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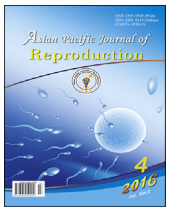

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Evaluation of polymorphonuclear (PMN) cells in cervical sample as a diagnostic technique for detection of subclinical endometritis in dairy cattle

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ABSTRACT

Objective: This study evaluated the cervical sampling as an easy and safe method for the diagnosis of subclinical endometritis in dairy cattle.

Methods: One hundred ninety seven lactating Holstein cows were examined at 26–32 d in milk (DIM) for diagnosis of endometritis. Differential cellular counts were also made from stained smears of the cervical mucosa. Using the Receiver/Response Operating Characteristics (ROC) curve, presence of >17.5% polymorphonuclear (PMN) cells was calculated for detection of subclinical endometritis with sensitivity and specificity of 56.5% and 83.3% respectively.

Results: Cows with subclinical endometritis had significantly more open days and all service conception rate than normal cows. The results of survival analysis showed that normal cows became pregnant at a significantly faster rate than cows with subclinical endometritis.

Conclusions: The results of the present study introduced the cervical smear sampling as an easy and suitable method for diagnosis of subclinical endometritis in dairy cattle.

1. Introduction

Nearly all cows experience some degree of endometrial inflammation in early postpartum that is associated with normal involution; however, the accurate identification of truly diseased cows for administration of appropriate treatment is so important [1]. Endometritis is defined as inflammation limited to the endometrium occurring at least 21 d after calving without signs of systemic illness. Observation of purulent discharge on the tail, detection of purulent material in the vagina by vaginoscopy and detection of an enlarged uterine horn following transrectal palpation of the uterus are some of the techniques that are used for detection of endometritis in cattle [2]. In new classification, endometritis has been subdivided into clinical and subclinical categories [3]. Clinical endometritis is defined as purulent or mucopurulent uterine discharge present after 21 or 26 d postpartum. Subclinical endometritis can be defined as endometrial inflammation of the uterus in the absence of purulent material in the vagina [1]. Despite the absence of purulent discharge, the severity of the disease is still considered sufficient to impair reproductive performance. Animals with subclinical disease have more days open, take longer to conceive, have lower conception rates and are culled more than normal animals. Typical conception rates are half that of normal animals [1,4]. Several techniques such as uterine biopsy [5], uterine lavage [6], cytobrush [7] and a guarded cotton swab [8] have been used for the diagnosis of the subclinical endometritis. Collection of endometrial and inflammatory cells is the base of all these techniques. Among these, uterine biopsy is the most invasive technique and can impair subsequent reproduction in biopsied cows [9]. Cytobrush and uterine lavage are less harmful techniques for the endometrium than the uterine biopsy; however, both techniques are time consuming. The fluid that is used for uterine lavage can be irritant and causes a 17% failure in attempts to recover fluid [7]. It has been suggested that sampling by uterine lavage

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can cause a tendency for lower first-service conception in primiparous cows [10].

Yavari *et al.* showed that the neutrophil percentage in cervical mucosa and uterine fluid of the cows affected by *Arcanobacterium pyogenes* and clinical signs of purulent uterine discharge were significantly higher than unaffected cows. The result of their study did not show any significant differences between neutrophils percentages of cervical mucosa and uterine fluid smear in cows with three classifications of clinical endometritis [11]. These authors also investigated there were no significant differences in cell densities between uterine and cervical mucosa in genital tracts of slaughtered cows affected with either acute or chronic endometritis [12]. These findings suggest that there is a reasonable correlation between uterine and cervical cytology. As regards cervical sampling, it is easier, requires less time and it is not irritant for uterine endometrium, it could possibly be a convenient alternative for uterine lavage and cytobrush in subclinical endometritis diagnosis.

The objective of this study was to evaluate cervical sampling as an easy and safe method for the diagnosis of subclinical endometritis in dairy cattle.

2. Material and methods

2.1. Animals and herd

The study was conducted on 197 lactating Holstein cows in a large commercial dairy herd in Shiraz, Iran (longitude 052°36′E and latitude 29°33′N). The area has a warm climate with four distinct seasons, with peak summer temperatures reaching above 40 °C and a winter minimum temperature below freezing. Throughout the year, cows were housed in a tie-stall barn with straw bedding. Cows were group fed a total mixed ration to meet production requirements.

2.2. Clinical examinations

Cows were examined at 26–32 d in milk (DIM) for diagnosis of clinical endometritis. During the examination, cows were first inspected for the presence of fresh discharge on the vulva, perineum or tail. If discharge was not visible externally, the cows were examined through the vagina. The cow's vulva was thoroughly cleaned with a dry paper towel and a clean, lubricated and gloved hand was inserted through the vulva. In each cow, the lateral, dorsal and ventral walls of the vagina were palpated, and the mucus contents of the vagina withdrawn manually for evaluation [2]. The vaginal mucus was assessed for color and proportion of pus. Cows with abnormal uterine discharge (discharge with flecks of pus or mucopurulent discharge) were diagnosed with clinical endometritis. Cows were also examined with transrectal ultrasonography (real time B-mode linear array scanner with a 5 MHz transducer, 500 V, Ami, Canada) with a 5 MHz linear-array transducer for presence of any amount of fluid in uterus.

Differential cellular counts were made from stained smears of the cervical mucosa. A clean 50 cm plastic uterine pipette was used for collection of cytological samples. The vulva was washed with antiseptic solution. The plastic uterine pipette was fixed at external os of the cervix by rectovaginal method. Cervical mucosa aspirated by 50 mL syringe. Once the sample had been taken, the mucosa was rolled on glass slides and air-dried and differential cellular count was carried out on Giemsa-stained smear of the mucosa. The cytology slides were fixed by methanol for five minutes then put on the Giemsa stain for 20 min and finally washed in distilled water and dried. Smear slides were evaluated by light microscope. At least 100–200 cells were counted in 20 microscopic fields (×900). The counted cells were epithelial cells and neutrophils.

2.3. Calculation of reproductive parameters

Parameters assessed for reproductive performance were interval from calving to pregnancy (days open, DO) (the mean number of days from calving to conception among the cows that became pregnant within 210 d postpartum), interval from calving to first service, all service conception rate (the number of cows that conceived within 210 d postpartum, divided by the number of all given services, multiplied by 100) and first service conception rate (the number of cows conceived with first AI within 210 d postpartum, divided by total number of cows that received AI, multiplied by 100).

2.4. Statistical analyses

A Receiver/Response Operating Characteristics (ROC) curve was plotted to determine the cut off percentage PMN for detection of subclinical endometritis using the visible abnormal uterine discharge as gold standard test for endometritis. Based on calculated cut off PMN in cervical discharge, cows were divided into two groups: with and without subclinical endometritis. Four indices of reproductive performance (days open, calving to first service, all service conception rate and first service conception rate) were compared between these two groups using the *t*-test of the SAS 9.2 (Statistical analysis system package, Cary, NC, USA: SAS Institute, Inc., 2005). Days open and days to first service were presented as least square means and the accompanying standard error of the mean. First service and all service conception rates were presented as percent. Significance was established at $P < 0.05$. The sensitivity and specificity values for the cervical cytology diagnostic test were calculated when it was used to predict pregnancy status at 100 d postpartum. To verify the assumption of proportional hazards of pregnancy for cows with and without subclinical endometritis, the Kaplan–Meier survival functions were estimated for each group using the LIFE TEST procedure in SAS. The number of open cows was plotted against the time to pregnancy.

3. Results

The study was conducted on 197 cows at 26–32 d after calving. Of these 197 cows, 121 (61.4%) cows were observed with abnormal uterine discharge and were diagnosed as clinical endometritis. All of the cervical cytology slides were readable and assessed successfully. Mean PMN percentage was 18.8% (range, 0%–95%). A Receiver/Response Operating Characteristics (ROC) curve was plotted using the sensitivity and specificity for each possible percentage PMN at 26–32 d postpartum for detection of subclinical endometritis. In this analysis, presence of abnormal uterine discharge was used as gold standard test for endometritis. The ROC curve identified >17.5% PMN at 26–32 d postpartum as the cut point (Figure 1). Dot histogram