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ULTRA STRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF ENDOMETRIAL BIOPSIES IN REPEAT BREEDER BUFFALOES

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ABSTRACT

Endometritis inflammation and unfavourable uterine environment is the most important etiological factor for infertility in bovines. Endometrial biopsy examination is the most reliable diagnostic tool for veterinarians to identify the nature of infertility for the present study; twelve endometrial biopsy samples were collected from infertile buffaloes for Ultra structural and immunohistochemical studies. Scanning electron microscopic examination of uterine biopsies collected from normal endometrium revealed surface epithelial cells with few ciliated and non ciliated cells. Loss of cilia and microvilli of surface epithelium was observed in acute endometritis cases. Sub acute endometritis cases revealed rod shaped bacteria adhering to surface epithelial cells and damaged epithelial cells with loss of microvilli. Destruction of surface epithelium and glandular structure leaving hole like spaces and fibrosis with thin long reticulin fibers were noticed in chronic endometritis cases.

Immunohistochemical studies of chronic endometritis biopsies revealed more number of CD3 positive cells (pan T lymphocytes) in stratum compactum. Six chronic endometritis biopsies revealed CD138 positive cells (plasma cells) in endometrial stroma.

Key Words: *Endometritis, Repeat Breeders, Immunohistochemistry*

INTRODUCTION

Poor reproductive efficiency is one of the major problems faced by buffalo breeders (Jainudeen 1986 and Singh *et al.*, 2000). Endometrial biopsy is the most reliable diagnostic tool and an important key to identify the nature of infertility. Electron microscopy has been an important tool to study morphologic features of uterine epithelium in cows (Hafez and Kanagawa, 1973 and Fathalla *et al.*, 1975).

Immunohistochemistry is an integral technique in many veterinary laboratories for diagnostic and research purposes (Ramos-Vara 2005). Endometritis is mediated by presence of T and B lymphocytes and plasma cells. Although bovine endometrium supports large number of immune cells (Vanderhoek, 1959) the diagnosis of chronic endometritis depends upon detection of plasma cells within inflammatory infiltrate in endometrium (Crum *et al.*, 1983 and Garner *et al.*, 2004).

Immunohistochemistry technique is more sensitive for detecting isolated plasma cells which are missed by standard screening techniques.

MATERIALS AND METHODS

To know the sub-cellular changes in endometrial biopsies, that were obtained from endometritis cases, the specimens were washed in phosphate buffer and the tissues were cut into 2 pieces and fixed for eight hours after collection in 3% glutaraldehyde. To remove glutaraldehyde, the uterine specimens were rinsed twice in phosphate buffer for 10 minutes each. The tissues were then fixed in 2% aqueous osmium tetroxide for four hrs and rinsed in 3 changes of distilled water for 10 minutes each rinse. Dehydration was accomplished by immersion in a graded series of ethanol solutions. Specimens were prosecuted for critical point drier for eight hours. The specimen was mounted to aluminum stub and coated with palladium gold alloy (350A°) in a sputter coater. The surface examined and photographed using SEM (6KV) Ferriera (Dias *et al.*, 1994).

Research Article

To know the presence of inflammatory cells in endometritis cases, histopathological sections obtained from biopsies were deparaffinized and subjected to antigen retrieval by enzymes. To block endogenous peroxidase, the sections were treated with hydrogen peroxide and were rinsed with deionised water. The sections were incubated with primary antiserum and then incubated with biotinylated secondary reagent (goat anti-rabbit immunoglobulins). The sections were rinsed with buffer and incubated with Avidin biotin complex. Sections were rinsed with buffer. To detect the immune reaction, the sections were treated with Diaminobenzene. Then the sections were counterstained with Mayer's hematoxylin (Ramos vara, 2005).

RESULTS AND DISCUSSION

Scanning electron microscopy of endometrial biopsies of fertile animals revealed few ciliated and many non ciliated cells of uterine surface epithelium. Microvilli and secretory blebs were noticed in surface epithelium (Fig. 1) and the observations were in accordance to Ferreira Dias *et al.*, (1994) and Al-Bagdadi *et al.*, (2004).

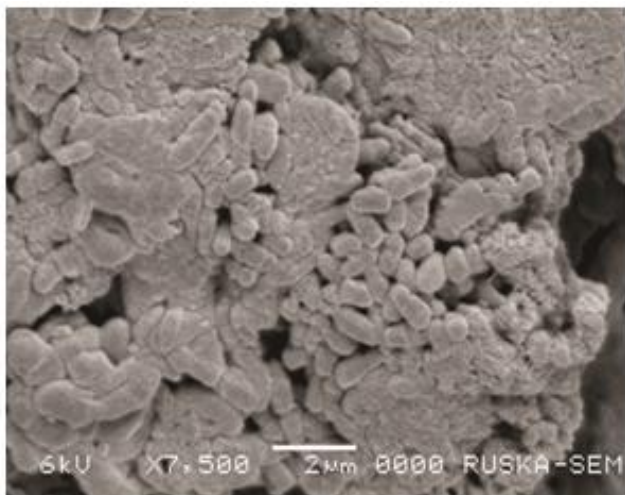


Figure 1: Scanning electron micrograph showing ciliated cells and non ciliated cells in luminal epithelial x 7500

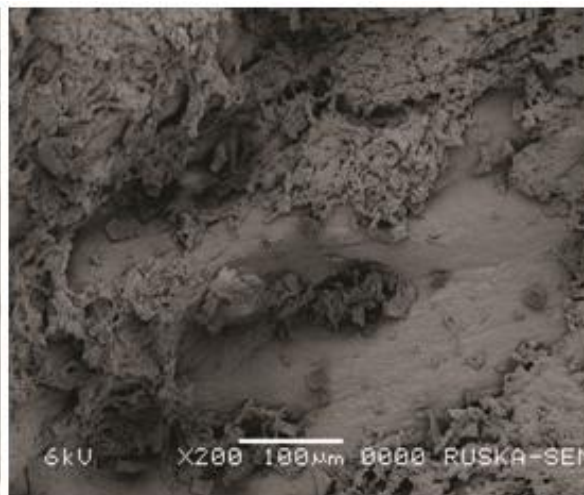


Figure 2: Scanning electron micrograph showing severe denudation of surface epithelium x 200

Scanning electron microscopic studies of endometrial biopsies of acute endometritis revealed loss of luminal epithelial cells and few swollen epithelial cells (Fig. 2). Few biopsies revealed rod shaped bacteria adhering to surface epithelium (Fig. 3). Similar observations were noticed by Yoshikazu (1985). Endometrial biopsies of sub acute endometritis revealed inflammatory changes with few smaller and damaged epithelial cells, loss of cilia and microvilli. Similar changes were made by Raju and Madhuri (2009).

Chronic endometritis biopsies revealed extensive damage of surface epithelium. Many cells lacked cilia and microvilli with destruction of glandular structures forming ulcer like holes (Fig. 4). Similar observations were made by Ferreira – Dias *et al.*, (1994) and Raju and Madhuri (2009). Thin long reticulum fibres with increased intercellular spaces were noticed. Similar observations were made by Yoshikazu (1985).

Demonstration of immune cells in endometrial biopsies by immunohistochemistry revealed the distribution and type of immune cell in endometritis cases. Immuno histochemical studies of six endometritis cases revealed significant increase in CD₃ (Pan T lymphocytes) Positive cells (Fig. 5) and Ham 56 (Macrophages). CD₃ positive cells were distributed in sub epithelial zone of stratum compactum

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and periglandular aggregates. In normal endometrium, CD₃ positive cells are present predominantly in endometrial stroma.

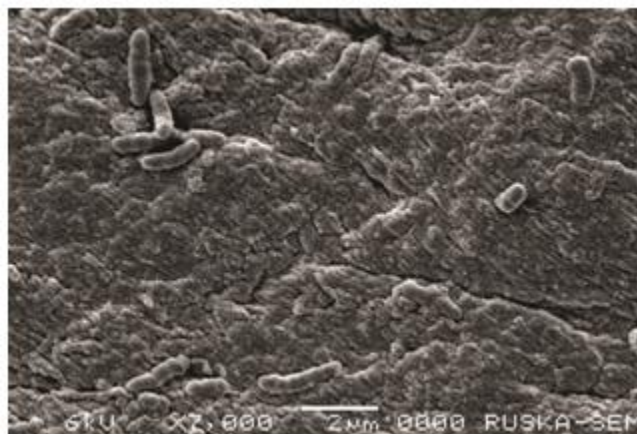


Figure 3: Scanning electron micrograph showing few bacilli adhering to luminal epithelium x 7000

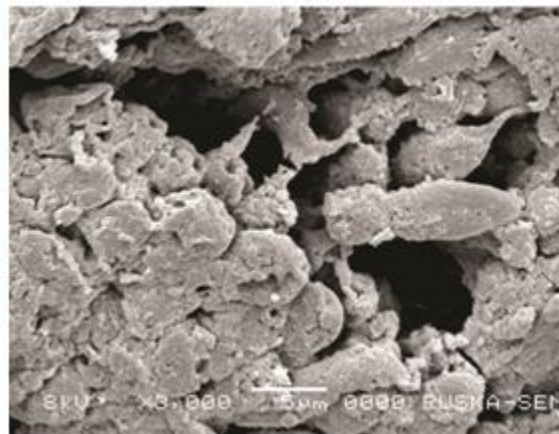


Figure 4: Scanning electron micrograph showing severe denudation of glandular epithelium showing ulcer like spaces x 7000

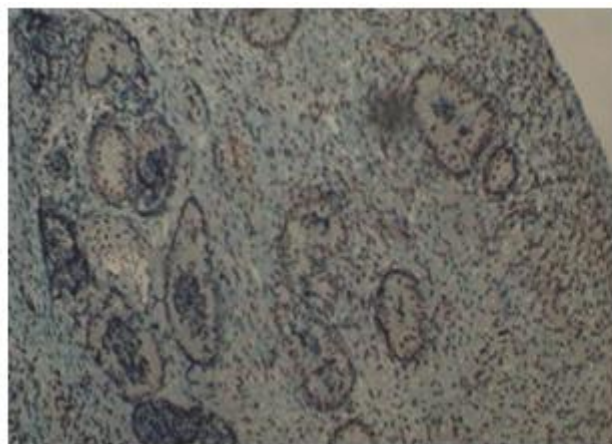


Figure 5: Immunostaining for CD₃ and positive cells in subepithelial zone of endometrium. x 70

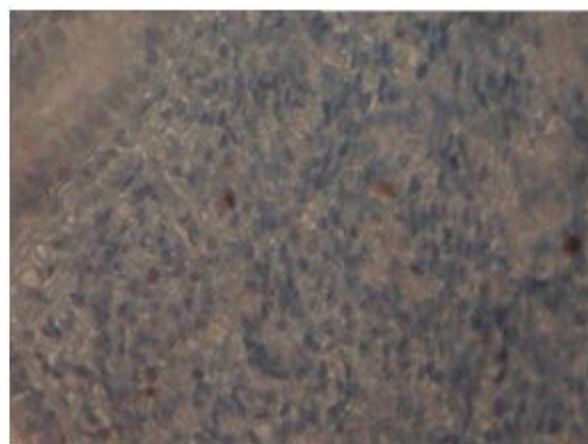


Figure 6: Immunostaining for CD₁₃₈ (plasma cells) in endometrial stroma immunohistochemistry x 280

Tawfik *et al.*, (1996) observed increase in CD₂₀ positive cells and CD₃ positive cells up to 50 folds and 3 fold respectively in endometritis cases. The observations are in accordance with Tawfik *et al.*, (1996). No difference in number of T lymphocytes in normal and endometritis cases was reported by Disep *et al.*, (2004). Six biopsies from chronic endometritis revealed plasma cells (CD₁₃₈ positive cells) immunohistochemically (Fig. 6). Similar finding were made by Tawfik *et al.*, (1996) and Illene *et al.*, (2001).

IL₂ secreted by T-cells after stimulation with antigen is followed by release of cytokines by T₂ cells and that induces humoral immunity by inducing proliferation of local and regional lymphnodes and cause increase in B cells and T cells. B cells proliferate and differentiate to produce plasma cell and then antibodies (Bondurant, 1991; Tawfik *et al.*, 1996 and Azawi, 2008). The increased number of immune cells in chronic endometritis has ability to produce variety of cytokines and growth factors that have harmful effect on pregnancy leading to abortion and infertility. Immunohistochemical detection of other

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immune cells can provide important information not otherwise obtained by conventional histological techniques according to Tawfik *et al.*, (1996).

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