



ORIGINAL RESEARCH PAPER

Gynaecology

EFFECT OF UTERO-TONICS (PROSTAGLANDIN) DRUG ON POSTPARTUM COW UTERUS- HISTOLOGY AND IMMUNOHISTOCHEMISTRY STUDY

KEY WORDS: Endometrial tissue, Histology, Immunohistochemistry and Postpartum.

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ABSTRACT

Normally calved 18 healthy Holstein Friesian crossbred cows aged between 2nd and 5th lactations were selected immediately after parturition. Day of parturition was considered as day 0 of the experiment. All the selected cows were randomly and equally divided into 2 experimental groups viz., group I (prostaglandin F2α) and IV (control). Endometrial tissue was collected on day 10 and 30 postpartum in all the two groups of animal through Albuchin's biopsy catheter. In group I on day 10 postpartum, histologically the endometrium showed involution process, with mild neutrophilic and mononuclear infiltration. When compared to day 10 postpartum, the regenerative changes of epithelium and endometrial glandular activities were predominant on day 30 postpartum in experimental groups especially in group I cows. Immunohistochemistry of endometrium showed mild expression for CD 20+ cells in groups I and moderate expression of CD20 +ve cells in control cows on day 10 and 30 postpartum. None of the cows in experimental and control groups expressed positive for CD3+ cells.

INTRODUCTION:

Use of prostaglandin is common during the early postpartum period to improve uterine involution and fertility in dairy cattle (Archbald et al., 1994). PGF₂ is widely used to manage postpartum reproductive efficiency through reduction of postpartum interval "backing up" (Elsheikh and Ahmed, 2004). The administration of exogenous PGF₂ during postpartum improved the conception rate (Michel et al., 1999) and reduced the calving to conception interval (Schofield et al., 1999). But both beneficial and a failing uterotonic effect have been reported with PGF₂ treatment in cows (Bajcsy et al., 2006).

The examination of the endometrial biopsy, inflammatory, periglandular fibrosis and cystic glandular degeneration were studied to provide prognosis for cow fertility (Singh et al., 1983). Uterine biopsy was a valuable diagnostic tool in identifying morphological alterations in the uterine endometrium (Prasad and Krishna, 2009).

Krishna et al. (2010) suggested that characteristic changes of endometrial infection were glandular hyperplasia with periglandular fibrosis of the endometrial layer and necrotic changes with cystic dilatation of endometrial glands. These changes indicated poor fertility.

Immunohistochemistry is used to confirm the identity of the immune cell types present in the endometrium. Positive immunostaining was detected for tyrosine protein phosphatase non-receptor type substrate 1 (CD172A, representing macrophages and granulocytes), CD14 cell surface antigen (a macrophage marker), CD2 molecule (T-cell and natural killer (NK) cell marker) and T-cell surface glycoprotein CD8 (Uthai et al., 2013).

Lower percentage of T lymphocytes and decreased CD2, CD4 and CD8 subsets of T lymphocytes were observed in the peripheral blood of healthy cows (Van Kampen and Mallard, 1997). CD8+ T lymphocytes are mainly of a cytotoxic nature during mid to late lactation, but they are mainly suppressive in postpartum animals. A decrease in CD2+, CD4+ and CD8+ T lymphocytes on 7 days after parturition, but subpopulations were observed (Shafer-Weaver and Sordillo, 1997).

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 (Samatha et al., 2013).

Endometritis is mediated by presence of T and B lymphocytes and plasma cells. Although bovine endometrium supported large number of immune cells, 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).

Tawfik et al. (1996) observed increase in CD20 positive cells and CD3 positive cells up to 50 and 3 folds respectively in endometritis cases. No difference in number of T lymphocytes in normal and endometritis cases was reported by Disep et al. (2004).

MATERIALS AND METHODS:

Normally calved 18 healthy Holstein Friesian crossbred cows aged between 2nd and 5th lactations were selected immediately after parturition. Day of parturition was considered as day 0 of the experiment. All the selected cows were randomly and equally divided into 2 experimental groups viz., group I and IV (control). Therefore each group consisted of 9 cows. On day 2 postpartum, cows of group I and IV were treated with an intramuscular injection of 25 mg PGF₂ (Dinoprost Tromethamine, 5ml, Lutalyse, Pfizer Animal Health India Ltd, India) and 5 ml normal saline (Parental drugs (India) limited, Indore, Madhya Pradesh, India), respectively.

ENDOMETRIAL BIOPSY:

The ideal endometrial tissue for interpretation was found to be at least 10-20 mm x 3 mm in both epithelial cell layer and the glandular architecture (Raja et al., 2012). Albuchin's uterine biopsy catheter was used to obtain endometrial biopsy samples as per the technique followed by Palanisamy (2012) with slight modifications. The closed, sterilized biopsy catheter was introduced into the uterus adopting aseptic technique. The biopsy catheter was advanced into one of the uterine horns of uterus and the catheter was opened. The uterine wall was pressed with the thumb against the opening of the catheter. The catheter was closed, rotated and retracted slowly. Slight pressure was applied against catheter to prevent haemorrhage before retracting the instrument. A piece of endometrium was released from the cutting edge of the catheter into a vial containing Bouin's fluid and stored for 24 hours and processed by routine paraffin technique and stained with haematoxylin and eosin as per technique described by Bancroft and Gamble (2008). Endometrial biopsy was taken from the all experimental and control cows on (i) day 10 and (ii) day 30 postpartum.

IMMUNOHISTOCHEMISTRY OF ENDOMETRIUM:

The endometrial tissue collected on day 10 and 30 postpartum in

all the four groups through Albuchins's biopsy catheter was processed by paraffin technique and sections were cut and mounted on coated slides for immunohistochemistry. These slides were tested for immunological expression of CD3+ and CD20+ cells to identify T and B lymphocytes, respectively in the endometrium by employing polymeric method using T and B lymphocytes antibodies as described by Samatha et al. (2013).

RESULTS AND DISCUSSION:

Histology of endometrium:

Histology of endometrium in the control cows on day 10 postpartum showed mild mononuclear infiltration and congestion in the endometrium along with few neutrophils. But, on day 30 postpartum, 2 cows in control group showed endometrial glandular proliferation with few neutrophils. In experimental cows (group I), on day 10 postpartum, the endometrium showed involution process, with mild neutrophilic and mononuclear infiltration (Plat 1). The mucin secretion was also noticed. The increased endometrial glandular activity was seen. Focal mild mononuclear infiltration and mild fibrosis were seen in few cases. Squamous metaplasia of uterine epithelium was noticed in one cow. These regenerative changes of endometrium on day 10 postpartum were prominent in group I (PGF₂ group) than control group. These proliferative changes were minimal in control group.

On day 30 postpartum, many uterine ecobolics treated cows had increased glandular activity with extensive lymphocytic infiltration and increased vascular spaces. The glandular secretion was predominant in few cases. Moderate neutrophilic and mononuclear infiltration was noticed. Few cows showed the presence of fibrous tissue. When compared to day 10 postpartum, the regenerative changes of epithelium and endometrial glandular activities were predominant on day 30 postpartum in experimental groups especially in group I cows (Plate 1). These regenerative changes were very minimal in group IV (control) on day 30 postpartum.

postpartum normally calved cows. These endometrial involution and regenerative changes clearly reflected on the conception rate in each treatment and control groups.

IMMUNOHISTOCHEMISTRY OF ENDOMETRIUM:

In uterotonics treated groups such as groups I, II and III, there was a mild expression for CD 20 positive cells (B Lymphocytes). Whereas none of the cow in experimental and control groups expressed positive for CD3 positive cells (T Lymphocytes). However, there was a moderate expression of CD20 +ve cells on day 10 and 30 postpartum in control cows (Plate 2). The results indicated bacterial infection might have been cleared in all the uterotonic drugs treated cows. Further no expression of CD3 +ve cells in control and experimental cows confirmed the absence of cytotoxic immunity due to viral infection in these cows.

In this experiment CD3+ and CD20+ markers were used to assess T lymphocytes and B lymphocytes, respectively in the uterine endometrium. In control and treatment groups, there was no expression of CD3 +ve cells on day 10 and 30 postpartum. This result concurred with the finding of Samatha et al. (2013) who found more number of D3+ ve cells in stratum compactum of endometritis affected cows. Absence of CD3+ ve cells in all the cows in this investigation indicated no cytotoxic immunity in the uterus. Moderate expression of CD20+ cells in control cows and mild expression in treatment groups of this study were in accordance with the reports of Tawfik et al. (1996) and Disep et al. (2004) in cows. The poor expression of CD20+ ve cells indicated the absence of cell mediated immunity and no clinical bacterial infection in all the cows of treatment groups as described by Tawfik et al. (1996). The CD20+ ve cells expression were higher in control group followed by groups II, III and I which concurred with the conception rate in each group of the study.

Histology of endometrium

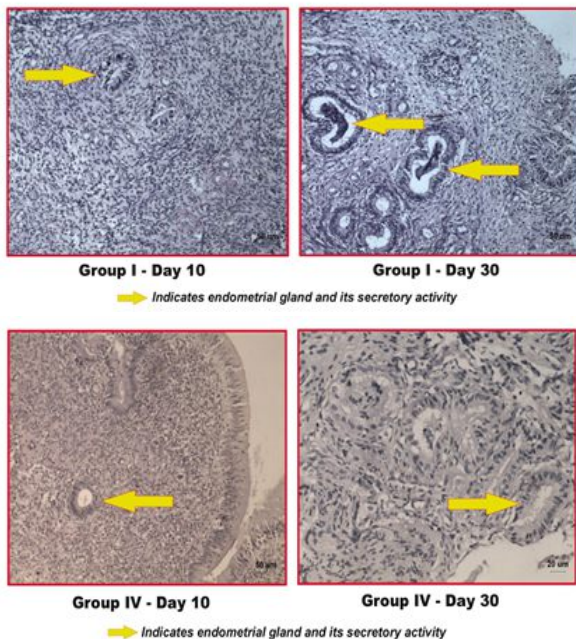


Plate- 1

In this study, on day 10, postpartum uterine lining epithelium was found to be normal with pseudostratified columnar type. Endometrium showed involution process with mild neutrophilic and mononuclear infiltration. The endometrial glandular activity was seen in few cases with increased vascular spaces. On day 30 postpartum there was an increased endometrial glandular activity with extensive lymphocytic infiltration. These observations were in accordance with the findings of Prasad and Krishna (2009) in

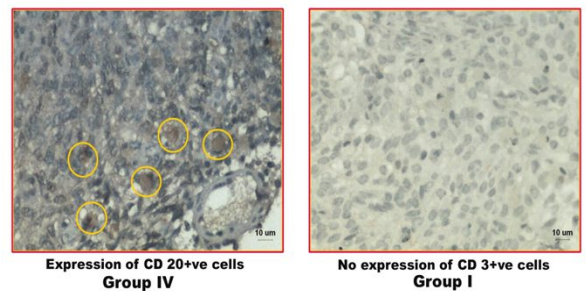


PLATE – 2 IMMUNOHISTOCHEMISTRY

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