

## Comparative cervical cytology and conception rate in postpartum dairy cows

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### ABSTRACT

During the early postpartum period, multiple bacterial species invade the uterus of cows. Phagocytosis by polymorphonuclear cells is a primary mechanism involved in the elimination of bacteria and improvement of conception rate. Therefore, a relationship could exist between endometrial cytology and conception rate of postpartum dairy cows. In this study 50 postpartum healthy Holstein Frisian dairy cows were selected. They had a normal parturition history and had no mucopurulent discharge from vulva or abnormality in rectal palpation. Cervical mucosal discharge was collected from all cows on days 25 to 30 and 55 to 60 postpartum. Blood progesterone levels were determined in all cows by radioimmunoassay (RIA). Differential cellular counts were carried out from a Giemsa-stained smear of the mucosa. Data were analyzed by Independent T test, one-way ANOVA, and Duncan's multiple range tests. There was no significant difference between cell percentages at different times or in number of postpartum artificial inseminations ( $P \geq 0.05$ ). However, when cows were divided into two groups to progesterone above 1 ng/mL and below 1 ng/mL, there were significant differences ( $P < 0.05$ ) between neutrophil percentages at different times after parturition and artificial insemination number (1 and 2 or 3). The result of this study showed that cytological evaluation of cervical smear is helpful for diagnosis and treatment of subclinical endometritis and prognosis of postpartum fertility.

**Key words:** postpartum, endometritis, neutrophil, cytology, dairy cow

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### Introduction

The decrease of calving to first ovulation, first ovulation to first oestrus interval and improvement of conception rate depend on several postpartum factors (NOAKES et al., 2001). A complex relationship exists between factors influencing uterine health and disease in the postpartum cow (GILBERT, 1992; NEBEL, 1999). Reproductive performance of dairy cows after the voluntary waiting period is highly related to the health status of the

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uterus after calving (DIJKHUIZEN and STELWAGEN, 1985; FERGUSON and GALLIGAN, 2000). The important factors are uterine involution, bacterial elimination, restoration of endometrium and return to cyclical activity. The complete uterine involution range is 26 to 52 days after calving, but the changes after 20 to 25 days after calving are generally almost imperceptible. Complete re-epithelialization of the caruncle is complete from 25 days onwards (NOAKES et al., 2001).

Endometritis is defined as inflammation of the endometrium without systemic signs and is associated with delayed uterine involution. Postpartum endometritis has a negative effect on reproductive performance as it increases services per conception, calving to first service interval and calving to conception interval (BUTT et al., 1991; HEUWIESER et al., 2000), reduces the risk of pregnancy (LEBLANC et al., 2002), and decreases the conception rate (FERGUSON and GALLIGAN, 2000).

During the early postpartum period, multiple bacterial species have been isolated from more than 90% of cows, but the prevalence of infection decreases with time (ELLIOT et al., 1968). The initial uterine defence against bacterial infection is phagocytosis by uterine leucocytes (mainly neutrophils). The abnormal puerperium affects uterine defence mechanisms adversely and prolongs the time to complete uterine involution (HUSSAIN, 1989; BUTT et al., 1991; LANDER CHACIN et al., 1991; WATSON et al., 1987).

The profile and function of blood and uterine leukocytes were evaluated in 14 dairy cows which spontaneously recovered from postpartum endometritis. These results suggest that a decrease in blood polymorphonuclear granulocyte (PMN) oxidative burst activity until the first week postpartum could be associated with an increased susceptibility to early postpartum endometritis. The later increase in this parameter, as well as the increase in the intrauterine fluid phago-PMN, might favour the spontaneous resolution of endometritis (MATEUS et al., 2002). The contamination grade was positively correlated with uterine PMN numbers and negatively correlated with blood PMN numbers (HUSSAIN and DANIEL, 1991; MATEUS et al., 2002).

The diagnosis of endometritis palpation per rectum or by fortuitous observation of a genital discharge is insensitive and non-specific (MILLER et al., 1980). To gain clarity of some of the questions surrounding bovine endometritis, a simple, rapid, inexpensive means of diagnosing the condition is essential. Endometrial biopsy has been studied in cows. It is neither simple nor inexpensive for use in large numbers of animals. In addition, it has been suggested to impair subsequent reproduction in biopsied cows. Endometrial cytology has proved exceptionally valuable in equine theriogenology (BALL et al., 1988; DIGBY, 1978; ROSZEL and FREEMAN, 1988; SLUSHER et al., 1984), and was investigated in endometrial cytology at endometritis diagnosis in cows (KASIMANICKAM et al., 2004).

There were negative and significant correlations between neutrophil percentages in cervical mucosa and days post-parturition (AHMADI et al., 2001). Conception rates were

53.7%, 51.2% and 42.5% in cows with <1%, 1 to 5% and >5% PMN at endometrial smear, respectively (RAAB et al., 2002).

The aim of this study was determination of cytological changes in cervical mucosa at 25 to 30 and 55 to 60 days after normal parturition and consideration of the relationship between conception rates of dairy cows.

### Materials and methods

*Animals.* In this experiment, 50 postpartum healthy Holstein Frisian dairy cows were selected from a large commercial dairy farm near Shiraz. They had normal parturition and had had no postpartum problems post-parturition. The cows had passed through 1 to 4 parturitions and were inseminated 60 days after postpartum, when body condition scores (BCS) were about 2.5 (scale 1-5). The authors observed no clinical signs of endometritis when cows of the dairy herds were examined during routine visits for monitoring fertility. The criteria for selection were that they had a normal parturition history and had calved 25 to 30 days before. They had no mucopurulent discharge from vulva nor abnormality in rectal palpation. The cows were evaluated on days 25 to 30 and days 50 to 60 post-parturition. The calving to first service, open days and service per conception rate of cows were  $66.71 \pm 23.46$ ,  $90.69 \pm 39.09$  and  $1.71 \pm 0.87$ , respectively. Also, the mean ( $\pm$  SD) of milk on days 25 to 30 and days 50 to 60 were  $23.16 \pm 6.94$  and  $22.91 \pm 6.52$ , respectively.

*Examination and sampling.* The health of all uteruses was confirmed by cytological evaluation of cervical mucosa smear. Differential cellular counts were made from Giemsa stained smears of the mucosa. We used a clean 50 cm plastic uterine pipette for collection of cytological samples. The vulva was washed with antiseptic solution. The plastic uterine pipette was covered by a larger plastic tube fixed at external os cervix by rectovaginal method. Cervical mucosa aspirated by 50 mL syringes vacuum. Cervical mucosa smear was prepared and dried (AHMADI et al., 2001). Blood samples were collected from the coccygeal vein in vacutainers containing no anticoagulant. The serum was separated following centrifugation for 15 min at  $750 \times g$  and any haemolysed samples were discarded. Serum samples were stored at  $-20^\circ\text{C}$  until analyzed.

*Progesterone assay.* Progesterone level was measured at the Namazi hospital laboratory by RIA (Orion Diagnostica, Finland) with sensitivity 0.2 ng/mL. The intra-assay and inter-assay was 10.2% and 6.5%, respectively.

*Cytology.* Cervical mucosal discharge was collected from all cows on days 25 to 30 and days 55 to 60 postpartum by gentle suction using a plastic pipette and 50 mL syringe. Differential cellular counts were carried out from a Giemsa-stained smear of the mucosa. The cytology slides were fixed by methanol for five minutes then put at the Giemsa stain for 20 minutes and finally washed in distilled water and dried. Smear slides were evaluated by light microscope. At least 100 to 200 cells were counted at 20 microscopical fields ( $\times 900$ ).

The counted cells were epithelial cells, large vacuolated epithelial cells, neutrophil (Fig. 1 and 2) and lymphocyte.

*Evaluation of results.* Data were analysed by SPSS software, version 11.5. Independent T test, one-way ANOVA, and Duncan's multiple range tests were used for detecting difference among means.

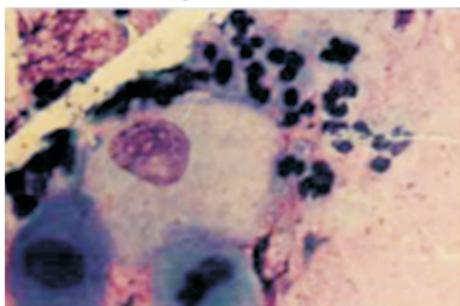


Fig. 1. Epithelial cells, large vacuolated cell and neutrophils in cervical mucosa smear. Giemsa stained;  $\times 900$

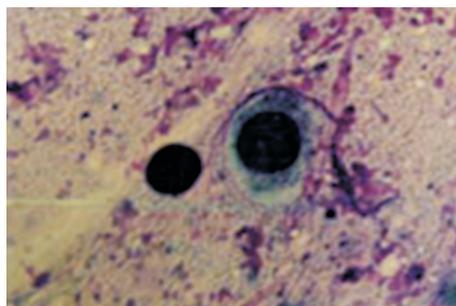


Fig. 2. Epithelial cell in cervical mucosa smear. Giemsa stained;  $\times 900$

## Results

Two cows were culled before 50 days post-parturition and were excluded from the study. There was no significant ( $P \geq 0.05$ ) difference between cell percentages at 25 to 30 days post-parturition and 50 to 60 days post-parturition (Table 1). But when cows were divided to progesterone above of 1 ng/mL and below of 1 ng/mL at 25 to 30 and 55 to 60 days after parturition, there was a significant difference ( $P < 0.05$ ) between neutrophil percentages at cows (Table 2). There was no significant difference between cell percentages in cows conceived with 1 and 2 or 3 artificial inseminations ( $P \geq 0.05$ ) (Table 3). But there was a significant difference in neutrophil percentages in cows conceived with 2 or 3 AI in cows with a progesterone below 1 ng/mL at 25 to 30 and 55 to 60 days post-parturition ( $P < 0.05$ ) (Table 4).

Table 1. Mean ( $\pm$  SEM) of cervical mucosa cells in postpartum period.

| Days of post partum | Cows N <sup>o</sup> . | EPC (%)          | LVEP (%)        | Neutrophil (%)  | Lymphocyte (%)   |
|---------------------|-----------------------|------------------|-----------------|-----------------|------------------|
| 25-30               | 48                    | 86.87 $\pm$ 0.84 | 4.52 $\pm$ 0.15 | 8.44 $\pm$ 0.45 | 0.17 $\pm$ 0.01  |
| 55-60               | 48                    | 89.13 $\pm$ 0.79 | 5.37 $\pm$ 0.19 | 5.44 $\pm$ 0.37 | 0.06 $\pm$ 0.005 |

EPC = epithelial cell; LVEP = large vacuolated epithelial cell

Table 2. Mean ( $\pm$  SEM) of cervical mucosa cells in postpartum period

| Days after calving | P4 ng/mL | Cows N <sup>o</sup> | EPC (%)          | LVEP (%)        | Neutrophil (%)                | Lymphocyte (%)  |
|--------------------|----------|---------------------|------------------|-----------------|-------------------------------|-----------------|
| 25-30              | >1       | 25                  | 89.84 $\pm$ 1.20 | 6.34 $\pm$ 0.29 | 2.03 $\pm$ 0.17 <sup>a</sup>  | 1.79 $\pm$ 1.56 |
| 25-30              | <1       | 23                  | 80.88 $\pm$ 1.92 | 2.88 $\pm$ 0.30 | 10.54 $\pm$ 1.23 <sup>b</sup> | 5.70 $\pm$ 2.94 |
| 55-60              | >1       | 27                  | 90.56 $\pm$ 0.64 | 5.15 $\pm$ 0.30 | 1.15 $\pm$ 0.08 <sup>a</sup>  | 3.14 $\pm$ 0.41 |
| 55-60              | <1       | 21                  | 74.50 $\pm$ 2.69 | 5.58 $\pm$ 0.67 | 16.33 $\pm$ 2.34 <sup>b</sup> | 3.59 $\pm$ 0.95 |

Values with different superscripts in each column are those that differ significantly ( $P < 0.05$ ).

Table 3. Mean ( $\pm$  SEM) of cervical mucosa cells in postpartum period

| Days after calving | AI N <sup>o</sup> | Cows N <sup>o</sup> | EPC (%)          | LVEP (%)        | Neutrophil (%)   | Lymphocyte (%)  |
|--------------------|-------------------|---------------------|------------------|-----------------|------------------|-----------------|
| 25-30              | 1                 | 27                  | 82.9 $\pm$ 1.36  | 5.83 $\pm$ 0.32 | 2.50 $\pm$ 0.25  | 8.77 $\pm$ 2.39 |
| 25-30              | 2 or 3            | 21                  | 79.33 $\pm$ 1.96 | 5.57 $\pm$ 0.53 | 10.28 $\pm$ 1.33 | 4.82 $\pm$ 2.35 |
| 55-60              | 1                 | 28                  | 85.66 $\pm$ 0.94 | 2.85 $\pm$ 0.14 | 4.00 $\pm$ 0.09  | 7.49 $\pm$ 0.97 |
| 55-60              | 2 or 3            | 20                  | 80.52 $\pm$ 6.88 | 8.36 $\pm$ 0.66 | 8.05 $\pm$ 1.53  | 3.07 $\pm$ 0.62 |

The different values in each column are not those that differ significantly ( $P > 0.05$ ).

Table 4. Mean ( $\pm$  SEM) of cervical mucosal cells in cows conceived with one AI and those conceived with 2 or 3 AI

| Days after calving | AI N <sup>o</sup> | P4 ng/mL | Cows N <sup>o</sup> | EPC (%)          | LVEP (%)        | Neutrophil (%)                | Lymphocyte (%)  |
|--------------------|-------------------|----------|---------------------|------------------|-----------------|-------------------------------|-----------------|
| 25-30              | 1                 | >1       | 16                  | 81.25 $\pm$ 2.28 | 7.62 $\pm$ 0.55 | 2.00 $\pm$ 0.28 <sup>b</sup>  | 9.13 $\pm$ 3.61 |
| 25-30              | 2 or 3            | >1       | 9                   | 92.00 $\pm$ 3.26 | 3.55 $\pm$ 0.38 | 1.11 $\pm$ 0.33 <sup>b</sup>  | 3.34 $\pm$ 3.57 |
| 25-30              | 1                 | <1       | 11                  | 87.37 $\pm$ 3.28 | 2.95 $\pm$ 0.66 | 3.00 $\pm$ 0.80 <sup>b</sup>  | 6.68 $\pm$ 3.91 |
| 25-30              | 2 or 3            | <1       | 12                  | 75.58 $\pm$ 3.97 | 3.25 $\pm$ 0.50 | 17.25 $\pm$ 3.00 <sup>a</sup> | 3.92 $\pm$ 3.79 |
| 55-60              | 1                 | >1       | 18                  | 91.27 $\pm$ 1.28 | 2.00 $\pm$ 0.16 | 1.16 $\pm$ 0.17 <sup>b</sup>  | 5.57 $\pm$ 1.02 |
| 55-60              | 2 or 3            | >1       | 9                   | 87.22 $\pm$ 1.62 | 9.33 $\pm$ 1.62 | 1.44 $\pm$ 0.20 <sup>b</sup>  | 2.01 $\pm$ 0.52 |
| 55-60              | 1                 | <1       | 10                  | 92.57 $\pm$ 4.31 | 4.42 $\pm$ 0.68 | 0.42 $\pm$ 0.09 <sup>b</sup>  | 2.59 $\pm$ 3.46 |
| 55-60              | 2 or 3            | <1       | 11                  | 70.22 $\pm$ 4.22 | 5.88 $\pm$ 1.23 | 21.77 $\pm$ 4.23 <sup>a</sup> | 2.13 $\pm$ 0.56 |

Values with different superscripts in each column are those that differ significantly ( $P < 0.05$ ).

## Discussion

The object of this study was the application of cytological evaluation of cervical smear in fresh cows. A significant difference was found between the mean of neutrophils of cervical mucosa smear at oestrus cycle phases in 10 Holstein dairy heifers. The means ( $\pm$  SD) of neutrophil percentages were  $0.10 \pm 0.10$ ,  $17.60 \pm 8.83$ ,  $0.50 \pm 0.30$  and  $3.60 \pm 1.44$  at oestrus, metestrus, diestrus and prestrus, respectively (AHMADI et al., 2000).

Endometritis in breeding cattle occurs during the postpartum period and is associated primarily with contamination of the reproductive tract involving *Arcanobacter pyogenes* together with Gram-negative anaerobes. Polymorphonuclear inflammatory cells (PMNs) contribute partly to the defence mechanisms against micro-organisms contaminating the vagina and uterine lumen, whose phagocytic activity depends on bacterial opsonisation by humoral antibodies; significant numbers of lymphocytes are also present (DHALIWAL et al., 2001). Abnormal parturition can be followed by persistent endometritis which can have deleterious effects on the cow's subsequent reproductive performance. Normal and active uterine defence mechanisms have been reported to be very important for the exclusion of bacterial infection from the uterus and recovery from endometritis developing post-parturition. The phagocytic activity of leucocytes in the uterine fluid seems to play a crucial role in halting the propagation and establishment of bacterial infection in the uterus immediately after calving and thereafter (HUSSAIN and DANIEL, 1992).

KLUCINSKI et al. (1990) showed that the inflammatory process in the uterus caused intensive migration of large numbers of PMNs into the lumen. A significant rise in phagocytic activity mediated by non-immunological receptors occurs simultaneously in this cell population. MATEUS et al. (2002) reported that there is a decrease in blood PMN oxidative burst activity until the first week postpartum which could be associated with increased susceptibility to early postpartum endometritis. The later increase in this parameter, as well as the increase in the intrauterine fluid phago-PMN and PI, might favour the spontaneous resolution of endometritis. The killing ability of bovine peripheral blood neutrophils did not vary during the oestrous cycle. Administration of sex steroids to ovariectomised cows reduced the intrinsic killing ability of neutrophils but enhanced the opsonising ability of serum. Exudate from experimental uterine infection with *Corynebacterium pyogenes* and *Fusobacterium necrophorum* impaired the neutrophil function, probably as a result of the action of bacterial leukotoxins (MORRIS, 1989). ZERBE et al. (2000) reported that altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows is associated with fatty liver.

The result of this study showed there was no significant difference between cell percentage at cervical mucosa when didn't evaluate progesterone level (Tables 1 and 3). It is therefore useful that cytological evaluation of cervical mucosa in the postpartum period

of dairy cows is compared with progesterone level. Therefore, if there is no progesterone measure we can use oestrus sign or oestrus date for the estimation of progesterone level.

There is a significant difference in neutrophil percentages between cows with progesterone above 1 ng/mL and below 1 ng/mL at 25 to 30 days post-parturition (Table 2). There was no significant difference between cows conceived with 1 artificial insemination (AI) and 2 or 3 AI. This result showed a significant difference in neutrophil percentages between cows conceived with 2 or 3 AI with high progesterone (P4) and low P4. The phase of oestrus cycle is therefore important for cytology sample collection. It is not useful cytological smear without any information about oestrus cycle period, especially in cows conceived with more than one AI. This result did not show any significant difference between cows conceived with one AI. There were no highly inflamed cells or endometritis in these cows. Therefore, cytological evaluation of cervical mucosa in cows that will conceive with one AI is useful. Because the low percentage of WBC at cervical mucosa smear is good character for evaluation of dairy cows.

Endometrial cytology and ultrasonography were used as diagnostic techniques for identification of subclinical endometritis. The endometrial cytology and ultrasonography findings were defined in terms of impact on actual reproductive performance. The results of this study indicate that endometrial cytology and ultrasonography will assist in the identification of those animals with subclinical endometritis that will benefit from early treatment (KASIMANICKAM et al., 2004).

SUBANDRIO et al. (2000) reported that the inherent differences in either peripheral or intrauterine neutrophil function did not support the hypothesis that the influence of the reproductive state of the cow to the resistance of the uterus to infection is mediated.

Progesterone induces an increase in the number of uterine neutrophils. Both in normal cyclic cows and after ovariectomy following progesterone, treatment may be a compensatory one due to reduced neutrophil phagocytosis and bactericidal activity, or to the suppression of other uterine defence mechanisms. Since this response is inconsistent with the long-recognized observation that the uterus of the cow is more susceptible to infection in diestrus than in oestrus (SUBANDRIO et al., 1997).

AHMADI et al. (2004) reported that there was no significant difference between PMNs (cells) percentages at cervical mucosa and uterine fluid smears. Cervical mucosa was taken without manipulation of cow uterus at commercial herd. There were no side effects on the uterus of cows. Therefore, this method is practical and applicable in all commercial herds.

In conclusion, it is suitable the cytological evaluation of cervical smear at fresh cow for diagnosis of subclinical endometritis, planning for treatment and prognosis of fertility after voluntary waiting period of dairy cows.

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**AHMADI, M. R., S. NAZIFI, H. R. GHAIASARI: Usporedna citologija cerviksa i stopa koncepcije u mliječnih krava nakon teljenja. Vet. arhiv 76, 323-332, 2006.**

**SAŽETAK**

U tijeku ranoga postpartalnoga razdoblja u maternicu krava dospiju mnogobrojne vrste bakterija. Primarni mehanizam njihova uklanjanja jest fagocitoza polimorfonuklearnih stanica, čime se postiže veći stupanj koncepcije. Zbog navedenoga može se očekivati povezanost između citoloških nalaza endometrija i koncepcije u krava u postpartalnom razdoblju. Za istraživanje je odabrano 50 zdravih krava holštajn-frizijske pasmine nakon teljenja. Krave su se normalno otelile, nisu imale mukopurulentni iscjedak iz stidnice, a u tijeku rektalnog pregleda u njih nije utvrđen patološki nalaz. Mukozni iscjedak iz grljaka prikupljen je od svih krava u razdoblju od 25 do 30 i 55 do 60 dana nakon teljenja. Razina progesterona u krvi utvrđivana je radioimunim testom. Diferencijalno brojenje stanica provedeno je na razmasku mukoze obojenom po Giemsi. Podatci su analizirani T testom za nezavisne uzorke, jednosmjernim ANOVA testom i Duncanovim testom. Nisu ustanovljene statistički značajne razlike između udjela stanica u promatranim vremenskim razmacima i broja postpartalnih umjetnih osjemenjivanja ( $P \geq 0,05$ ). Međutim, statistički značajne razlike ( $P < 0,05$ ) ustanovljene su za postotak neutrofila u različitim vremenskim razmacima nakon teljenja te za broj umjetnih osjemenjivanja (1 i 2 ili 3), nakon što su krave prema razini progesterona bile podijeljene u dvije skupine, iznad i ispod 1 ng/mL. Rezultati istraživanja pokazuju da citološki pregled razmaska iz cerviksa može pomoći pri postavljanju dijagnoze i pri liječenju supkliničkih endometritisa, te pri prognozi postpartalne plodnosti.

**Cljučne riječi:** postpartum, endometritis, neutrofil, citologija, mliječne krave

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