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Short Communication

Comparison of the Cytology Brush and Cotton Swab in the Cytological Evaluation of the Endometrium in Mares with Regard to Fertility

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ABSTRACT

The aim of this study was to compare the cytology brush (CB) and cotton swab (CS) in the cytological evaluation of the endometrium in mares with regard to fertility. The study was conducted on 26 mares displaying spontaneous estrus. Samples for cytological evaluation were taken from each mare by using commercially available CS and CB. After sample collection, all mares were mated in the same estrus, and pregnancy diagnosis was performed 14-18 days after last mating. No vaginal cells were found in smears, and the CB technique yielded significantly more cells/high-power field (HPF) than the CS technique. Additionally, more cases of endometritis were diagnosed using the CB than the CS. It was also shown that the degree of inflammation is more important in diagnosis of infertility in the mare than the mere presence or absence of inflammation. In conclusion, further studies are needed to establish more precise criteria for the interpretation of inflammation, especially if samples are collected using the CB.

1. Introduction

Diagnosis of endometritis is based on the mare's history, external examination, vaginal examination, palpation, and ultrasonography of the genital tract, as well as laboratory diagnostics including uterine culture, cytology, endometrial biopsy, and occasionally endoscopic examination of the mare's reproductive tract [1]. Cytological evaluation of the endometrium is one of the most commonly applied diagnostic methods. An advantage of this method is the ability to receive results quickly after specimen collection. This technique is also relatively inexpensive and easy to perform, although it does not provide much information about a cause of the inflammation. It was shown that a positive cytology is twice as common as a positive culture, and the degree of inflammation is more important in the diagnosis of infertility in the mare than the presence or absence of inflammation [2]. Samples for cytological examination from the endometrium can be harvested with the use of cotton swab (CS), uterine cytological brush (CB), and low-volume uterine flush and through preparing smears from the biopsy material [1-10]. It is important to collect samples from the uterus to avoid contamination by collection of cells from other parts of the reproductive tract. This may lead to false diagnosis due to a higher number of neutrophils being found in other areas of reproductive tract than in the endometrium [11]. Although all the above-mentioned methods of sample collection enable acquisition of uterine material, low-volume flushing can take more time, but some authors consider it a rapid, accurate and practical method [12], and biopsy could be more invasive. Additionally, endometrial cytology from a low-volume uterine flush may not reflect a degree of inflammation [12]. This is due to dilution and centrifugation of the

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efflux, which may disrupt cell walls [12]. On the other hand, a disadvantage of the CS is that they contact a small area of the endometrium, and smears made using the CS yield fewer endometrial cell per high-power field (HPF) than smears made using the CB [4,7-9]. The CB has been used more and more often recently; however, there are no precise criteria for interpretation of inflammation in the cytological smears obtained using the CB [4,8].

The objective of this study was to compare the CB and CS in the cytological evaluation of the endometrium in mares with regard to fertility. In particular, we tested whether commercially available CB are superior to commercially available CS in diagnosis of endometritis in estrus mares.

2. Materials and Methods

2.1. Animals

Twenty four multiparous warmblood mares aged 6-18 years and 2 warmblood mares aged 4 years that had given birth to one foal were used. Twelve of these mares were barren, and 14 were foaling. The material for the study was collected from mares in spontaneous estrus in March or in April.

2.2. Estrus Detection

Estrus was detected in the presence of a stallion based on external signs shown by the mare (i.e., raising the tail, urination, repeated eversion of the labia with exposure of the clitoris [winking], and tolerance to the stallion). Samples were collected only from mares showing estrus, and based on ultrasonographic examination of the reproductive tract, in each mare the endometrial edema was classified as 3 (moderate edema throughout the whole uterus) based on a scale of 1-4 as described by Pycock et al. [13]. There was no fluid in the uterine lumen, and a follicle with a diameter more than 3.5 cm was found. Foaling heat was not taken into consideration in this study.

2.3. Sample Collection

Prior to the sample collection, the mare's tail was bandaged and vulva and perineum were scrubbed with povidone-iodine and dried with a paper towel. Samples for cytological evaluation were taken from each mare by using a commercially available cotton swab (Equivet uterine culture swab; Kruuse, Denmark) and then a uterine cytological brush (Cytology Brush; Minitube GmbH, Germany). The instruments were passed through the vagina and cervix into the uterus with a sleeved and lubricated arm. Cellular material was always collected from the uterus by rotating the CS and by rotating the CB. All samples were collected by students.

2.4. Cytology

Following the sample collection, a smear was prepared immediately on a glass slide by using a gentle rolling motion. The smear was fixed with Cytofix (Samko, Dobczyn, Poland), stained using Diff-Quick stain (Medion Diagnostics AG, Düdingen, Switzerland), and then evaluated by light microscopy at $\times$400 magnification. Cellularity was assessed by counting the total number of cells in 10 HPFs, and then averages were taken. In addition to counting the epithelial cells and polymorphonuclear cells (PMNs), smears were also analyzed for the presence of vaginal cells (large polygonal or oval cells with central round nuclei) [11]. In order to evaluate the inflammation, two criteria were assumed, as follows. In criterion I, the percentage of PMNs was quantitated and scored (a total of 300 cells were counted [8,14]); that is, a score of (−) indicated no PMNs were seen; (±) indicated <0.5% PMNs; (+) indicated 0.5%-5% PMNs; (++) indicated 5%-30%; and (+++) indicated >30% PMNs. A mare was considered suffering from endometritis if the result was at least (+). In criterion II, the number of PMNs/HPF was determined [2]; that is, 0-2 PMNs/HPF indicated no inflammation; 2-5 PMNs/HPF indicated moderate inflammation; and >5 PMNs/HPF indicated severe inflammation. Ten HPFs were chosen at random and evaluated in each smear, and the number of PMNs was averaged.

2.5. Mating

After sample collection, all mares were naturally mated in the same estrus with stallions with proven fertility. No treatment was applied before sample collection and after sample collection until the first pregnancy diagnosis (14-18 day after last mating). Second pregnancy diagnosis was performed 4-5 weeks later.

2.6. Statistical Analysis

Statistical analysis was carried out with the use of the Student t test for comparison of the number of cells in smears obtained using CB and CS, and the analysis of variance (ANOVA) test was used for other analyses. All data indicated normal distribution. Level of significance was set at a P value of <.05. All statistical analyses were carried out with Statistica version 9.1 software (StatSoft, Kraków, Poland).

3. Results

3.1. Cytological Findings

The number of cells in smears obtained from the CB amounted to 41.15 ± 21.12/HPF, whereas smears obtained from the CS amounted to 19.58 ± 12.83/HPF ($P < .05$). Vaginal cells were not found in any smears.

It was shown that endometritis (+, ++, +++) was significantly more frequently diagnosed if a specimen was collected using the CB (54% and 38% cases of endometritis, respectively, for smears obtained using the CB and CS, $P < .05$) (Table 1). Applying the criterion based on the number of PMNs/HPF, (criterion II) no significant differences in the frequency of diagnosis of endometritis was found between smears obtained using the CB or CS (Table 2).

3.2. Pregnancy Results

Pregnancy was found in 12 mares at the first examination (pregnancy rates of 46%), and at the next examination, the number of pregnant mares did not change. No mare was pregnant if there was more than 5% PMNs in the