Clinic for Ruminants, Clinical Unit for Herd Health Management, Vienna, Austria

ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 13 February 2014
Accepted 1 March 2014

Keywords:
Endometritis
Cattle
Cytology

ABSTRACT

Endometrial cytology as a reliable diagnostic technique has been established for the diagnosis of subclinical endometritis (SE) in cows. Several counting techniques have been used to determine polymorphonuclear neutrophils (PMN) in endometrial samples. Information on the agreement between different techniques, however, is limited. Therefore, the objective of this study was to analyze the degree of variation in the percentage of endometrial cells and PMN determined by six different counting techniques. A second objective was to evaluate the interobserver reproducibility of the cell counting by two different examiners. One hundred samples were examined by the different counting techniques. The applied methods counted a total of 100, 300, or 500 cells (C100, C300, C500), respectively. In addition, method HPF100 and HPF300 counted 100 and 300 cells in 10 high-power fields per slide. Finally, one method estimated (EST) the percentage of PMN by screening the slide under the microscope. The interobserver reproducibility between two examiners was analyzed for method C300. The comparison between the six different methods showed a strong compliance ($r = 0.77–0.90$) with greatest correlation coefficient between C100 and C300. The results of Kappa statistics revealed agreement between methods varying from $\kappa = 0.30–0.85$, with the greatest agreement between HPF300 and EST. Furthermore, the impact of the different methods on the resulting prevalence of SE was calculated, with the greatest prevalence determined by C100 (33.0%) and the least by HPF300 (10.0%). The results of the interobserver reproducibility showed good correlation and agreement ($r = 0.86$, $\kappa = 0.79$). In conclusion, all examined methods were suitable for the cytological evaluation of PMN, with method C100 showing lowest agreement with the other methods. This confirms the hypothesis that a suitable threshold for PMN is not only influenced by, for example, time of sampling postpartum, but also by the diagnostic method. A threshold of 5% PMN seems to be useful when C300 and HPF100 are used, whereas counting 100 cells or estimating the percentage of PMN seems to overestimate or underestimate the prevalence of SE, respectively. In conclusion, method C300 and HPF100 can be recommended as methods of choice for evaluating the percentage of PMN in endometrial samples to diagnose SE.

1. Introduction

A regular uterine function is an important factor for reproductive performance of cows and for the economic success of dairy farms. An inflammation of the postpartum endometrium is a risk factor for subfertility in the subsequent breeding period. Pathogenic bacteria can be isolated

* Corresponding author. Tel.: +43 1 25077 5296; fax: +43 1 250 77 5290 .
E-mail address: marc.drillich@vetmeduni.ac.at (M. Drillich).
[1] Present address: Center for Experimental Medicine, Medical Faculty, University of Cologne, Cologne, Germany.

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.03.003

from the uterus of most cows postpartum [1]. Uterine infection causes delayed uterine involution, histological lesions of the endometrium, severe inflammatory responses, and perturbed embryo survival [1,2]. These effects result in prolonged intervals from calving to first service, lower conception rates, prolonged days open, and more cows culled because of infertility [1–3].

Uterine diseases within the first 3 weeks after calving can be defined as postpartum and clinical metritis. For uterine diseases later in lactation, the terms postpartum or clinical endometritis (CE) and subclinical endometritis (SE) have been established. On the basis of a generally accepted definition by Sheldon et al. [1], vaginal discharge is a criterion for the diagnosis of CE. Clinical endometritis manifests either a purulent (≥50%) uterine discharge in the vagina ≥21 days postpartum (dpp) or a mucopurulent (50% pus, 50% mucus) discharge in the vagina after 26 dpp [1]. Recent research, however, showed a lack of specificity of this criterion and suggested to use results of endometrial cytology for the diagnosis of endometritis [4]. Subclinical endometritis is characterized by the absence of purulent material in the vagina but by the inflammation of the endometrium determined by cytology [5,6].

Lymphocytes, macrophages, eosinophil leukocytes, and neutrophil leukocytes are the most important cells related to inflammatory processes [7,8]. Polymorphonuclear neutrophils (PMN) represent the major proportion of cells for an elimination of infection in the uterus based on their ability for phagocytosis [9,10]. The criterion for an inflammation of the endometrium is the percentage of PMN determined in cytological samples from the uterus [2,11]. Although an infiltration of PMN can be regarded as physiological during estrous [12,13], Madoz et al. [14] found that there is no significant effect of different stages of the estrous cycle on the variation of percentage of PMN if used for the diagnosis of SE.

Different methods can be used to collect endometrial and inflammatory cells from the uterus, including biopsy, low-volume uterine lavage, and the cytobrush technique [1,4–6,8,11,14–19]. The cytobrush technique has been described as a reliable and easy to perform method that provides results quickly [15]. The cytobrush technique was described in the late 1970s to detect malignant degeneration in the cervical canal of women [8,20]. Almost 10 years ago, Kasimanickam et al. [16] described the cytobrush technique as a useful method for the evaluation of bovine endometrial cells. The cytobrush is screwed on a metal rod, protected by a disposable plastic catheter, inserted into the uterus and rolled alongside the uterine wall [5,6,8,11,14]. Afterward, the cytobrushes are rolled onto a microscopic slide, fixed, stained, and evaluated under the microscope [21]. The number and type of cells counted, the magnification of the microscope, the number of high-power fields, and other criteria for the evaluation of the samples are not standardized and vary between studies [4,6,11,22,23]. Not only the criteria for evaluating cytological smears, but also thresholds of PMN for defining SE are still under discussion. Frequently used thresholds range from 5% to 18% PMN (Table 1).

There is no generally accepted gold standard for the evaluation of cytological smears for the diagnosis of SE. Therefore, the objective of this study was to evaluate the degree of variation of the percentage of PMN determined by six different counting techniques. The hypothesis was that a threshold of 5% PMN for the diagnosis of SE is influenced by the counting method. A second hypothesis was that a high interobserver agreement indicates that the evaluation of the cytological smears is a reliable technique for the diagnosis of SE. The results should help to define a generally accepted standard for determining the percentage of PMN for the diagnosis of SE in cows.

Table 1
Different thresholds for PMN to define SE.

Reference	PMN threshold	dpp	Justification for threshold
Barlund et al. [4]	8%	28–41	Based on literature
Baranski et al. [24] (2012)	18%	21–28	Based on literature
	8%	21–28	
	5%	21–28	
	10%	35–42	
Galvao et al. [17]	4.0%	42	Threshold based on ROC analysis
	6.5%		
	8.5%		
Gilbert et al. [2]	5%	40–60	Threshold was set arbitrarily
Hammon et al. [25] (2006)	25%	28 (±3)	Threshold was set arbitrarily
Kasimanickam et al. [6]	18%	20–33	Threshold based on ROC analysis
	10%	34–47	
Madoz et al. [14]	8%	21–33	Threshold based on ROC analysis
	6%	34–47	
	5%	21–62	
	4%	48–62	
McDougall et al. [26]	9%	28	Threshold based on highest quartiles
	7%	42	of PMN% in the population
Plöntzke et al. [18]	5%	18–38, 32–52	Threshold was set arbitrarily
Sens and Heuwieser [21]	10%	21–27	Threshold based on ROC analysis
	10–18%		
	18%		

Abbreviation: ROC, receiver operating characteristics.