The role of centrifuged liquid-based cytology in the evaluation of the endometrium in mares

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ABSTRACT

Endometritis is frequently encountered in brood mare practice and is one of the main causes of infertility. Several methods, including among others cytology, bacteriology and histopathology, are used for diagnosis, however, even the combined use of these methods may not produce a definitive result. The objective of this study was to make an assessment of the diagnostic efficiency of centrifuged liquid-based cytology (LBC) in comparison to the determination of: 1) the presence of polymorphonuclear cells (PMNs) in the uterine epithelium and stratum compactum by histopathology; 2) the presence of PMNs by cytology and 3) the presence of uterine infection by microbiology. Fifty mares of varying age, which belonged to the Jockey Club of Turkey, were used in this study. Endometrial samples were harvested using the cytobrush (CB) and endometrial biopsy (EB) techniques for microbiological, Cytological and histopathological examinations. The comparison of liquid-based cytology (LBC) with histopathological diagnosis (HD), which is considered to be the best standard, showed that the sensitivity (SE), specificity (SP), predictive value of positive test (PVP), and predictive value of negative test (PVN) of LBC were 0.5000, 0.4000, 0.7692 and 0.6087, respectively. No coherence was detected between the results of these two methods (P = 0.0166). Furthermore, it was ascertained that LBC failed in detecting degenerative and fibrotic alterations in the endometrial glands. LBC slides provide sharp images of well-dispersed cells, and enable greater diagnostic sensitivity and certainty. Thus, as the area examined is small, the identification of the target cells is relatively easy.

Key words: liquid-based endometrial cytology; endometrial biopsy; cytobrush; bacteriology; mare

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List of abbreviations

EB = Endometrial biopsy, CB = Cytological brush, HE = Haematoxylin and Eosin stain, LBC = Liquid-based cytology, PMNs = Polymorphonuclear leukocytes, PAP = Papanicolaou stain, PAS= Periodic acid-Schiff, SE = Sensitivity, SP = Specificity, EC = Endometrial changes, HD = Histopathological diagnosis, PVP = Predictive value of positive test, PVN = Predictive value of negative test, MGG = May-Grünwald Giemsa, SDA = Sabouraud's dextrose agar.

Introduction

In equine practice, endometrial lesions are diagnosed by several methods, including gynaecological examination, cytology and/or bacteriology and biopsy (NIELSEN, 2005; LE BLANC, 2010; NIELSEN et al; 2010; SNIDER et al., 2011; COCCHIA et al., 2012; WALTER et al., 2012; NIELSEN et al., 2012; OVERBECK et al., 2013; BOHN et al., 2014; FERRIS et al., 2015; KILGENSTEIN et al., 2015; RUA et al., 2018).

It is agreed that endometritis is most accurately diagnosed by the histopathological detection of PMNs in the endometrial luminal epithelium and stratum compactum. Furthermore, histopathology also enables the diagnosis o degenerative (KENNEY, 1978; KENNEY and DOIG, 1986; SNIDER et al., 2011) and glandular differentiation disorders (SNIDER et al., 2011).

In 1978, KENNEY categorized equine endometrial biopsies under three grades (I, II, and III) on the basis of inflammation and fibrosis, with grade I involving clinically insignificant alterations and grade III involving severe pathological changes (KENNEY, 1978). The category of a mare is ascertained in view of the severity of endometrial fibrosis and endometritis, the presence of endometrial atrophy in the breeding season, the time of barrenness and the presence of moderate to marked lymphatic lacunae (SNIDER et al., 2011; KENNEY and DOIG, 1986).The system described by KENNEY and DOIG (1986) is an internationally accepted evaluation scheme used to predict the ability of a mare to conceive and carry a foal to term.

Several sampling methods have been described for use in endometrial cytology in mares (DEFONTIS et al., 2011; COCCHIA et al., 2012; WALTER et al., 2012; OVERBECK et al., 2013; BOHN et al., 2014; FERRIS et al., 2015; CHRISTOFFERSEN et al., 2015; RUA et al., 2018). Literature reports indicate that the cytobrush technique is more efficient than other sampling methods currently in use (RIDDLE et al., 2007; NORIMATSU et al., 2008; LE BLANC and CAUSEY, 2009; DEFONTIS et al., 2011; OVERBECK et al., 2011; COCCHIA et al., 2012; DWIVEDI et al., 2012; WALTER et al., 2012; KOZDROWSKI et al., 2013; REMONDI et al., 2013; BOHN et al., 2014; BUCZKOWSKA et al., 2014; FERRIS et al., 2015).

Generally, endometritis are associated with bacterial infection (RIDDLE et al., 2007; NIELSEN et al., 2012; RUA et al., 2018). Several studies have demonstrated a

strong correlation between bacteriological and cytological findings (OVERBECK et al., 2011; WALTER et al., 2012; OVERBECK et al., 2013; BUCZKOWSKA et al., 2014). Nevertheless, there is always a risk of misinterpreting false-negative and false-positive culture results (NIELSEN, 2005; LE BLANC and CAUSEY, 2009). Furthermore, positive cultures may not always be associated with endometritis or low fertility rates (DE AMORIM et al., 2016; COCCHIA et al., 2012; RUA et al., 2018). The results of a gold standard, applied to determine the presence or absence of disease, would enable the comparative assessment of bacterial culture and cytology (BLUE, 1987; NIELSEN, 2005; BUCZKOWSKA et al., 2014a; KOZDROWSKI et al., 2015). Uterine fungal infections occur more frequently in older mares with weaker defense mechanisms receiving prolonged antibiotic therapy. Fungal species most frequently isolated from the uterus are Candida spp., primarily C. albicans and A. Fumigatus. Several other yeasts and fungi have also been isolated (BLUE, 1987). In both humans and equine species, the cytobrush technique has been ascertained to yield better results in the collection of cervical and endometrial cells. Uterine brushes have been developed for use in human and equine uterine cytology (COCCHIA et al., 2012).

The sensitivity and specificity of different methods available for the detection of endometrial inflammation and/or infection have been previously investigated (NIELSEN, 2005; RIDDLE et al., 2007; NIELSEN et al., 2010; NIELSEN et al., 2012; WALTER et al., 2012; OVERBECK et al., 2013; BOHN et al., 2014; BUCZKOWSKA et al., 2014a;).

Cytology and culture samples can be collected using swabs, brushes or low-volume lavage. Thus, the examination of CB samples both cytologically and bacteriologically provides a higher sensitivity in endometritis diagnosis. The use of cytological examination provided a more direct diagnosis of acute endometritis and increased the accuracy of the interpretation of the bacteriological findings. The combined use of cytological and bacteriological diagnostics is suggested to increase the ability to detect endometritis (OVERBECK et al., 2011).

Moreover, cytology makes its possible to obtain results as early as the day of collection, whereas bacteriological examination results are obtained within 48-72 h of sample collection. To determine the relative significance of cytology vs. bacterial culture, it is helpful to have a gold standard for the presence/absence of disease against which the results can be compared (OVERBECK et al., 2011).

In a recent study, the identification of polymorphonuclear neutrophils (PMNs) in endometrial cytology smears was found to have a sensitivity of 0.77 when compared to the identification rate of PMNs in histological preparations from the same animals (NIELSEN, 2005). In the same study, endometrial cytology was shown to have a specificity and a positive predictive value of 1.00 and a negative predictive value of 0.62. This indicates that endometrial cytology offers a relatively higher reliability in the

diagnosis of endometrial inflammation, although false negative cases are to be expected (NIELSEN, 2005). Previous research suggests that the combined use of cytological and bacteriological examinations increases diagnostic sensitivity (NIELSEN, 2005; OVERBECK et al., 2011; WALTER et al., 2012).

Positive cytology is reported to be twice as common as positive cultures, and in mares, the degree of inflammation is more important in the diagnosis of infertility than the presence or absence of inflammation (COCCHIA et al., 2012).

Liquid-based cytology (LBC) techniques used for the gynaecological examination of women have been developed with an aim of maintaining cell morphology and improving the quality of cellular diagnosis (NORIMATSU et al., 2008). Previous research on cervical cytology demonstrated that the use of LBC reduced difficulties encountered in smear preparation as well as the rate of false-negative results. When compared to conventional smears, LBC not only provides greater sensitivity and specificity, but also more material for ancillary techniques, and thus, enables further investigation, such as by the use of immunohistochemistry (DWIVEDI et al., 2012).

To date, the cytocentrifugation technique has been used only on samples taken from Holstein cows by low-volume uterine flushing (GILBERT et al., 2005). The researchers reported that there is no effect of fertility on the prevalence of cytologically diagnosed endometritis. GILBERT et al. (2005), argue that cytological diagnosis can be used as a basis for studies on the prevention and treatment of endometritis. They mention that endometritis can be defined as cytological evidence of inflammation by cytocentrifuge technique.

To the authors' knowledge, there is no previous report published on the diagnosis of equine endometritis by the use of LBC on cytobrush samples. Thus, the present study was designed with an aim to investigate the efficiency of the cytospin technique in the diagnosis of equine endometritis using endometrial cytobrush samples.

Materials and methods

Animals. This study was carried out between February and September 2014, in mares raised at the Karacabey Stud farm of the Jockey Club of Turkey (TJK). The age, live foaling rate and reproductive data of the 49 mares from the preceding breeding season were recorded. At the time of sampling, all the mares were inoestrus. The sampling of each animal started with a detailed reproductive examination, including anamnesis, physical examination of the perineum, transrectal palpation and ultrasonographic control None of the mares included in the study presented with uterine fluid accumulation.

The endometrial sampling of the mares used in the present study was performed pursuant to the approval of the Local Ethics Board for Animal Experiments of Erciyes University (Approval Reference Number:11.07.2012/12-75).

Gynaecological examination and uterine cytobrush and biopsy sampling. Diagnostic samples were collected first with a modified endometrial cytobrush (CB) for cytological evaluation, then a cytobrush (CB) for culture, and finally by means of an endometrial biopsy for histopathological evaluation (RUA et al., 2018). For gynaecological examination and sample collection, the mares were restrained, their tails were covered with a disposable examination glove, tied to their neck.

During all procedures, the mares were restrained in standing stocks. Sterile equipment was used for sampling. Before the collection of samples, the tail was taken aside, the perineum and anal sphincter were first cleaned with a dry paper towel and then with a disinfectant solution, and lastly dried with a paper towel. During the sampling process the tip of the swab was protected and held securely under the thumb as it was introduced through the vulvar lips, vestibule, and vagina.

In this study, a commercial disposable plastic cytobrush (CB) (Cytological brush, Gynobrush® Plus, Germany) was used for cytobrush sampling (MAKSEM et al., 2001; BUCCOLIERO et al., 2007; BUCZKOWSKA et al., 2014a; PHOOLCHAROEN et al., 2017). The plastic cytobrushes were modified for use in large animals (Fig. 1A).

Fig. 1. Instruments for collection of uterine samples for bacteriological, cytological and histopathological examination. A) Modified commercial disposable plastic cytobrush (top) and inner protective tube sealed with sterile plastic cap of cytobrush (bottom) B) Biopsy punch of the type Equi - Vet, Kruuse

To prevent contamination, the tip of the external guard of the brush was closed with a gelatine cap. The cap was only removed just before the harvest of samples within the uterus and in so doing precaution was taken to avoid cervical and/or vaginal contamination with cells and debris. The catheter was directed into the cervix by the vaginal method, and once inside the cervix, it was advanced as far as possible into the corpus uteri. The cytobrush in the pipette was pushed forward and placed in the cornu uteri. Then, by rotating the catheter around its axis, cell samples were collected from the uterus. The endometrial surface was gently scraped for the collection of cells. The cellular material was always collected from the base of the uterine horns by a rolling motion of the cytobrush. Cytobrush samples were immediately transferred into 10 mL of a fixing solution (Cytospin collection fluid, Thermoshandon 6768001, USA) for cytological examination. The cytobrush samples,

which were collected for a second time for microbiological examination, were stored at
+4 °C until being inoculated onto growth media.

For histopathological examination, uterine tissue samples, measuring approximately
4 × 15 mm in size, were taken from the cornu uteri of the animals, while they were
restrained in standing stocks, using the vaginal method. The endometrial biopsy samples
were taken from the cornu uteri using a sterile biopsy punch (Denmark; Equi-Vet, Kruuse),
(Fig. 1B). When applying the vaginal technique the closed instrument was introduced
through the cervix, 2.5-3 cm beyond the tip of the index finger into the uterus. The tip of
the punch was moved forward with the basket opened an additional 4-6 cm. Using the
index finger in the cervix as a fulcrum, the punch was swung laterally in order to insert the
tip into the endometrial fold. Finally, the jaw was closed and the instrument withdrawn.
The endometrial biopsy punch was maintained in a disinfectant solution, sterile gloves
were worn, and ultrasound gel was used for lubrication.

The tissue samples were divided into two portions, and half were stored at +4 °C to
be used later for isolation.

Cytological examination

Cytospin technique. Once the harvesting was completed, the brushes were transferred
into a fixing solution (Cytospin collection fluid, Thermo Shandon 6768001, USA), The
brushes were stored in the fixing solution for 1 day at +4 °C and spun at 1500 rpm for 15
minutes. The endometrial cells, collected via brush cytology, were deposited onto poly
L-lysine-coated glass slides by cytocentrifugation (Cytospin; Thermo Shandon Southern
Ltd. Cheshire, England). Wet cytocentrifuged slides were air-dried and stained with
hematoxylin-eosin (HE), Papanicolaou stain (PAP), Diff-Quick, and May-Grunwald-
Giemsa (MGG), and later mounted with mounting medium (Merck).

The Papanicolaou stain provides perfect nuclear detail, and is particularly useful
in the identification of viral inclusions. Although it stains fungal hyphae and yeasts
reasonably well, it stains bacteria less effectively. This stain is affected more by excessive
blood, inflammation, and necrosis than the Diff Quik stain.

Diff Quik staining was performed on air-dried material to achieve increased cell
adhesion to the glass slides and maximum recovery of material. Although nuclear detail
was less distinct on Diff Quick-stained slides, the bacteria, as well as cytoplasmic and
extracellular inclusions, were well stained. While the staining of fungal hyphae with
Diff Quik varies, the staining of yeasts is better and consistent. Hematoxylin-eosin
stained slides were useful in evaluating the quality of the samples and their suitability
for specific stains, such as immunoperoxidase stains. May-Grünwald-Giemsa enables
the complementary visualisation of the cytoplasmic components, as well as an improved
background, and better mucus and intercellular matrix examination.

Evaluation of LBC quality. The Papanicolaou stain was used for cell morphology; whilst the Diff Quik and May-Grünwald-Giemsa stains were used for detection of blood and microbial colonies. Hematoxylin eosin was used for determination of cell distribution, cellularity and cellular clumping.

The stained smears were compared for sample quality with respect to cell morphology, cell distribution, cellularity, cellular clumping, and the presence of blood and microbial colonies. Grading and scoring were performed for each criterion as described by DWIVEDI et al. (2012) with some modifications explained below. Three hundred cells were counted in ten different fields of each smear (BUCZKOWSKA et al., 2014). Several cell depositions, which could not be identified, were considered to be cell deformations. The smears of each mare were first assessed for quality on the basis of cellularity, cell morphology, number of inflammatory cells/ 400x field, and any other distinct feature. Overall, cellularity was subjectively ranked as 1 (poor), 2 (moderate), or 3 (high), in terms of the areas with the greatest number of cells on the slides. Cell clumps, accumulation of unidentified cells, and severely deformed single cells that could not be allocated to an identifiable cell type were all considered as cell deformations. The diagnostic value of cell morphology was categorized as follows: high (<10% of cells deformed); moderate (10-20% of cells deformed); poor (20-50% of cells deformed) and none (>50% of cells deformed) (WALTER et al., 2012). The samples were also ranked for the presence of neutrophil leukocytes, lymphocytes, and macrophages (2 = severe 1 = present; 0 = not present) using either of the 2 slides per sample, and the highest number of lymphocytes and the range of neutrophil leukocytes/400× field were determined (BOHN et al., 2014). "Cytological scores" were generated by means of individual cytological grading (lymphocytes, macrophages, polymorphonuclear leukocytes).

Next, all the slides were grouped according to the mares they belonged to and, making sure the evaluator was blinded to the sampling technique, they were re-evaluated for quality, cell integrity, and diagnostic utility. Diagnostic quality was scored as adequate (2), minimally adequate (1), or inadequate (0). Ten different fields (×400) were assessed in each smear, and the epithelial cells and PMNs/ inflammatory cells were counted. Smears with fewer than 35 cells were not included in the analysis (inadequate), those with more than 35 cells were adequate, and those with as many as 35 cells were considered to be minimally adequate (WALTER et al., 2012). The six slides belonging to a single mare (2 for each sampling method tested) were subjectively ranked with respect to sample quality (BOHN et al., 2014). All the slides were photographed using a digital camera (Olympus DP71) and digital programmers (DP Controller and the DP Manager) fitted to a microscope (BX-51, Olympus) (at least 4 fields per slide, using ×10, ×40 and ×100 objectives).

Histopathological examination (Best Standard). First, the endometrial biopsy samples were transferred in 4% buffered formalin. Tissue samples were fixed in 10% neutral buffered formaldehyde, embedded in paraffin and cut into 4 µm thick-sections (one section from each block). The staining of the sections was performed with Crossmon's trichrome, HE, Periodic Acid-Schiff (PAS), and the Brown and Brenn modification of the Gram stain, prior to their examination by light microscopy. The hematoxylin and eosin (HE) method was used for the routine staining of the endometrial histology sections, as it provides sufficient contrast and detail in most biopsy cases. Masson's trichrome staining was useful to highlight the connective tissue, and it also aids in detecting fibrosis. Periodic acid-Schiff (PAS) highlights polysaccharide-rich substances found in the glandular lumen or extracellular matrix. Stains are also available for potential infectious agents (SNIDER et al., 2011).The infiltration of the endometrial luminal epithelium and the stratum compactum with one or more PMNs in five fields at high magnification (400×) was considered to be indication of endometritis (NIELSEN, 2005; RICKETTS and ALANSO, 1991).The samples were rated according to Kenney's classification method, on the basis of the morphological characteristics of the endometrial changes (KILGENSTEIN et al., 2015; KENNEY and DOIG, 1986).

The biopsy samples were examined for glandular differentiation disorders (SCHOON et al., 1999) and the presence of inflammatory (endometritis, vasculitis /perivasculitis) and degenerative (endometrosis, angiosclerosis) lesions (KENNEY and DOIG, 1986; SCHOON et al., 1999). Endometritis was classified as either acute suppurative, subacute suppurative or nonsuppurative (SCHONIGER et al., 2013). Siderocyte and eosinophil counts were recorded semi quantitatively, whereby siderocyte infiltrates with cells restricted to the luminal epithelium and the stratum compactum were evaluated as being superficial. The severity and distribution of the degenerative lesions in the endometrial vessels before mating were evaluated and correlated to the age and reproductive status of the mares (KENNEY and DOIG, 1986).

Bacteriological and mycological analyses. Endometrial cytobrush and biopsy samples were placed in screw-capped tubes containing trypticase soy broth for aerobic bacteriological analyses, and in tubes containing anaerobic basal broth for anaerobic bacteriological analyses. Samples collected using the CB and EB techniques were inoculated onto both Mueller-Hinton agar supplemented with 5% sheep blood and Sabouraud's dextrose agar (SDA). For bacterial isolation, the plates were incubated at 37 °C for 24 hours under aerobic and microaerophilic conditions. For fungal isolation, the SDA plates were incubated at 24 °C for a week.

Statistical analysis. The statistical analysis of the findings obtained in this study was performed using the SPSS® (SPSS, 14.0) software and a DAG_Stat Excel spread sheet. For calculation of the Chi-Square Test, sensitivity, specificity, negative/positive predictive

values and Cohen's Kappa (95% CI) were used Diagnostic & Agreement Statistics pages. The Chi-Square Test was used for comparison of the percentages of the different groups. Statistical significance was set at P<0.05. The Golden Test was made using the Yates Correction Chi-Square Test (MACKINNON, 2000).

Results

Cell types detected in the cytospin preparations. The numbers of the different types of cells collected with the CB technique (endometrial epithelial cells, neutrophil leukocytes and other inflammatory cells) indicated the good cellularity in all the slides examined (Fig. 2-5). The term "good cellularity" is used to describe the presence of an adequate number of cells in a microscopic field, when examined at ×400 magnification. The diagnostic value of cell morphology was categorized as follows: high (<10% of cells deformed); moderate (10-20% of cells deformed); poor (20-50% of cells deformed) and no (>50% of cells deformed) (WALTER et al., 2012).

Histopathological findings - Endometrial changes (EC).

The histopathological examination of the biopsy specimens revealed the highest number, 49% (24 mares) to be Category I.

The group of mares classified as Category IIA constituted 8.2% (4 mares) of all the animals included in this study.

The group of mares classified as Category IIB constituted 32.7% (16 mares) of all the animals.

Lastly, the mares classified as Category III constituted 10.02% (5 mares) of all the animals.

Correlation of endometrial changes with age. Among the 49 mares examined in this study, none of those up to 6 years of age (8 mares) showed any endometrial changes, while those aged 7-12 years (20 mares) and older, and those aged 13-30 years (21 mares) presented with endometrial changes.
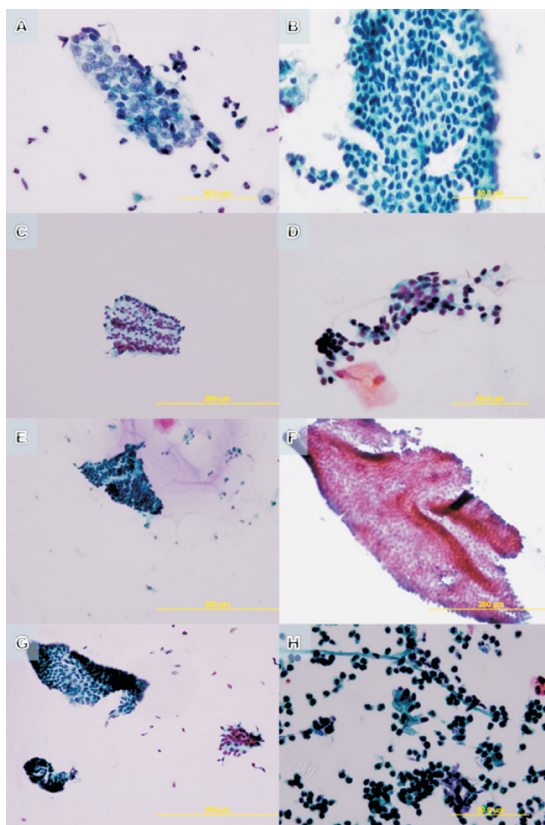
Fig. 2. Cytological preparation of normal endometrial cells. LBC (Cytospin, PAP stain). A - This sheet of histiocyte like cells represents endometrial stromal cells. There are many neutrophils with a few lymphocytes. The LBS (Liquid base slides) contain a concentrated number of cells per same high-power field. B - Normal endometrial cytology includes healthy appearing simple stromal cells, often in clumps and no neutrophils. The clusters of well preserved endometrial glandular cells show small nuclei with stippled chromatin. C - E - This cytological preparation shows endometrial cells observed with considerable nuclear overlapping. F - Organoid configurations can usually be noted with direct endometrial sampling. Proliferative endometrial glands are found intact on the slide. G - Endometrial glandular cells and spindle stromal cells Endometrial smear from a normal oestrous mare, showing normal endometrial epithelial cells and no PMNs. H - Many columnar epithelial cells with oval, basal nuclei, containing finely strippled chromatin can be seen. The cytoplasm is columnar and finely vacuolated. A few small lymphocytes are seen in the background. No neutrophils or infectious agents are visible. The cytological features are consistent with normal reproductive status and active cycling. It is important to know this since the cytology indicates that the mare is ready to breed and does not need any treatment prior to breeding. Scale bars: 50 μm (A, B, D, H), 200 μm (C, E, F, G).
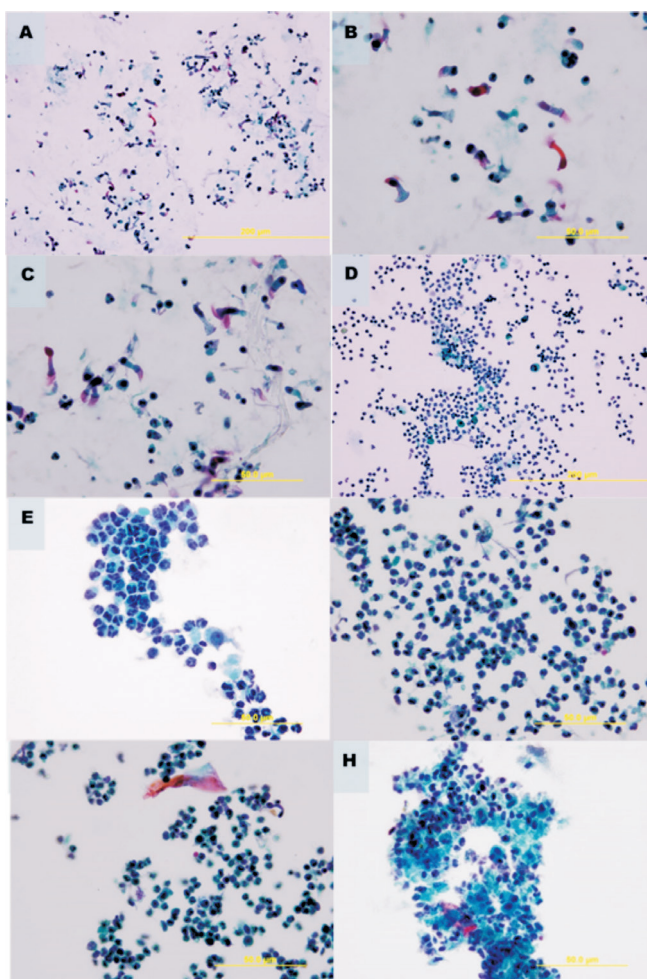
Fig. 3. Uterine LBC (Cytospin) with cytobrush of a mare with endometritis, PAP stain.
A - There are low columnar to cuboidal epithelial cells occurring singly and in groups. The nuclei are relatively bland and chromatin stippling is not present. Note the numerous neutrophils and lymphocytes in this preparation. B and C - Higher magnification shows red to red-orange staining(orangophilic) ciliated columnar epithelial cells with a basal pyknotic cytoplasm.
D - Severe infectious inflammation consisting of neutrophils and some mononuclear cells.
E to G - Higher magnification. H - The clusters of well preserved uterine epithelial cells nucleus without cytoplasm and a few small lymphocytes are seen in the background. Scale bars: 50 μm (B, C, E, F, G, H), 200 μm (A, D).
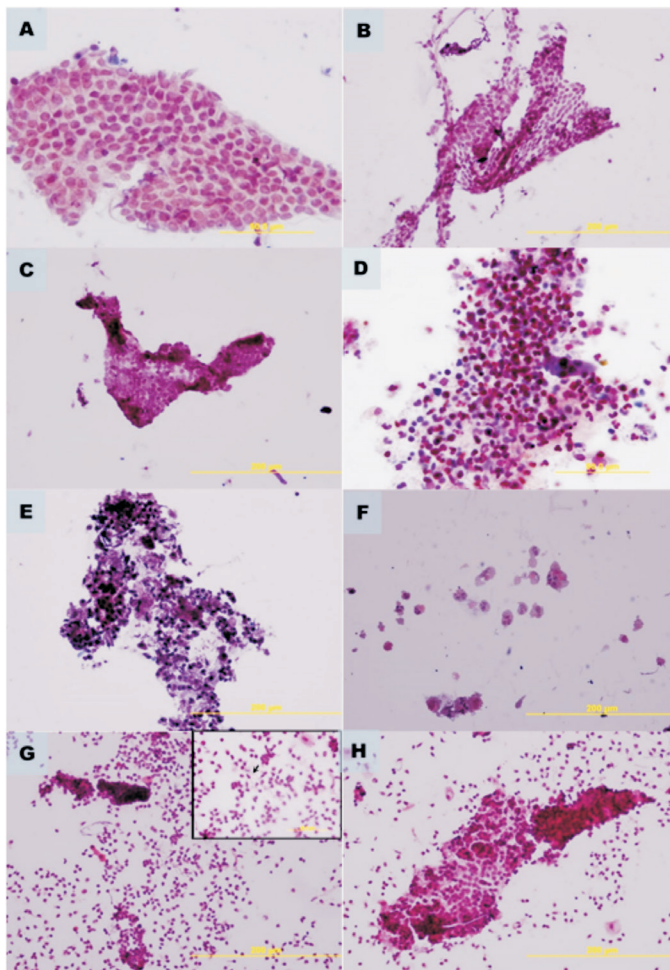
Fig. 4. LBC stained with H&E. A - This sheet of histiocyte like cells represents endometrial stromal cells. B and C - Each of these images shows a large tissue particle composed of densely packed small cells. This cytological preparation shows endometrial cells and considerable nuclear overlapping is seen. Organoid configurations can be commonly noted with direct endometrial sampling. Sheets of endometrial cells obtained from a brush sample. The small cells have a paucity of cytoplasm with round nuclei, and a slightly hyperchromatic chromatin pattern. D to H - Cytological preparation from mares with acute endometritis yields numerous neutrophil. Some of the cells have some cytophagia. Yellow arrow: yeast. Inset: Gram negative cocci (arrow). Scale bars: 50 μm (A, B, D), 200 μm (C, F, G, H), 500 μm (E).
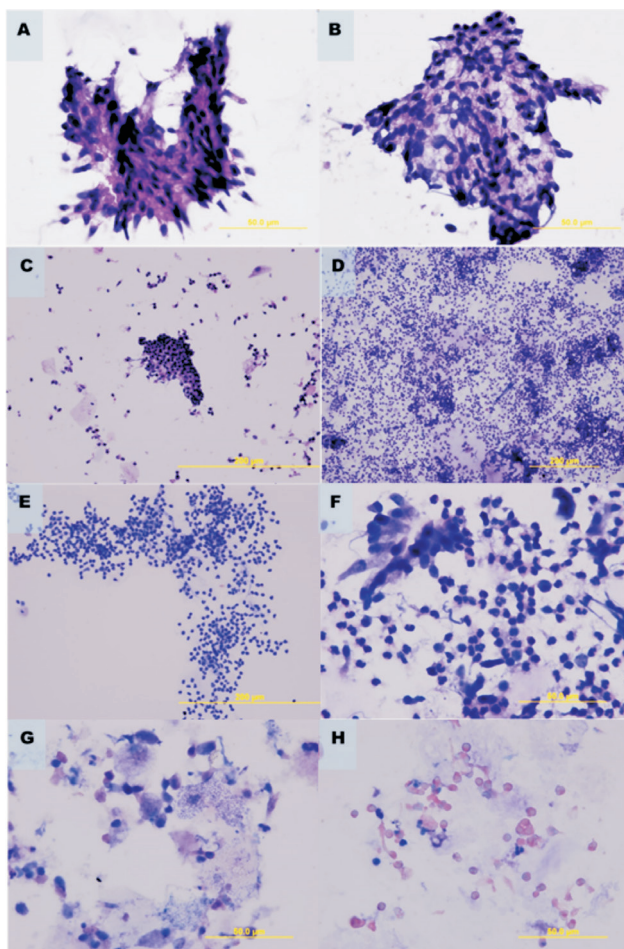
Fig. 5. LBC stained with MGG A-E and Diff-Quick F-H. A - These deep endometrial stromal cells lie in clusters or in loose aggregate, and feature hyperchromatic uniform spindle-shaped nuclei B - Normal appearing simple stromal cells often in clumps and no neutrophils. C - The clusters of well preserved endometrial glandular cells show small nuclei with stippled chromatin and polymorphonuclear leucocytes (PMNs). D and E - Cytological preparations from mares with acute or subacute endometritis yield numerous neutrophils (F-H). F - Many columnar epithelial cells and PMN's. G and H - Common pathogens found on endometrial cytology G - They are often degenerate and may contain phagocytosed bacteria along with many singular degenerate epithelial cells H - Yeast organisms (identified as *Candida albicans*). Scale bars: 50 μm (A, B, F, G, H), 200 μm (C, D, E).

Bacteriological findings. The results of bacteriological and fungal isolation are summarized in Table 1. The most common facultative pathogenic bacteria isolated from BC samples were haemolytic *E. coli*, which were detected in 19 mares. Furthermore, facultative pathogens including *Pseudomonas aeruginosa*, *Corynebacterium pyogenes*, *Klebsiella pneumonia*, and *Staphylococcus aureus* and *Streptococcus zooepidemicus* were identified. The brush culture technique yielded mixed bacterial cultures (composed of 2 bacterial species) in 8 mares, pure cultures in 32 mares, and no bacterial growth in 9 mares.

EB and CB Cultures. Facultative pathogens were identified by biopsy culture in 17 mares. These included haemolytic *E. coli*, *Pseudomonas aeruginosa*, *S. zooepidemicus*, *Staphylococcus aureus*, and *Truperella pyogenes*. All the mares with positive biopsy culture results for *E. coli* and *S. aureus* also produced positive results with the use of the brush culture method.

The results of the biopsy culture method demonstrated mixed bacterial growth in 1 mare, and pure bacterial growth in 22 mares, whilst no bacteria were cultured in the remaining 25 mares. *S. zooepidemicus* was often cultured together with other species. A pure growth of *S. zooepidemicus* was detected in only 1 mare with the use of both the brush and biopsy culture methods. Furthermore, *E. coli* was also often cultured together with other bacteria.

The most common fungal species isolated with the use of the brush culture technique was *Cryptococcus albidus* (30.6%). Furthermore, *Aspergillus flavus* (8.2%) *Cladosporium* spp. (6.1%) *Paecilomyces* spp. (6.1%), and *Sporobolomyces salmonicolor* (6.1%) were also isolated and identified. No fungi were cultured from the remaining 21 mares (42.9%). The most common fungal species isolated with the use of the biopsy culture technique was again *Cryptococcus albidus* (24.5%). Other fungi that were identified by biopsy culture included *Aspergillus flavus* (6.1%), *Cladosporium* spp. (4.1%), and *Paecilomyces* spp. (6.1%) *Sporobolomyces salmonicolor* was not isolated, and no fungi were cultured from the remaining 30 mares (59.2%). For each sample, the correlation between the bacterial species identified from the positive brush cultures and the cytological results was determined. Out of the 26 mares with positive LBC smears, only 16 produced positive brush culture results, and only 10 gave positive biopsy culture results. The bacteria isolated from the 39 pure cultures were *E. coli* in 11, *Staph. aureus* in 8, *Klebsiella pneumonia* in 3, *Streptoccocus zooepidemicus* in 1, *Pseudomonas* spp. in 4, and *Arcanobacterium pyogenes* in 4. The bacteria isolated from the mixed cultures were *E. coli* and *Klebsiella* spp. in 2, *E. coli* and *Str. zooepidemicus* in 1, *E. coli* and *Staph. aureus* in 2, and *E. coli* and *Arcanobacterium* spp. in 1. The bacteria isolated from the 14 pure biopsy cultures were *E. coli* in 1, *Staph. aureus* in 7, *Klebsiella pneumonia* in 1, *Streptoccocus zooepidemicus* in 1, *Pseudomonas* spp. in 2, and *Arcanobacterium pyogenes* in 2 (Table 1).

Table 1. Bacteriological growth results from brush and biopsy cultures from mares

		Count (%)	Total (%)
Brush- and biopsy-	No Growth	8	49
Brush+ and biopsy-	*E. coli*	10	22
	Klebsiella pneumonia	3	22
	Streptococcus pyogenes	2	22
	Staphylococcus aureus	2	22
	Arcanobacterium pyogenes	2	22
	Pseudo. aeruginosa	3	22
Brush- and biopsy+	Staphylococcus aureus	1	49
	Pseudo. aeruginosa	1	49
Brush+ and biopsy+	Pseudo. aeruginosa	1	14
	Klebsiella pneumonia	1	14
	Staphylococcus aureus	4	14
	Arcanobacterium pyogenes	4	14
	Streptococcus	1	14
Total			

Diagnosis: Pure Culture Growth (+). No Growth /Mix Growth (-).

Correlation of bacteriological and histopathological examination results. Out of the 12 mares diagnosed with suppurative endometritis, 7 were determined to be infected with facultative pathogenic bacteria, including *K. pneumonia, S. aureus T. pyogenes*, *E. coli*, and *S. zooepidemicus*. Of the remaining mares, 2 presented with mixed growth, whilst 3 did not present with any bacterial growth. *S. aureus* and *T. pyogenes* were isolated with the brush culture technique from 5 mares, which produced positive results with the biopsy culture method. Endometritis was of nonsuppurative nature in 13 mares. *K. pneumonia*, *E. coli* and *S. aureus* were isolated by brush culture from 13 mares with endometritis, whilst *S. aureus* and *T. pyogenes* were isolated by both brush and biopsy culture from 3 mares. In 3 mares, no bacterial growth was detected with either of the two culture methods. From the brush cultures of the mares, which did not have endometritis (n = 20), *E. coli* was isolated in 3, *T. pyogenes* in 2, *P. aeruginosa* in 2 and *S. aureus* in 3. Out of the three mares from which *S. aureus* was isolated by brush culture, two were also detected to be *S. aureus*-positive by biopsy culture. In two mares, the isolation and histopathological results of the EB samples were coherent.

The SE, SP, PVP and PVN values were determined to be 0.5000, 0.4000, 0.6538 and 0.2609, respectively. The p value was 0.7369, and no coherence was detected between the different methods used in the study.

Correlation between the bacteriological results of the samples collected by CB and EB. The CB technique was found to be more efficient in terms of bacterial isolation (P = 0.000). While the number of samples, from which *E. coli* was isolated purely, was 11 (22.45%) with the CB technique, this number was much lower with the use of the EB technique (n = 4, 8.16%) (Tables 1-4).

The efficiency of the different methods tested, in comparison to the best standard (HD), was 0.694 ± 0.066 for LBC, 0.111 ± 0.105; for PVP, 0.350 ± 0.076 for PVN, 0.650 ± 0.075 for SE, and 0.889 ± 0.105 for SP. The coherence of two different techniques was found to be at a moderate level (Cohen's Kappa = 0.346 ± 0.115, Fisher's Exact X2 = 8.62, P = 0.007).

Evaluations made with LBC and histopathological diagnosis (golden test: the efficiency of the different methods tested) revealed negative sample numbers of 23 (46.94%) and 20 (40.82%) for LBP and EB, respectively. Furthermore, the sensitivity and specificity of the cytological test were ascertained as 0.690 (0.492-0.847 95%CI), 0.700 (0.457-0.881 95%CI) respectively. When compared to the golden test (EB), the efficiency of cytology was determined to be 0.694 (0.546-0.818 95%CI). Furthermore, the false-positive and false-negative values of cytology were ascertained as 0.300 (0.119-0.543 95%CI) and 0.310 (0.153-0.508 95%CI), respectively. The coherence of the two techniques was found to be at a moderate level (Cohen's Kappa = 0.381 (0.123-0.639 95%CI), Pearson's X2 = 5.74 (Yates Correction) P = 0.017) (Table 2).

Table 2. Relationship between histopathological examination and cytological evaluation

Golden Test X2 = 5, 74P = 0, 0166 Yates correction			PMN / HPF (LBC)		
			Positive	Negative	Total
Endometritis (HD)	Positive	Count	20	9	29
		% within HD	69.0%	31.0%	100.0%
		% within LBC	76.9%	39.1%	59.2%
		% of Total	40.8%	18.4%	59.2%
	Positive	Count	6	14	20
		% within HD	30.0%	70.0%	100.0%
		% within LBC	23.1%	60.9%	40.8%
		% of Total	12.2%	28.6%	40.8%
Total		Count % of Total	26 53.1%	23 46.9%	49 100.0%

Table 3. Cross-tabulation of bacterial growth from brush and biopsy cultures

| | Bacteria | Biopsy Culture | | | | | | | | Total |
		No growth	S. aureus	E. coli	Klebsiella	S. zooepidemicus	Arcano	Pseudomonas	E coli & Klebsiella	
Cytobrush Culture	No growth	9[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	9
	S. aureus	3[a]	5[b]	0[a]	1[b,c]	0[a,c]	0[a]	0[a,c]	0[a,c]	9
	E. coli	7[a,b]	0[b]	1[a,b]	0[a,b]	0[a,b]	2[a]	1[a,b]	0[a,b]	11
	Klebsiella	2[a]	0[a]	0[a]	0[a,b]	0[a,b]	0[a]	0[a,b]	1[b]	3
	S. zooepidemicus	1[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	1
	Arcanobacterium	2[a]	0[a]	0[a]	0[a,b]	1[b]	1[a,b]	0[a,b]	0[a,b]	4
	Pseudomonas	2[a]	0[a,b]	1[a,b]	0[a,b]	0[a,b]	0[a,b]	1[b]	0[a,b]	4
	E. coli & Klebsiella	1[a]	0[a]	1[a]	0[a]	0[a]	0[a]	0[a]	0[a]	2
	E. coli & S. aureus	2[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	2
	E. coli & S. zooepidemicus	1[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	1
	E. coli & Arcanobacterium	0[a]	0[a,b]	1[b]	0[a,b]	0[a,b]	0[a,b]	0[a,b]	0[a,b]	1
	E. coli & Pseudomonas	2[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	2
Total		32	5	4	1	1	3	2	1	49

Within a row, rates without a common letter ([a, b, c]) differ (P<0.05).

The sensitivity and specificity of LBP were determined as 0.500 (0.324-0.676 95%CI) and 0.400 (0.163-0.678 95%CI), respectively. When compared to the golden test, the efficiency of LBP in the assessment of degenerative findings was calculated as 0.469(0.325-0.617 95%CI). The false-positive and false-negative values of EC were found to be 0.600(0.323-0.837 95%CI) and 0.500(0.324-0.676 95%CI), respectively. There was no coherence between the two techniques (Cohen's Kappa = -0.087 (-0.349-0.174 95%CI); Pearson's X^2 = 0.11, (Yates Correction) P = 0.737).

The number of samples which did not produce any bacterial growth was 18 (36.73%) with the use of CB, and 33 (67.35%) with the use of EB. The SE and SP values of the CB technique were 0.8125 and 0.4545, respectively. The cytological method was found to be more efficient in terms of bacterial isolation. The CB technique was observed to yield a

higher level of success in the isolation of *E. coli* from pure cultures, and *Arcanobacterium* spp. and *Pseudomonas* spp. from mixed cultures of *E. coli* (P = 0.1330).

The isolation methods were found to differ significantly for the fungal species that were identified (P = 0.000). The comparison of the CB and EB sampling techniques for fungal growth in the samples showed that no fungal growth occurred in 21 (42.86%) CB samples and 40 (81.64%) EB samples (P = 0.019). Thus, the CB technique was found to be more efficient in terms of fungal isolation. The CB technique was considered to be highly differentiating with respect to the isolation of *Cryptococcus* spp. Given the challenge of performing a biopsy in the field, the CB technique offers a safe alternative to EB for identification of both bacteria and fungi. The results of the present study also suggest that the CB technique is the best diagnostic method for differentiation of bacterial and fungal species. These methods were observed not to differ statistically for fungal growth (P = 0.79). The SE and SP values were determined as 0.5556 and 0.4250, respectively. The number of samples that showed no fungal growth was 9 (18.37%) for CB and 22 (49%) for EB.

Correlation between LBC and histopathological diagnosis (Best Standard). The number of samples with negative LBC results was 23 (46.94%). Furthermore, 20 (40.82%) samples produced negative HD results. It was determined that 68.97% of the cases, which were endometritis-positive according to HD, were also positive according to LBC (Table 2).

Discussion

The aim of the present study was to investigate the efficiency of the LBC technique in bacterial and fungal isolation, and the collection of endometrial and inflammatory cells from the endometrium of 49 race horses. Previous cytological research in humans has shown that LBC is a highly sensitive diagnostic method, owing to the homogenously distributed and clearly imaged cell populations in cytospin smears (BLUE, 1987; NORIMATSU et al., 2008; DWIVEDI et al., 2012; REMONDI et al., 2013). It is reported that cytocentrifugation with liquid fixation also enables interpretable microbiopsies (JOHNSON et al., 2000).

The diagnostic techniques that are currently used as standard tools in mares are the cytological examination and culture of uterine flush, endometrial brush and swab samples (LE BLANC et al., 2007; NIELSEN et al., 2010; DEFONTIS et al., 2011; OVERBECK et al., 2011). The cytobrush technique, which yields well-preserved cells representative of a large uterine surface area, without causing harm to the reproductive tract, is required for consistent and reliable cytological results (BLUE, 1987; BUCCOLIERO et al., 2009; KOZDROWSKI et al., 2013; BOHN et al., 2014; BUCZKOWSKA et al., 2014a).

This study shows that the cytobrush technique can be used successfully and reliably to obtain endometrial samples.

The cytobrush technique resulted in less distortion of cells (Figs 2-5). Even though the cytobrush technique requires specialized equipment, sample collection by this method was easier, more consistent, and produced rapid results.

In the field of veterinary pathology, there are no standardized criteria yet for cytological interpretation. Currently, there is no efficient screening and diagnostic method or common lesion classification system that can be used for cytological examination of the equine endometrium. Which cytological techniques are more efficient in the diagnosis of the cause of infertility in mares remains controversial. Therefore, it is suggested that uterine samples be evaluated both bacteriologically and cytologically for diagnosis of endometritis (OVERBECK et al., 2011; BOHN et al., 2014; FERRIS et al., 2015).

The present study demonstrated that the CB technique was more efficient in bacterial isolation. CB yielded a higher level of success, particularly in the isolation of *E. coli* from pure cultures and the isolation of *Arcanobacterium* spp. and *Pseudomonas* spp. from mixed cultures of *E. coli*. The diagnostic methods tested were observed to differ significantly in relation to the fungal species identified (P = 0.019). The cytological method was found to be highly efficient in the isolation of *Cryptococcus albidus*, on the basis of the number of positive samples recorded (n = 15, 30.61%). The results of the present study suggest that CB could be used safely for bacterial and fungal isolation as an alternative to EB, which is challenging to perform under field conditions.

It is well known that the severity of uterine inflammation is greater with the detection of a higher number of PMN/BBA at higher magnification. Non-infectious endometritis can be differentiated from infectious endometritis on the basis of case history, positive cytology results and the use of other diagnostic methods. Positive microbiological results are not always associated with the cytological detection of inflammatory cells (FERRIS et al., 2015). NIELSEN et al. (2010), reported a cytological positivity rate of 50% for *E. coli*-positive cultures and 70% for other microorganisms. In the present study, cytological positivity rates detected for *S. aureus, E. coli, P. aeruginosa, K. pneumonia, A. pyogenes,* and *S. zooepidemicus*-positive cultures of CB samples were 15%, 31%, 0%, 8%, 4%, and 4%, respectively, whilst positivity rates for EB samples were 5%, 4%, 2%, 1%, 3%, and 1%, respectively (Tables 1, 3).

In previous studies, uterine samples belonging to cases diagnosed with endometritis were determined to contain PMNs (NIELSEN, 2005; LE BLANC and CAUSEY, 2009), and the culture results were reported to be negative (WALTER et al., 2012). DIGBY (1978) attributed such cases to non-infectious uterine irritation caused by the use of antimicrobials. In the present study, the negative culture results obtained for the EB samples were likewise attributed to uterine irritation.

Table 4. Cross-tabulation of yeast growth from brush and biopsy cultures

	Fungus	Biopsy culture						Total
		No growth	*Aspergillus*	*Cladosporium*	*Cryptococcus*	*Paecilomyces*	*Sporobolomyces*	
Cytobrush culture	No Growth	17[a, b]	0[b]	0[a, b]	0[b]	2[a]	2[a]	21
	Aspergillus	4[a]	0[a]	0[a]	0[a]	0[a]	0[a]	4
	Cladosporium	1[a]	1[b]	1[b]	0[a, b]	0[a, b]	0[a, b]	3
	Cryptococcus	13[a]	1[a]	0[a]	1[a]	0[a]	0[a]	15
	Paecilomyces	3[a]	0[a]	0[a]	0[a]	0[a]	0[a]	3
	Sporobolomyces	2[a]	0[a, b]	0[a, b]	1[b]	0[a, b]	0[a, b]	3
	Total	40	2	1	2	2	2	49

Within a row, rates without a common letter ([a, b, c]) differ (P<0.05)

The microbial species isolated from uteri in the present study are similar to those reported to have been isolated in previous research (ALBIHN et al., 2003; RIDDLE et al., 2007; LE BLANC et al., 2007; BUCZKOWSKA et al., 2014a; PASOLINI et al., 2016). In the research conducted by LEBLANC et al. (2007), RIDDLE et al. (2007) and BUCZKOWSKA et al. (2014a), mostly non-pathogenic bacteria were reported to have been isolated. Furthermore, LEBLANC et al. (2007) and RIDDLE et al. (2007), also reported that in mares, some cases, from which β-*haemolytic Streptococci* and other pathogenic species were isolated, were associated with positive cytological results. In the present study, pure cultures of *Staphylococcus aureus, Klebsiella proteus, Escherichia coli, Pseudomonas aeuroginosa* and mixed cultures with *Aspergillus flavus, cladiosporidium* spp*.,* and *Crptococcus albidus* were found to be associated with positive cytological results. However, no correlation was detected between positive culture results and histological examination results. In the present study, very high levels of fungal isolation were obtained for both CB and EB samples. BUCZKOWSKA et al. (2014a), attributed fungal isolation from mares to a case history of frequent intrauterine antibiotic administration in the preceding breeding season. In agreement with previous studies, the most common pathogenic bacteria isolated from both EB and CB samples in the present study were β-haemolytic *Streptococci*. Both the pure cultures of β-haemolytic *Streptococci* and their mixed cultures with *Candida* spp. were always found to be associated with positive cytological results (ALBIHN et al., 2003; RIDDLE et al., 2007; NIELSEN

et al., 2012). While BUCZKOWSKA et al. (2014a) reported a similar correlation for *Corynebacterium* spp. isolation, they demonstrated that such a correlation was invalid for *E. coli* isolation. Similarly, in other studies, while the highest level of association with positive cytological results was detected for the isolation of β-haemolytic *Streptococci*, the isolation of *E. coli* and other Gram-negative bacteria was not found to be associated with positive cytological results (RIDDLE et al., 2007; NIELSEN et al., 2010). While WALTER et al. (2012), as certained a statistically significant correlation between the number of β-haemolytic *Streptococcus* colonies and the number of PMNs in smears, such a correlation was not detected for other microorganisms. LEBLANC et al. (2007), reported that the pathogenicity of *E. coli* and *β-haemolytic Streptococci* differed, and indicated that the inflammatory response of the uterine tissue to different microorganisms also varied. WALTER et al. (2012), detected a dense population of PMNs and isolated a high level of β-h*aemolytic Streptococci* from CB smears. In the present study, the pure cultures of *E. coli, Staphylococci,* and *Klebsiella pneumonia* and their mixed cultures with *Cladosporium* spp*., Cryptococcus albidus,* and *Aspergillus flavus* were found to be associated with positive cytological results. Furthermore, the bacterial species isolated from EB and CB samples in the present study were found to be in agreement with the species reported to have been isolated in the previous studies referred to above. However, the high level of *E. coli* isolation and the absence of β-haemolytic *Streptococci* isolation in the present study contradict the results of previous research (NIELSEN, 2005; OVERBECK et al., 2011; KOZDROWSKI et al., 2013; BUCZKOWSKA et al., 2014; RIDDLE et al., 2007). Several studies have demonstrated pathogenic bacteria to have been isolated from clinically healthy mares with negative cytological results (LEBLANC and CAUSEY, 2009; LE BLANC, 2010; OVERBECK et al., 2011). In these studies, researchers have attributed this situation to the use of aseptic sampling methods, bacterial contamination (OVERBECK et al., 2011), peracute infection or the temporary bacterial colonization of the endometrium with no concurrent imflammatory reaction. RIDDLE et al. (2007), reported a positive culture rate of 36% for negative cytological results, and a negative culture rate of 65% for positive cytological results. In agreement with these results, the present study demonstrated that, out of the 26 cytologically positive mares, 21 (16 of which displayed pure growth) had positive culture results, whilst out of the 23 cytologically negative mares, 20 (13 of which displayed pure growth) had positive culture results. REDAELLI and CODAZZA (1977), reported that fungal and mold infections are always associated with bacterial infections, do not cause primary infection, and are uncommon in the non-gravid uterus, whilst LEBLANC (1997), suggested that fungi and molds are increasingly isolated as a result of the extensive use of antibiotics, intensive breeding and reproductive manipulation. *Candida* spp. and *Aspergillus* spp. are reportedly the most common yeast and mold species isolated from uterine cultures (DASCANIO et al., 2001; PASOLINI et al., 2016). In their study on infertile horses, DASCANIO et al

(2001), reported that they isolated *C. albicans*, *A. Fumigates* and *Rhizopus equi* from the cervix and uterus of the animals they examined. AMARAL et al. (2007), indicated that they most frequently isolated *Penicillium* spp. (35.4%), *Aspergillus* spp. (20.3%) and *Candida* spp. (13.9%) from uterine cultures. The yeast and fungal species isolated in the present study are similar to those isolated in previous research (CODAZZA et al., 1973; PATGIRI and UPPAL, 1983; DASCANIO et al., 2001; PASOLINI et al., 2016). In the present study, *cladosporium* spp. and *Sporobolomyces salmonicolor* were isolated for the first time from mares with endometritis. It is believed that further, more detailed research is needed to elucidate the true aetiological role of such opportunistic fungal pathogens in the development of infertility (VERMA et al., 1999. The histopathological diagnosis of endometritis was performed as described by KENNEY and DOIG (1986), SCHOON et al. (1997) and SCHOON et al. (1999). The microscopic examination of endometrial biopsy samples enables the detection of degenerative alterations (endometrosis, angiosclerosis) and glandular differentiation disorders, which makes it possible to assess all factors that contribute to subfertility (KENNEY and DOIG, 1986; SCHOON et al., 1997; SCHOON et al., 2003).

In this study, endometrial degeneration was classified as described by Kenny (LE BLANC, 2010; NIELSEN et al., 2010; BUCZKOWSKA et al., 2014b). In the present study, pathological endometrial changes were defined as mild (Category II A: 8.2%) and severe (Category IIII: 10.2%), except for mares (49%) classified as normal under Category I. The findings of this study demonstrate that endometrial biopsy is a useful diagnostic and adjunctive diagnostic tool in the evaluation of endometrial lesions. The type and incidence of altered pathological changes were similar to those reported by RICKETTS and ALONSO (1991).

While FIALA et al. (2010), reported that a single biopsy sample could not be considered representative of the entire uterus, OVERBECK et al. (2013), determined that the number of PMNs in necropsy samples taken from different parts of the post-mortem uterus did not differ significantly. On the other hand, NIELSEN et al. (2012), reported the presence of PMNs in the endometrium to be strongly correlated with gestation, but also indicated that cytological examination results were not correlated with pregnancy. In view of the presence of PMNs in the endometrium, researchers have suggested that cytological examination is more relevant than microbiological isolation for the diagnosis of endometritis (BUCZKOWSKA et al., 2014; RIDDLE et al., 2007). In this context, researchers have claimed that, since it is considered as an indication of endometritis, cytological examination is more efficient than bacteriological isolation, and that a positive cytological result is a more accurate tool for the diagnosis of endometritis, although it does not provide any information on the aetiology of inflammation (RIDDLE et al., 2007; LEBLANC, 2010).

In the present study, the fact that the positive correlation detected between the cytological and microbiological findings was not observed between the histopathological and microbiological results was attributed to the brush technique that enabled the collection of cells from a much larger surface in comparison to the biopsy technique. The examination of a small area of cellular material reduces the time required for detection of differentiating cells on which the diagnosis is based, and suggests that the CB technique may be used to complement cytological findings and to support a diagnosis. According to previous research, although EB is considered the gold standard for evaluation of uterine health, the present study showed that, owing to the quality of the LBC samples, this method could be used as a screening tool for gynaecological examination of mares. Endometrial cytology provided sufficient diagnostic material at a significantly higher level than biopsy. Thus, we propose the use of endometrial cytology for routine diagnosis, either alone or together with other diagnostic procedures to improve diagnostic accuracy (BUCCOLIERO et al., 2009). Cervicovaginal samples are relatively less cellular than non-gynaecological samples. Therefore, it is very difficult to achieve an adequate level of cellularity in endometrial samples. Furthermore, the transfer of these cells on to glass slides at the highest concentration possible is rather difficult. False-negative cervical cytology results have been determined at the stage of the collection of the differentiating cells (FIALA et al., 2010; DEFONTIS et al., 2011; OVERBECK et al., 2011; COCCHIA et al., 2012; WALTER et al., 2012). This study demonstrated the diagnostic distinction and superiority of liquid-based cytology based on investigation of cytological smears, which were prepared from samples containing low numbers of cells and different staining techniques were applied.

LBC provides better cytological evaluation with improved sensitivity and specificity through the better preservation of nuclear details, with good fixation.

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CAKIR BAYRAM, L., K. GÜRBULAK, K. S. GÜMUSSOY, O. KUTSAL: Uloga centrifugirane tekućinske citologije u procjeni endometrija kobila. Vet. arhiv 89, 743-770, 2019.

SAŽETAK

Endometritis je čest u rasplodnih kobila i jedan je od glavnih uzroka njihove neplodnosti. Za dijagnostiku ovoga stanja primjenjuje se nekoliko metoda, uključujući citološke, bakteriološke i histopatološke metode, no čak ni njihova kombinirana primjena ne mora dati konačan rezultat. Cilj je ovoga istraživanja bio procijeniti dijagnostičku učinkovitost centrifugirane tekućinske citologije (LBC) kako bi se usporedila prisutnost polimorfonuklearnih stanica (PMN) u materničnom epitelu i vanjskom sloju endometrija (stratum compactum): 1. histopatološkim metodama, 2. citologijom, 3. mikrobiološki (s obzirom na prisutnost uzročnika upale). U istraživanje je uključeno pedeset kobila različite dobi, uzgojenih u konjičkom klubu Jockey Club of Turkey. Uzorci endometrija za mikrobiološku, citološku i histopatološku pretragu dobiveni su CB i EB tehnikama. Usporedba tekućinske citologije (LBC) s patohistološkom dijagnostikom (HD), koja se smatra najboljom metodom, pokazala je da su osjetljivost, specifičnost i pozitivna prediktivna vrijednost testa (PVP) te negativna prediktivna vrijednost testa (PVN) tekućinskom citometrijom bile 0,5000, 0,4000, 0,7692 i 0,6087. Rezultati ovih dviju metoda nisu se podudarali (P = 0,0166). Nadalje, potvrđeno je da LBC nije pouzdana u otkrivanju degenerativnih i fibroznih promjena u žlijezdama endometrija. U pozitivnom smilu LBC pruža jasnu sliku dobro disperziranih stanica te omogućuje veću dijagnostičku osjetljivost i pouzdanost. Također, kako je ispitivano područje malo, identifikacija ciljanih stanica relativno je lagana.

Ključne riječi: centrifugirana tekućinska citologija endometrija; biopsija endometrija; ispirak stanica; bakteriologija; kobila