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Cytological changes during certain reproductive conditions in *in-vitro* uterine flushing in buffaloes (*Bubalus bubalis*)

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ABSTRACT

Cytological changes during follicular, luteal phase, inactive ovarian stage and infected uterine flushings *in-vitro* were studied in buffalo uteri (n-54) collected from local abattoir. The overall total nucleated and epithelial cell count was significantly ($P<0.01$) higher in infected uteri compared to follicular phase and inactive ovarian stage, however, differance was non-significant during luteal phase but these cells varied significantly ($P<0.01$) between late-luteal phase and infected uteri. Absolute lymphocyte counts, and lymphocyte percentages varied non-significantly between two uterine horns as well as during different stages of uterine flushings, however, absolute neutrophil count and neutrophil percentage showed significant ($P<0.01$) increase in the infected uteri as compared to other stages. Cytological studies could be used as an adjunct to diagnose sub-clinical uterine infections. However, *in-vivo* study on large number of bovines, particularly in buffaloes is warranted.

Key Words - Buffalo, uterus, cell cytology

The study of various cell types lining the endometrium and the exfoliated cells within the uterine lumen has long been practiced in human medicine as an adjunct to diagnose fertility (Cary, 1943). The types of cells and their number usually vary depending on physio-pathological state of reproduction in cow (Hussain *et al.*, 1992). The percentage of polymorphonuclear cells and the exfoliated epithelial cells have been reported to increase during oestrus and dioestrusin uterine flushings in mares (Knudsen, 1964). In the present paper, an attempt has been made to evaluate the uterine cytological changes during various reproductive phases in buffaloes *in-vitro*.

MATERIALS AND METHODS

Fifty-four genital tract were collected within 30-60 minutes of slaughter of buffaloes, irrespective of their age, breed, parity and body weight at Bareilly abattoir. The genital organs were ligated at cervix and utero-tubal junction, kept in a separate polythene bags and were transported to the laboratory on ice in a thermosflask.

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In the laboratory, the genitalia were examined grossly for any apparent abnormalities and were categorised into follicular phase (Luktuke and Rao, 1962; Dobson and Kamonpatana, 1986), luteal phases (Ireland *et al., 1980*), inactive ovarian stage and infected uteri. Each horn was then flushed with 10 ml of sterile normal saline using Folleys catheters (INMED Corporation, USA) as per the method of Boos *et al.* (1988) within 4-5 hours of collection after cleaning the genitalia with normal saline and then with 70% ethyl alcohol. Equal volumes of fluids recovered from each horn were centrifuged at 1500 rpm for 15 min. The cell pellet was reconstituted to a final volume of 500 µl with chilled tissue culture media (RPMI, sigma) depending upon the cell concentration. The smears of reconstituted pellet were prepared on greese free glass slides, fixed and stained with Giemsa stain (2%, Glaxo Laboratories, Bombay). A total of 500 nucleated cells which included leukocytes (lymphocyte, neutrophil, monocyte and eosinophil), epithelial and epithelial cell rafts (group of 4 or more epithelial cells in close apposition) were counted in all the four primary square of haemocytometer after diluting the cell suspension with CSF diluting fluid in a white cell

diluting pipette. The cell count obtained was finally multiplied by 50,000 to get the cell concentration/ml of reconstituent. Dunkan's multiple range test (dmrt) was applied to compare the mean±se as per the method of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Mean±SE of different cytological parameters (overall) in uterine flushings of buffaloes have been presented in Table-1. The infected uteri had shown significant (P<0.01) rise in overall total nucleated and epithelial cell counts as compared to follicular phase and inactive ovarian stage, however, during luteal phase difference was non-significant but these cells varied significantly (P<0.01) between late-luteal phase (10.29±1.40 and 8.97±1.20 millions/ml, respectively) and infected uteri (19.50±1.57 and 16.84±1.47 millions/ml, respectively). On the other hand, difference between two uterine horn flushings was non-significant. Increased cell counts in infected uteri could possibly be due to the adverse effect of microbial toxins. In an study, Ambrose and Pattabiraman (1989) in bovines and Couto and Hughes (1985) in mare have also reported similar findings.

Absolute lymphocyte counts and lymphocyte percentages varied non-significantly between two uterine horns as well as during different stages of uterine flushings. In sheep, Gottshall and Hansen (1992) reported the role of progesterone in migration and proliferation of lymphocytes, thus modifying uterine immune function. On the other hand, absolute neutrophil count and neutrophil percentage showed significant (P<0.01) increase in the infected uteri as compared to other stage uterine flushings. Increased neutrophils in this study might be due to the chemotatic effect of the infection to facilitate phagocytosis. In mare also, Staab and Ely (1985) reported a positive correlation between infection and polymorphonuclear leukocytes. Furthermore, uterine leukocytes were reported to vary as per the stage of oestrous cycle in cows (Hussain *et. al.*, 1992). In buffaloes, Ahmed *et al.* (1993) reported higher leukocytes during endometritis and significantly (P<0.05) higher leukocytes in uterine flushing during follicular phase as compared to the luteal phase. However, in our finding these leukocytes were non-significantly higher in luteal phase.

Though the uterine leukocyte numbers vary during various phases of reproduction it could be used as an adjunct to diagnose sub-clinical uterine infections.

Table 1. Mean (±Se) values of different cytological parameters in uterine flushings of follicular phase, luteal phases inactive ovarian stages and infected uteri of buffaloes

Parameters	Follicular Phase	Luteal Phase	Inactive Ovarian Stage	Infected Uteri
Total nucleated cell count (millions/ml)	10.89±1.79^{b} (n=7)	16.98±1.97^{a} (n=24)	6.59±1.17^{b} (n=6)	19.50±1.57^{a} (n=6)
Absolute epithelial cell count (millions/ml)	10.75±1.88^{b} (n=7)	14.58±1.64^{a} (n=24)	5.76±1.05^{b} (n=6)	16.84±1.47^{a} (n=6)
Absolute lymphocyte count (millions/ml)	1.82±0.56^{a} (n=7)	2.11±0.45^{a} (n=24)	0.77±0.17^{a} (n=6)	2.22±0.23^{a} (n=6)
Absolute neutrophil count (millions/ml)	0.02±0.01^{b} (n=7)	0.04±0.02^{b} (n=24)	0.02±0.01^{b} (n=6)	0.36±0.12^{a} (n=6)
Epithelial cell per cent	85.56±3.11^{a} (n=10)	88.06±1.21^{a} (n=29)	87.65±1.67^{a} (n=7)	83.58±1.99^{a} (n=8)
Lymphocyte per cent	14.09±3.01^{a} (n=10)	11.46±1.22^{a} (n=29)	11.89±1.54^{a} (n=7)	14.43±1.94^{a} (n=8)
Neutrophil per cent	0.19±0.09^{b} (n=10)	0.25±0.05^{b} (n=29)	0.25±0.15^{b} (n=7)	1.99±0.43^{a} (n=8)

In rows, figures with different superscripts differ significantly (P<0.01).
N = Number of observation.

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