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Comparison of the Cytobrush Technique, Vaginoscopy and Transrectal Ultrasonography Methods for the Diagnosis of Postpartum Endometritis in Cows

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Abstract: In the present study, cytobrush (endometrial cytology) technique, vaginoscopy and transrectal ultrasonography methods were used for the diagnosis of endometritis in cows and their diagnostic values compared. The study involved 31 Brown Swiss cows aged between 4 and 6 years. The cows were chosen from infertile cows at 45-180 days of parturition. Vaginoscopically, vaginal discharge scores were +3 in 17 cows, +2 in 4 cows, +1 in 3 cows and 0 in 7 cows. Transrectal ultrasonographic examination of the cornu uteri revealed a fullness of the ≥5 cm in 15 cows, 3-5 cm in 3 cows and ≤3 cm in 13 cows. On the endometrial cytologic examination, the presence of Polymorph Nuclear Neutrophil Leucocytes (PMNLs) number ≥5% were regarded as positive for the diagnosis of endometritis. Statistically, there was no significant difference between clinical diagnostic methods and cytobrush technique (p = 0.658). This study showed that endometrial cytology could be used in cows safely and effectively for the diagnosis of the infertility particularly in cases of subclinical endometritis. Furthermore, it was showed that cytobrush technique could also be used during the follow up of the recovery.

Key words: Endometritis, endometrial cytology, ultrasonography, vaginoscopy, cow, brown swiss

INTRODUCTION

Uterus is vulnerable to trauma and infection during and after parturition in cows. Pathogenic bacteria pass through the cervix and contaminate the uterus (Kasimanickam *et al*., 2005; Sheldon, 2004, 2007) resulting endometritis. Postpartum uterine infection has an adverse effect on reproductive performance and causes economic loss (Dolezel *et al*., 2008; Foldi *et al*., 2006; Javed and Khan, 1991; Kasimanickam *et al*., 2005; Sheldon, 2007). The incidence of the disease is high (20-40%) and it ranges from 10-80% depending on various internal or external factors as well as diagnostic methods (Dolezel *et al*., 2008).

Many methods are used for the diagnosis of endometritis, including inspection of vaginal discharge, transrectal palpation, transrectal ultrasonography, uterine bacterial culture, uterine biopsy and endometrial cytology (Ahmadi *et al*., 2006; Blanchard *et al*., 1981; Dolezel *et al*., 2008; Kasimanickam *et al*., 2005; Lewis, 1997; Meanspeaker, 2003; Sheldon *et al*., 2006). Clinically, vaginoscopy and transrectal ultrasonography are commonly used for the diagnosis of the endometritis. Accurate and early diagnosis is essential for successfull treatment of this disease (Lewis, 1997; Sheldon *et al*., 2006).

The evaluation of vaginal discharge is commonly used for the diagnosis of the clinical endometritis in cows. Vagina should be inspected by vaginoscopy for the presence of pus. The character of vaginal mucus can be scored to produce a clinical endometritis score. Vaginal discharge score is based on the color and volume of pus. If the vaginal discharge shows clear translucent mucus character, the score is 0 and when it shows clear mucus containing flecks of white pus character, the score is 1 and when it contains ≤50% white or yellow white pus character, the score is 2 and when it contains ≥50% white, yellow white or bloody pus character, score is 3 (Sheldon *et al*., 2006). Transrectal ultrasonography helps to diagnose endometritis by representing accumulation of fluid in uterus (Dolezel *et al*., 2008).

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Cytological examination of reproductive tract is commonly used to evaluate reproductive lesions in humans and domestic animals (Ahmadi and Nazifi, 2006; Ahmadi *et al*., 2006; Kasimanickam *et al*., 2005). Endometrial samples are collected by using, including cotton swabs, uterine biopsy, uterine lavage and cytobrush technique for cytological examination as an aid in the diagnosis of acute and subclinical endometritis in cows (Ahmadi *et al*., 2005, 2006; Bonnett *et al*., 1991; Kasimanickam *et al*., 2004, 2005). Bacteriology and determination of quantity and spectrum of microbes could be helpful for the diagnosis of endometritis, but these methods are not accurate for the diagnosis of puerperal endometritis as findings are similar in early postpartum physiological puerperal discharge (Bonnett *et al*., 1991; Dolezel *et al*., 2008). Cotton swabs are routinely used to evaluate the vagina cytologically in bitch. Cytobrush technique is used in both humans and equine species for the diagnosis of reproductive tract disorders by collecting cervical and endometrial cells (Kasimanickam *et al*., 2005).

The objective of this study was to compare the cytobrush technique, vaginoscopy and transrectal ultrasonography methods for the diagnosis of endometritis in cows.

MATERIALS AND METHODS

Cattle and experimental design: In this study, 31 Brown Swiss cows, 4-6 years old, were used. Cows, 45-180 days of postpartum, were examined by vaginoscopy and transrectal ultrasonography and then endometrial cytology was carried out. All examinations were done by the same operator. Vaginoscopical examination was evaluated in accordance with vaginal discharge scores (0, +1, +2, +3) (Sheldon *et al*., 2006). Vaginal discharge scores +1≥ were regarded as endometritis. A real-time ultrasound scanner equipped with a 8 MHz linear-array transducer (ESAOTE® 410477, Pie MEDICAL) was used for the study. Transrectal ultrasonographic examination was used to evaluate accumulating of fluid in the uterine lumen (Fig. 1a, b). Intrauterine fluid accumulations were categorized as uterine size at the base of uterine horn diameter (≤3, 3-5 and ≥5 cm, respectively) (Kasimanickam *et al*., 2004). Uterine horn diameters >3 cm were regarded as endometritis.

Cytobrush technique: Endometrial samples were collected by using cytobrush for cytological examination (Kasimanickam *et al*., 2004, 2005) (Fig. 2). Slides for cytological examination were prepared by rolling the cytobrush onto a clean glass microscope slide, air dried and fixed in methanol for 2 min and stained with Giemsa stain for 15 min. These stained smears were examined by counting inflammatory cells and other cell types. Cytological assessment was done by counting 100 cells at 1000X magnification (Olympus CX21FS1, Olympus corp, Japan) to determine percentage of inflammatory

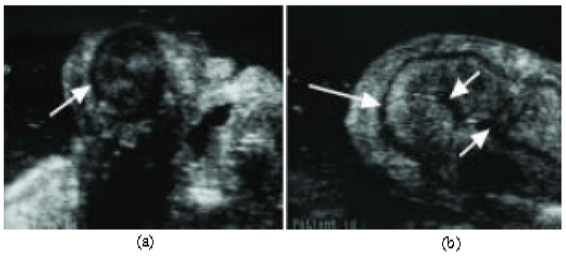

Fig. 1: Ultrasonographic evaluations normal uterus (arrow), uterus with chronic endometritis. Accumulation of uterine fluid (small arrows). Uterine wall (long arrow)

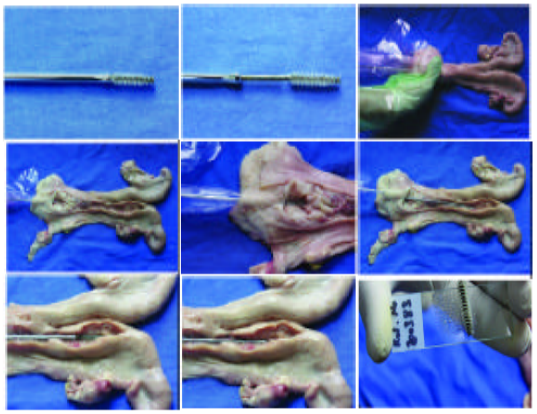

Fig. 2: Cytobrush technique

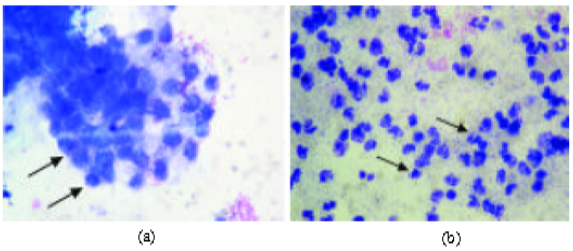

Fig. 3: Endometrial cytological evaluations a): Slide from cytobrush technique shows normal endometrial cells. X 1000. Giemsa stain and b: Slide from cytobrush technique shows diffuse infiltration of PMNLs. X 1000. Giemsa stain

cells. Cows with >5% PMN in the samples were regarded as affected by endometritis (Drillich *et al*., 2005) (Fig. 3a, b).

Statistical analysis: Differences between the groups were tested by analysis of variance and Duncan test using SPSS for Windows (version 12.0, 2005). Data were presented as mean±standard deviations and $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The objectives of this study were to compare the cytobrush technique, vaginoscopy and transrectal ultrasonography methods used for the diagnosis of endometritis, which adversely effects reproductive performance and causes economic loss.

Vaginoscopic examination revealed vaginal discharge scores as +3 in 17, +2 in 4, +1 in 3 cows and 0 in 7 cows (1 of 7 cows was evaluated as subclinical endometritis) (Mean±SE: 2.00±0.227). Transrectal ultrasonographic examination of the cornu uteri revealed a fullness of the ≥5 cm in 15 cows, 3-5 cm in 3 cows and ≤3 cm in 13 cows (Fig. 1) (7 of 13 cows were evaluated as subclinical endometritis) (Mean±SE: 2.06±0.173). In the endometrial cytology, the presence of PMNLs ≥5% were regarded as positive for the diagnosis of endometritis (Mean±SE: 2.26±0.217) (Table 1). Cytologically 6 of 31 cows were diagnosed non-endometritic. Statistically, there was no significant difference between clinical diagnostic findings and cytobrush technique ($p = 0.658$).

This study showed that combination of vaginal discharge score and transrectal ultrasonography methods and cytobrush technique were useful for the diagnosis of endometritis in cows.

Ultrasonography and vaginoscopy methods are generally used for the diagnosis of endometritis. Recently, a new technique, endometrial cytology (cytobrush), have been introduced for the diagnosis of endometritis. The combination of these diagnostic methods and cytobrush technique have been reported to be useful for the diagnosis of endometritis, especially of subclinical endometritis.

Transrectal palpation is not reliable method for the diagnosis of all types of endometritis. Therefore, vaginal inspection and evaluation of vaginal discharge methods are useful for the diagnosis of clinical endometritis but not for subclinical endometritis. The absence of uterine discharge by vaginoscopic examination is not truly indicative of absence of uterine inflammation (Kasimanickam *et al*., 2004). Similarly, in this study, seven cows were evaluated by vaginoscopy as non endometritic but one of these seven cows was diagnosed subclinical endometritis by cytobrush technique.

Table 1: Findings of vaginoscopy and transrectal ultrasonography methods and cytobrush technique for all cows

Findings	Cows (n: 31)
Vaginoscopy (vaginal discharge score)	
Score	
0 (Metritis -)	7 (1 of 7 cows, evaluated as subclinical according to cytobrush)
1 (Metritis +)	3
2 (Metritis +)	4
3 (Metritis +)	17
Transrectal ultrasonography	
Uterine diameter	
≤3 cm (Metritis -)	13 (7 of 13 cows, evaluated as subclinical according to cytobrush)
3-5 cm (Metritis +)	3
≥5 cm (Metritis +)	15
Cytobrush	
(PMNLs%)	
<5 (Metritis -)	6
≥5 (Metritis +)	25

The uterine size and its content could be determined by transrectal ultrasonography in clinical endometritis. Kasimanickam *et al.* (2004) concluded that ultrasonographic examination and endometrial cytology were measuring 2 different causative factors; ultrasonography measuring the clearance mechanism and endometrial cytology measuring cellular response of the uterus. In our investigation, 13 cows were evaluated non metritic by ultrasonographic examination of the cornu uteri. However, 7 of 13 cows were found out subclinical endometritis by endometrial cytology.

Although, many methods are used for the diagnosis of endometritis few of them may be of use in practice, especially in diagnosis of subclinical endometritis. Subclinical endometritis can be diagnosed accurately by either cytologically (cytobrush and uterine lavage technique) or histologically (biopsy) (Blanchard *et al*., 1981; Javed and Khan, 1991; Kasimanickam *et al*., 2005; Lewis, 1997). Although, biopsy method is frequently used by some practitioners, use of this procedure is practically questionable (Lewis, 1997). Because this method is invasive, expensive and time consuming. Furthermore, biopsy may be associated with delayed conception becauseof induced damage of uterine tissue (Dolezel *et al*., 2008; Lewis, 1997). Kasimanickam *et al.* (2005) suggested that cytobrush technique may also cause irritation to the endometrium but this is arguable. Furthermore, according to Kasimanickam *et al.* (2005), this technique was reliable, easy and not time consuming. The results obtained in this study were also similar.

CONCLUSION

This study showed that endometrial cytology could be used in cows safely and effectively for the diagnosis of the infertility particularly in cases of subclinical endometritis that results in poor reproductive performance. Especially, diagnosis of subclinical

endometritis by this technique will lead to commencement of early and effective treatment. Additionally, cytobrush technique could also be used during the follow up of the recovery period.

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