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The evaluation of endometrial cytology in cows with acute and chronic endometritis

Ertan Oruc[1]*, Yavuz S. Saglam[1], Bulent Polat[2], Mehmet Cengiz[2], Armagan Colak[2], Serdar Altun[1], Orcun Cannazik[2], and Kubra A. Terim Kapakin[1]

[1]*Ataturk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum, Turkey*

[2]*Ataturk University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Erzurum, Turkey*

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ABSTRACT

The aim of the present study was to evaluate the findings of endometrial cytology in cows with acute and chronic endometritis. For this purpose samples were collected from 217 Brown Swiss and Holstein cows, housed on the Atatürk University Dairy Research Farm between the years 2010 and 2012, and they were stained with Giemsa for cytological examination. In the evaluation, overall 100 cells were counted in the microscopic area and the cells were classified as polymorph nuclear leukocyte, macrophage, lymphocyte and epithelial cells. The cytopathological classification was done according to the percentages of inflammatory cells. Briefly, 126 (58.06 %) samples had extensive inflammatory cells, and of the uterine samples 91 (41.94 %) had normal exfoliation. According to the cellular density results, acute, and chronic and subacute endometritis were described in 68 (31.33 %), 23 (10.60 %) and 35 (16.13 %) cases, respectively. In conclusion, endometrial cytology was found to be an applicable and reliable diagnostic method in diagnosis and diffentiation of acute and chronic endometritis.

Key words: cow, endometrial cytology, endometritis

Introduction

Uterine infections generally begin as endometritis and become complicated with other forms in a short period. While epithelial desquamation and slight cellular infiltration are presented in cows with mild endometritis, lymphocytes and plasma cells become predominant in moderate forms. Similarly, leukocytes masses on the mucosal surface are

*Corresponding author:

Ertan Oruc, Ataturk University, Faculty of Veterinary Medicine, Department of Pathology, 25240, Erzurum, Turkey, Phone: +90 442 231 2299; Fax: +90 442 2360 881; E-mail: eoruc@atauni.edu.tr; ertanoruc@hotmail.com

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associated with purulent metritis and the chronic form consists of lymphocytes, plasma cells and fibrosis (SCHLAFER and MILLER, 2007). A purulent or muco-purulent vaginal discharge may be detected in cows suffering from clinical endometritis. In contrast, clinical and systemic signs, such as abnormal vaginal discharge, fever or anorexia are not observed in cases of subclinical endometritis, although inflammatory cells are present in the uterine lumen. Due to the absence of these noticeable clinical signs, subclinical endometritis cases are seen by fertility problems in dairy herds (CHAPWANYA et al., 2009; WALTER et al., 2012; ZOBEL, 2013). Therefore, postpartum uterine diseases, which cause production losses due to decreased fertility and increased treatment costs, become persistent and costly in dairy herds (SHELDON et al., 2006; LEBLANC, 2008). ZOBEL (2013) reported that the rates of clinical endometritis and subclinical endometritis were 15.31 % and 7.77 % in cows, respectively. As ultrasonography, which is the most common diagnostic tool in animal reproduction, is inadequate for detection of subclinical cases (BARLUND et al., 2008), endometrial cytology is the preferred laboratory method in mares (CARD, 2005; RIDDLE et al., 2007; OVERBECK et al., 2011; COCCHIA, 2012), and cows (KASIMANICKAM et al., 2004; GILBERT et al., 2005; KASIMANICKAM et al., 2005; BARLUND et al., 2008; DUBUC et al., 2010) despite its application difficulties in field conditions. Although many of these studies, which were based on PMNL, defined various PMNL percentages as a diagnostic cut-off value of about 5-6 % (ORAL et al., 2009; DUBUC et al., 2010; COCCHIA, 2012), the cytological diagnosis of subacute or chronic endometritis, based on the predominance of lymphocytes, is not a common criterion.

The objectives of the present study were to define the cytological characteristics of endometrial smears in terms of polymorph nuclear leukocyte (PMNL), macrophage (M), lymphocyte (L) and epithelial cells (EC), and to improve the pre-diagnostic criteria in the differentiation of acute and chronic endometritis in cows.

Materials and methods

Cows and herd. Overall, 217 Brown Swiss (n = 157) and Holstein (n = 60) cows, which were housed on the Atatürk University Dairy Research Farm (39°54'E; 41°13'N; altitude of 1980 m) between 2010 and 2012 were introduced to the study.

Sample collection and fixation. Endometrial cytobrush samples were collected using a cytobrush (Plasti-med®, Istanbul, Turkey) in the dioestrus stage of the reproductive cycle. Stainless steel catheters which were placed into the plastic examination gloves were changed for each animal, in order to prevent contamination via perineal and vaginal content. The cytobrush sampling was performed as previously described by KASIMANICKAM et al. (2004). In short, after cleaning the vulva with soap and water, the catheter was inserted into the vagina up to the external cervical orifice, where the glove was punctured and the catheter inserted through the cervical canal into the uterine lumen.

When the catheter reached the uterine corpus, it was rotated in a clockwise direction and retracted into the cover, removed from the uterus, and the obtained samples were smeared onto glass. Samples were fixed using methylene for 5 minutes and sent to the laboratory (Pathology Department, Faculty of Veterinary Medicine, Ataturk University). The same researcher performed all sampling procedures in the study.

Staining, microscopic examination and assessment. Giemsa staining was performed on all slides and the samples were examined under a light microscopy (Olympus, BX51 with DP72 camera system). For cytological diagnosis, all slides were evaluated in terms of exfoliation quality, cellularity, background material and exfoliative cells.

For evaluation, all areas were examined under ×4 and ×10 objectives on each slide and the regions in which there was homogeneous spreading were chosen. Then, the exfoliated cells were counted at a magnification of ×40. Finally, 100 cells were counted and classified as PMNL, M, L or EC, according to their morphological characteristics. Under the guidance of previous reports (ORAL et al. 2009; DUBUC et al. 2010; COCCHIA et al. 2012), we accepted ≥5 % of inflammatory cells in ten microscopic areas at ×40 magnification as positive result. Additionally, the percentages of PMNL and L were used for identification of acute, chronic and subacute inflammations and cytological description was made according to the cellular proportion.

Statistical analysis. Statistical significance in the occurrence of inflammation types was evaluated by chi-square analysis. Statistical significance was assessed at $P<0.05$.

Results

The results are presented in Figs 1-3 and Table 1. Normal exfoliation was diagnosed in 91 (41.94 %) samples having epithelial clusters and clumps in the microscopic areas. These cellular groups were compact (Fig. 1a-d) with round (Fig. 1c) or bullet shaped (Fig. 1d) EC and with scant cytoplasm.

Inflammatory cells (PMNL, L and M) were determined in 126 (58.06 %) samples. In cases of PMNL, which had 2-5 segmented nuclei and pink stained cytoplasm, we diagnosed both mild and moderate endometritis in the cows (Fig. 2a and b). The presence of a significantly higher number of PMNL in cows led to the diagnosis of severe endometritis (Fig. 2c and d). L, which had round shaped nuclei without cytoplasm (Fig. 3a and b) and M, had much larger cells and large-abundant cytoplasm and sometimes consisted of phagocytic materials (Fig. 3c and d). According to the percentage of PMNL and L, the type of endometritis was described as acute (n = 65, 31.33 %, $P<0.05$), chronic (n = 23, 10.60 %) and subacute (n = 35, 16.13 %) as shown in Table 1.

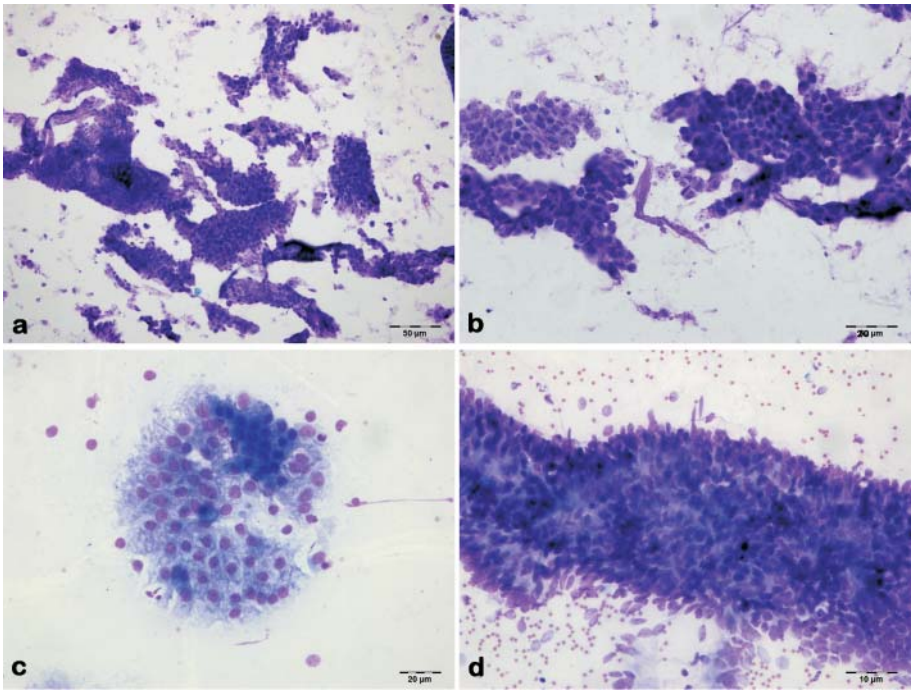

Fig. 1. Normal endometrial exfoliation of healthy uterine. Epithelial clusters (a) and close view (b) epithelial cells with scant cytoplasm (c) and finger print view from uterus in proliferative phase (d). Giemsa stain.

Table 1. The percentage of inflammatory cells and cytological proportion

Cytological description	N = 217	% PMNL	% L	% M	mucus
Normal endometrial cytology (NEC)	91	0-4	0-4	variable	variable
Acute endometritis	68*	³5	£5	variable	variable
Chronic endometritis	23**	£5	³5	variable	-
Subacute endometritis	35	5³	5³	variable	variable

PMNL = Polymorph nuclear leucocytes, L = Lymphocytes, M = Macrophages. *Superscript represents statistical significance at the level of P<0.05 depending on PMNL proportion. **Superscript represents statistical significance at the level of P<0.05 depending on L proportion.

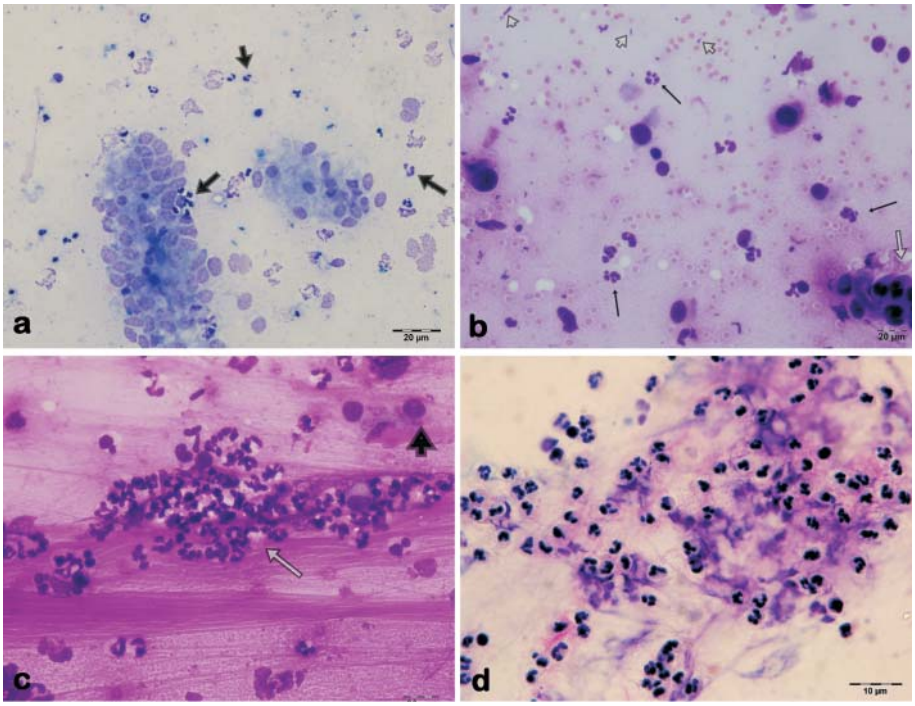

Fig. 2. Limited PMNL (arrows) and epithelial clusters (a). Increasing PMNL (black arrows) and more less epithelial clusters (white arrow) (b). Diffuse PMNL (white arrow), mucus on the background and a few epitheliums (c). Diffuse PMNL in all microscopic areas (d). Giemsa stain.

Background staining was variable, depending on the cellular composition of the smears. Mucous substances (Fig. 2c-d), necrotic debris and bacteria were observed in many samples, which were collected from acute or subacute endometritis.

Cytological description and cellular morphology are presented in Table 1.

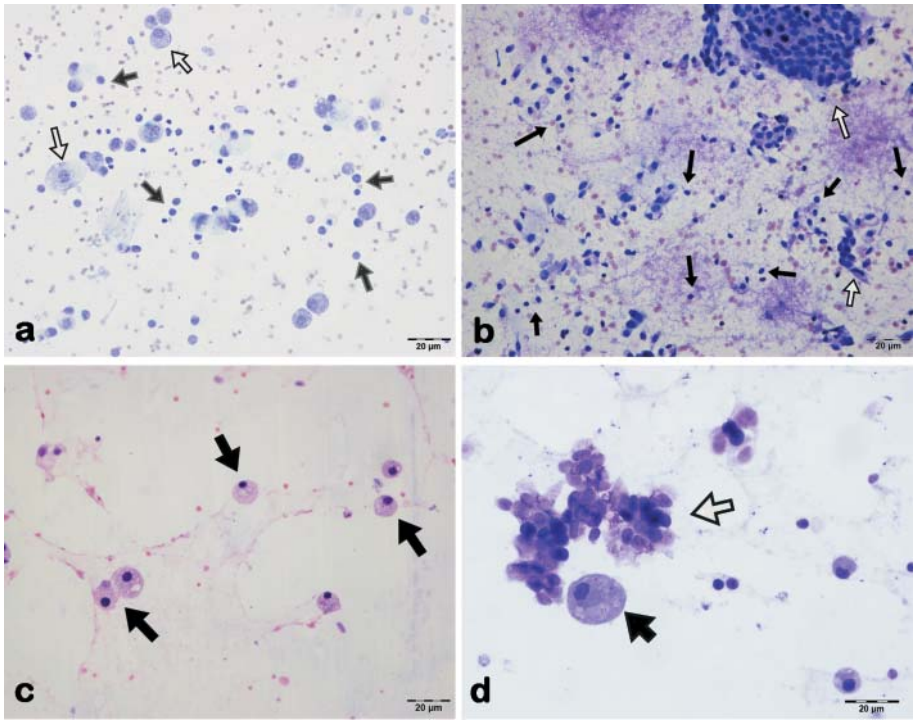

Fig. 3. Chronic endometritis. Lymphocytes are dominant in the microscopic area (black arrows) and phagocytic macrophages (white arrow) (a). Similarly, diffuse lymphocytosis (black arrows), epithelial clusters and fibrous material on the background (b). Typical phagocytic macrophages (arrows) (c). Phagocytosis (black arrow) and epithelial clusters (white arrow) (d). Giemsa stain.

Discussion

Due to absence of clinical signs, such as vaginal discharge or systemic illness, subclinical endometritis causes problems such as infertility, sustainability, and production losses (CHAPWANYA et al., 2009; WALTER et al., 2012; ZOBEL, 2013). Therefore, postpartum uterine diseases, which could not be detected early, become persistent and cause economic losses. Some diagnostic methods such as vaginoscopy (KASIMANICKAM et al., 2004; BARLUND et al., 2008; ORAL et al., 2009; DUBUC et al., 2010), ultrasonography (USG) (KASIMANICKAM et al., 2004; BARLUND et al., 2008; ORAL et al., 2009), microbiologic culture (NIELSEN, 2005; RIDDLE et al., 2007; OVERBECK et al., 2011) and biopsy (NIELSEN, 2005; RIDDLE et al., 2007) have been used alone or together with endometrial cytology for diagnosis of endometritis. In particular methods based on endometrial sampling, such as

cytology and biopsy, were found to be more reliable than other diagnostic tools. Although endometrial biopsy had the most definitive results, this method was not suggested due to its negative effects on future pregnancies (ETHERINGTON et al., 1988; BONNETT et al., 1993). Besides, in some studies, it has been shown that uterine biopsy did not affect the reproductive performance of dairy cattle (GOSHEN et al., 2012). Endometrial lavage, another intra uterine sampling procedure, was not preferred due to application difficulties in field conditions and at the cow's side (BARLUND et al., 2008). Finally, the cytobrush technique was accepted to be the most reliable and applicable diagnostic procedure for detection of subclinical endometritis (KASIMANICKAM et al., 2004; PRIETO et al., 2012). The sensitivity and specificity of the method was higher than those mentioned above. Additionally, the use of cytology together with ultrasonography was also suggested to increase the sensitivity and specificity of these methods by previous authors (BARLUND et al., 2008; OVERBECK et al., 2011; ZBYLUT et al., 2012). In the present study, we tried to demonstrate lymphocyte and macrophages apart from PMNL. According to the cytological exfoliation results, an initial assessment could be done for acute, chronic or subacute endometritis.

As shown in Table 1, PMNLs were dominant in 62 samples (31.33 %). The number of PMNLs varied between 5 and 100 (not evaluated below 5) according to the severity of inflammation and these samples were described as acute endometritis.

Lymphocytes were predominantly observed in 23 samples (10.60 %), which we defined as chronic endometritis. Significantly, PMNL and lymphocytes were seen together in varying degrees in 35 samples (16.13 %) and this was described as subacute endometritis.

As mentioned above, some clinical symptoms, such as vaginal discharge or systemic illness, are seen in acute or clinic endometritis. Chronic or subacute endometritis often does not present clinical signs and it cannot be diagnosed (or misdiagnosed) in palpation per rectum (SHELDON et al., 2006). Determination of lymphocytic exfoliation in smears would be useful for the diagnosis of chronic and subacute endometritis.

In the present study, a varying number of macrophages was seen as both lymphocytes and PMNLs. Therefore, macrophages, although associated with inflammation, were not evaluated as a criterion for distinguishing acute or chronic inflammation.

In conclusion, the cytological morphology of endometritis was clearly described and classified as acute or chronic endometritis. Endometrial cytology based on the presence of L might be a reliable diagnostic criterion in the diagnosis of chronic and subclinical endometritis in cows. These findings should be supported by future studies and confirmed with pregnancy rates.

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SAŽETAK

Cilj istraživanja bio je procijeniti citološke nalaze u endometriju krava s akutnim i kroničnim endometritisom. U tu su svrhu između 2010. i 2012. godine bili prikupljeni uzorci od 217 krava smeđe i holštajnske pasmine, uzgajanih na pokusnoj mliječnoj farmi na Sveučilištu Atatürk. Uzorci su bili obojeni Giemsinim bojenjem. Pri procjeni je u vidnom polju mikroskopa bilo izbrojeno ukupno 100 stanica koje

su razvrstane u polimorfononuklearne leukocite, makrofage, limfocite i epitelne stanice. Citopatološko razvrstavanje bilo je provedeno na osnovi postotka upalnih stanica. Ukratko, upalne stanice bile su ustanovljene u 126 (58,06 %) uzoraka, dok je u 91 (41,94 %) uzorku maternice ustanovljeno normalno ljuštenje stanica. Na osnovi stanične gustoće, akutni endometritis bio je dokazan u 68 (31,33 %) uzoraka, kronični u 23 (10,60 %), a subakutni u 35 (16,13 %) uzoraka. Zaključno, citologija endometrija pokazala se primjenjljivom i pouzdanom metodom u dijagnostici odnosno razlikovanju akutnog od kroničnog endometritisa.

Ključne riječi: krava, citologija, endometrij, endometritis