

ENDOCERVICAL CYTOLOGICAL STUDIES IN ENDOMETRITIS AFFECTED MURRAH GRADED BUFFLOES (*Bubalus bubalis*)

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ABSTRACT

Objective of the study was to develop endometrial cytology as a tool to diagnose endometritis in field situations. A total of 65 cyclic Murrah graded buffaloes consisting of 25 normal healthy in Group 1 and 40 infertile in Group 2 were utilized. For cytological studies at day 0 and 4 of the cycle endocervical secretions by aspiration technique and lavage technique, respectively, were collected. At day 4 of the cycle endometrial biopsies were obtained for histopathological studies.

In the present study, the aspiration at day 0 was successful in 100% attempts but cells were found 92.3% smears. The lavage at day 4 too was successful in 100% attempts and yielded cells in 96.93% smears. At two different sampling intervals both neutrophil count and lymphocyte count differed significantly ($P \leq 0.05$) between Groups 1 and 2. In Group 2, the neutrophil count differed significantly between sampling intervals ($P \leq 0.05$), but not in Group 1. Lymphocyte count did not differ between sampling intervals in both groups, but differed significantly between groups.

In the present study, based on histopathological lesions in Group 2 33, 28 and 39% biopsies were classified into acute, sub-acute and chronic endometritis, respectively. The mean neutrophil and lymphocyte counts did not differ between subgroups. Changes in the endometrium

could be related to cytological findings at day 0 and 4 of the cycle. It was concluded that the cytological studies in genital secretions might conveniently be implemented in field situations.

Keywords: Murrah buffalo, endometritis, endometrial cytology, infertility

INTRODUCTION

Subfertility is a worldwide concern as herd fertility rates have been declining at approximately 1% annually (Royal *et al.*, 2000). In spite of advances endometritis a form of subfertility still remained an economically important cattle fertility problem. One of the problems in tackling endometritis is the lack of a well defined method of diagnosing. Commonly the condition is diagnosed by clinical symptoms, vaginoscopy, rectal palpation, bacteriology and endometrial histopathology. Though the rectal examination is the most preferred method in field conditions, very often it is arbitrary, insensitive, erroneous and inaccurate. The later procedures are laborious and time consuming and required infrastructure and expertise. In the recent past endometrial cytology, which is based on the migration of leucocytes to the site of infections, has been tried elsewhere to rapidly diagnose endometritis. For harvesting

leucocytes from uterine secretions different methods *viz.* direct swab, cytobrush, aspiration and lavage have been described (Kasimanickam *et al.*, 2005; Barlund *et al.*, 2008). The objective of the present work was to develop endometrial cytology as a tool for diagnosing endometritis in field conditions.

MATERIALS AND METHODS

A total 65 cyclic Murrah graded parous buffaloes consisting of 25 at their first post partum estrus with normal discharge and reproductive organs on rectal examination constituted Group 1 (control) and 40 buffaloes that had the history of infertility ranging from abnormal discharge to repeat breeding were assigned to Group 2. The buffaloes had no history of periparturient complications. The lactation length ranged from 4 months to >1 year. For cytological studies, aspiration technique at day 0 and uterine lavage at day 4 of the cycle were adopted (Kasimanickam *et al.*, 2005; Azawi *et al.*, 2008). The estrus stage of the cycle was diagnosed by symptoms and clinical examination. Estrual discharge was aspirated through a sterile A.I. sheath connected to a 20 ml disposable syringe and was smeared on a clean glass slide. Further, on day 4 of the cycle, 20 ml normal saline solution was infused into uterine lumen through a sterile A.I sheath connected to a 20 ml syringe and allowed to remain there for a few seconds before it was withdrawn by aspiration. The fluid was centrifuged at 1000 rpm for 15 minutes. After discarding the supernatant, cytological smears were prepared from the sediment. Smears were stained with hematoxyllin and eosin stain and examined under oil an immersion lens of a microscope (Singh and Sulochana, 1996).

At day 4 of the cycle, endometrial biopsies

were collected from caudal one-third portion of uterine horns by Albuchin's uterine biopsy catheter. The biopsy samples were placed in 10% neutral buffered formalin, processed by routine procedures and finally stained with haematoxyllin and eosin stain (Singh and Sulochana, 1996). Statistical analysis of the data was done by adopting computer software programmed for Windows XP (Version 9.0, SPSS Inc. Munich) and Excel (Version 2003, Microsoft).

RESULTS AND DISCUSSION

Cytological Studies

In the present study, the aspiration at day 0 was successful in 100% attempts but cells were found in 92.3% smears. The lavage at day 4 too was successful in 100% attempts and yielded cells in 96.93% smears. In the lavage technique, Kasimanickam *et al.* (2005) failed to obtain samples in 17% attempts, when animals were sampled during the early post partum period. Whereas Gilbert *et al.* (2005) and Barlund *et al.* (2008) did not report failure of sampling with the lavage technique in early postpartum cows.

Neutrophils. The neutrophil count in cytological smears obtained at day 0 of the cycle was 4.60 ± 0.64 and $45.69 \pm 3.88\%$ and at day 4 of the cycle was 5.6 ± 0.78 and $57.82 \pm 3.50\%$ in Groups 1 and 2, respectively (Table 1). The neutrophil count significantly differed ($P \leq 0.05$) between groups at two different sampling intervals. In Group 1, the neutrophil count did not differ between sampling intervals, while it differed ($P \leq 0.05$) in Group 2. The significant differences between groups at different sampling intervals were in line with Azawi *et al.* (2008). In cyclic Iraqi buffalo, Azawi *et al.* (2008) reported the neutrophil count to be $41.1 \pm 11.91\%$ at

estrus in repeaters, which was significantly higher than $14.0 \pm 2.02\%$ in the normal control group. In the present study, increased neutrophil (Figure 1) count observed in Group 2 might indicate the presence of infection (Azawi *et al.*, 2008).

In Group 2, the elevated neutrophils count observed between sampling intervals could be attributed to the effect of rising progesterone. During the progesterone dominant phase of the cycle, down regulation of the immune function makes the diestrus uterus susceptible to infections (Lewis, 2003; Azawi *et al.*, 2008). Elsewhere, it was stated that the immune suppressive effect of progesterone might have been compensated for by increased neutrophil influx into the uterine lumen (Subandrio *et al.*, 2000). These authors reported neutrophil influx into the luteal phase uterine lumen when cows were challenged with intrauterine immunomodulators. They also recorded a similar phenomenon when ovariectomised cows were challenged with progesterone treatment.

Lymphocytes. The mean lymphocyte count was 1.64 ± 0.47 and $6.46 \pm 1.32\%$ at day 0 and 1.72 ± 0.51 and $6.68 \pm 1.10\%$ at day 4 of the cycle in Groups 1 and 2, respectively (Table 1). At the two sampling intervals, the counts differed ($P \leq 0.05$) significantly between groups. Further, the lymphocyte counts did not differ between sampling intervals. However, in both groups it followed largely a similar pattern between sampling intervals as observed by the positive correlations in Groups 1 ($r=0.428$) and 2 ($r=0.523$).

The perusal of literature on cytological investigations in the genital tract did not yield related publications on lymphocyte populations. Of course, Ahmadi *et al.* (2006) and Yavari *et al.* (2009) reported prevalence of lymphocytes in the early postpartum uterus. Ahmadi *et al.* (2006) reported $1.7 \pm 0.01\%$ lymphocyte count in early

post partum cows. Similarly, Yavari *et al.* (2009) demonstrated lymphocytes $<1\%$ in cervical and up to 4.5% in uterine smears of different degrees of endometritis affected cows.

The uterus is supplied with ample lymphocytic drainage and contains the full range of lymphohaemopoietic cells and molecular regulators required to generate and elicit humoral and cell mediated immunity. Of course, the uterus is exceptional among mucosal tissues, in that ovarian steroid hormones have considerable effect on humoral and cell mediated immunity (Sheldon *et al.*, 2009). The presence of lymphocytes in uterine fluids definitely throws light on the prevalence of uterine infections.

HISTOPATHOLOGY IN RELATION TO CYTOLOGICAL STUDIES

In Group 1, the common endometrial changes characterized by mild to moderate cellular infiltration and slight increase in connective tissue were found. In addition, in a few biopsies. the presence of inspissated material in the glandular lumen and mild periglandular fibrosis without affecting the glandular architecture were noticed and were in line with Moulukrishna *et al.* (2010).

Based on histopathological lesions, in Group 2 biopsies were classified in to acute, sub acute and chronic endometritis (Sheldon *et al.*, 2006; Azawi *et al.*, 2008; Chapwanya *et al.*, 2009). In acute endometritis the lesions were characterized by severe congestion, stromal edema, hemorrhages, denudation of luminal epithelium, infiltration of inflammatory cells predominantly neutrophils and few lymphocytes in the stratum compactum and stratum spongiosum.

In subacute endometritis, commonly the

Table 1. Cytological observations in endocervical smears at Day 0 and 4 of the cycle (Mean±SE).

| Group | Neutrophils (%) | | | Lymphocytes (%) | | |
|-----------|----------------------|---------------------|--------------------|---------------------|---------------------|--------------------|
| | Day 0 | Day 4 | 't' Value | Day 0 | Day 4 | 't' Value |
| 1 | 4.6 ± 0.64 (25) | 5.6 ± 0.78 (25) | 0.99 ^{NS} | 1.64 ± 0.47 (25) | 1.72 ± 0.51 (25) | 0.11 ^{NS} |
| 2 | 45.69 ± 3.88 (35) | 57.82 ± 3.5 (38) | 2.32 * | 6.46 ± 1.32 (35) | 6.68 ± 1.1 (38) | 1.13 ^{NS} |
| 't' value | 10.45* | 14.54* | | 3.43 * | 4.08 * | |

Figures in parenthesis indicate the number of observations.

*P≤0.05 NS: Non significant

Table 2. Cytological findings in endometrial impression smears collected from different endometritis sub groups at Day 0 and 4 of the cycle (Mean±SE).

| Group | Day 0 (%) | | Day 4 (%) | |
|----------------|----------------------------|---------------------------|----------------------------|---------------------------|
| | Neutrophils | Lymphocytes | Neutrophils | Lymphocytes |
| Acute (15) | 30.53 ± 5.04 ^{NS} | 4.13 ± 1.45 ^{NS} | 50.4 ± 5.88 ^{NS} | 5.6 ± 1.92 ^{NS} |
| Sub acute (13) | 42.85 ± 7.31 ^{NS} | 6.93 ± 1.57 ^{NS} | 54.69 ± 6.48 ^{NS} | 9.07 ± 1.80 ^{NS} |
| Chronic (18) | 33.72 ± 7.90 ^{NS} | 4.55 ± 2.21 ^{NS} | 42.16 ± 8.42 ^{NS} | 3.56 ± 1.17 ^{NS} |

NS: Non significant

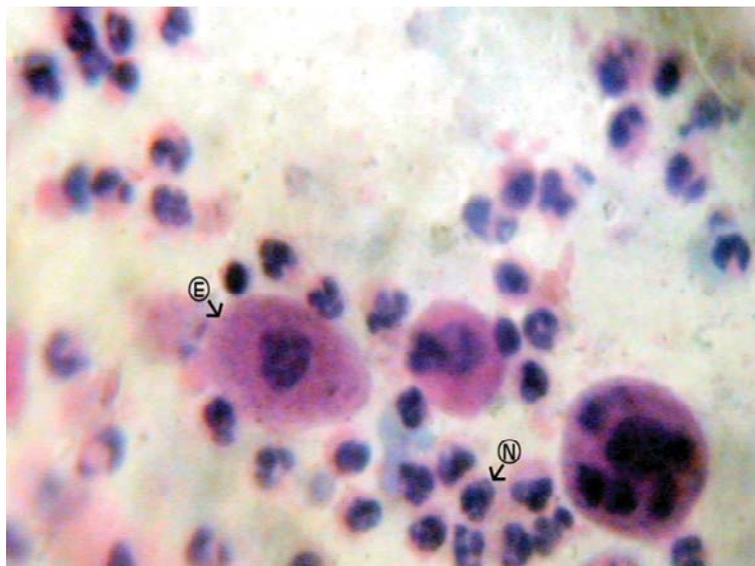


Figure 1. Neutrophils (N) and epithelial cells (E) in endocervical secretions stained with Hematoxylin and eosin (x40).

lesions were characterized by extensive denudation of the luminal epithelium, stromal edema and mild infiltrations of neutrophils, lymphocytes and few macrophages in the stratum compactum and stratum spongiosum, vascular hyalinisation, and peri vascular edema were found. Proliferation of fibroblasts, histiocytes and lymphocytes in the sub epithelial zone and nesting of glands in a few sections, mild periglandular fibrosis and focal lymphoid aggregates were observed. Periglandular fibrosis characterized by 2-3 concentric layers of spindle shaped fibroblasts around uterine glands was recorded.

In chronic endometritis, the lesions were characterized by extensive desquamation of luminal epithelium, severe infiltration by lymphocytes, plasma cells and macrophages, connective tissue proliferation and vascular hyalinization were observed. Fifty percent of the biopsies revealed irreversible degenerative changes characterized by glandular atrophy, pyknotic nuclei in the glandular epithelium, and perivascular and periglandular fibrosis and cystic glands with inspissated material in the glandular lumen were recorded.

In Group 2 33, 28 and 39% of the biopsies were found to have acute, sub acute and chronic inflammatory changes, respectively. Further, the mean neutrophil count was 30.53 ± 5.04 , 42.85 ± 7.31 and $33.72 \pm 7.9\%$ at day 0 and 50.4 ± 5.88 , 54.69 ± 6.48 and $42.16 \pm 8.42\%$ at day 4, in acute, sub acute and chronic endometritis, respectively (Table 2). Neutrophil counts did not differ between sub groups of endometritis. This implied that neutrophil influx into luminal contents was by and large found to be similar in sub groups of endometritis.

In case of lymphocytes too, the mean counts at day 0 were 4.13 ± 1.45 , 6.93 ± 1.57 and $4.55 \pm 2.21\%$ and at day 4 the counts were $5.6 \pm$

1.92 , 9.07 ± 1.8 and $3.56 \pm 1.17\%$ in acute, sub acute and chronic endometritis, respectively, and these did not differ between groups. Of course, the pattern of influx of neutrophils and lymphocytes was in agreement with the established principles of decline as the stage of inflammatory process advances (Cole *et al.*, 1997).

Based on the findings in the present study, it was concluded that cytological investigations in genital secretions might conveniently be implemented even in field situations for drawing appropriate conclusions on the status of uterine health. Further, it was confirmed that the samples obtained from the cervix might provide mirror image of the uterine environment.

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