



CYTOLOGY AND HISTOLOGICAL STUDIES OF NORMALLY CALVED POSTPARTUM UTERUS TREATED WITH OXYTOCIN IN COWS

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ABSTRACT

The puerperal uterine soundness is essential for the re-establishment of postpartum ovarian cyclicity and next pregnancy. 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). Cytology samples were collected on day 2, 10, 20 and 30 days postpartum and the endometrial tissue collected on day 10 and 30 postpartum in all the two groups through Albuchins's biopsy catheter. There was a reduction in percentages of PMN and increase in lymphocytes in groups I from day 2 to day 30 postpartum. These changes were predominant in group I than control group cows. 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. These regenerative changes were very minimal in group IV (control) on day 30 postpartum.

KEYWORDS : Endometrial tissue, Histology, Cytology, Crossbred cows, oxytocin and Postpartum.

INTRODUCTION

Reproductive performance of dairy cows after the voluntary waiting period is highly related to the health status of the uterus after calving (Dijkhuizen and Stelwagen, 1985 and Ferguson and Galligan, 2000). 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. Some microorganisms were associated with an influx of neutrophils and fluid into the uterine lumen while others were associated with only heavy debris on cytological specimens (LeBlanc, 2008). The PMN cells influx was the maximum at 24 hours and that too in cows treated with 0.5 per cent Lugol's iodine (Singh *et al.*, 2010) and also they stated that the percentage of neutrophils present in an endometrial cytology samples could be used to determine the degree of inflammation present.

Pathak and Bansal (2012) observed that the endometrial glands were simple or branched tubular glands of which few were coiled distally. They were lined with simple columnar epithelium. These glands were more active during the follicular phase as compared to the luteal phase of the oestrous cycle. But the secretory activity was more pronounced during the luteal phase in cows.

Oxytocin injection in the postpartum period did not improve production and reproduction traits in buffaloes and cows (Mustafa *et al.*, 2008 and Palomares *et al.*, 2010), whereas according to Qureshi and Ahmad (2008) the uterine involution was complete after an appropriate period of time after oxytocin treatment.

Intravenous injection of oxytocin (5 IU) resulted in a strong increase in uterine contractility on day 2 postpartum, however, the amplitude and duration of the response decreased by day 4 postpartum in cows (Kundig *et al.*, 1990). At days 10, 20 or 30 postpartum, intravascular treatments with 30, 150 and 300 IU oxytocin evoked markedly elevated plasma levels of 15-ketodihydro-prostaglandin F₂α (PGF₂α metabolite), although the magnitude of the response decreased with advancing puerperium (DelVecchio *et al.*, 1990).

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 50 IU oxytocin (10 ml, Syntophar[®], Interphar Healthcare Pvt. Ltd., Chandigarh, India) and 5 ml normal saline (Parental drugs (India) limited, Indore, Madhya Pradesh, India), respectively.

CYTOLOGY OF UTERUS

In all the cows, cytological studies of uterus were carried out on day 2, 10, 20 and 30 postpartum. All the slides were fixed with methanol and stained with modified Giemsa stain for 20 minutes (Singh *et al.*, 2000) and the cells were counted and percentages of differential count were recorded (Schalm *et al.*, 1975).

ENDOMETRIAL BIOPSY

The ideal endometrial tissue for interpretation was found to be at least 10-20 mm × 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. Endometrial biopsy was taken from the all experimental and control cows on (i) day 10 and (ii) day 30 postpartum.

RESULTS AND DISCUSSION

Cytology of uterine fluid: The mean (±SE) percentages of polymorphonuclear leucocytes (PMN), lymphocytes (L) and monocytes (M) found in the uterus of experimental and control cows are presented in Table 1 and Fig. 1. No eosinophil and basophil could be detected in uterine fluid in all the groups of this study. The results indicated that there was a reduction in percentages of PMN and increase in lymphocytes in group I, than control group from day 2 to day 30 postpartum indicating the absence of bacterial infection in uterine ecobolics treated cows.

TABLE-1 CYTOLOGY OF UTERINE FLUID IN COWS TREATED WITH UTERINE ECBOLIC

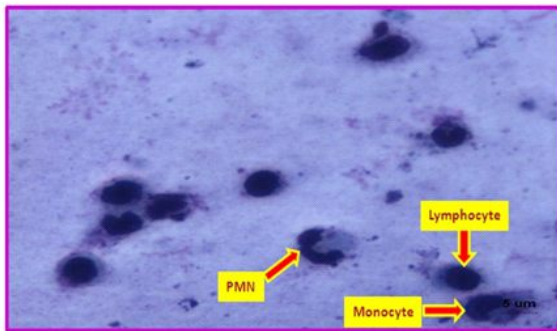
S. No.			Differential leucocyte count			
			Day 2 postpartum	Day 10 postpartum	Day 20 postpartum	Day 30 postpartum
1.	Group I	PMN	92.47 ^a ±1.34	88.68 ^b ±1.30	72.33 ^c ±1.20	73.72 ^c ±0.98
		L	6.38 ^a ±0.36	5.89 ^a ±0.39	26.33 ^b ±0.78	24.29 ^c ±0.19
		M	1.15±0.24	1.88±0.26	1.34±0.24	1.99±0.34
2.	Group IV (Control group)	PMN	86.44 ^a ±0.38	84.56 ^a ±0.56	91.67 ^b ±0.50	88.22 ^c ±0.74
		L	11.89 ^a ±0.48	14.33 ^b ±0.47	6.56 ^c ±0.50	10.22 ^d ±0.43
		M	1.67±0.17	1.11±0.26	1.77±0.18	1.56±0.22

Means bearing different superscript between column (a,b,c) within each row differed significantly ($p < 0.05$).

PMN - polymorphonuclear leucocytes, L - Lymphocytes, M - Monocytes.

Group I- Oxytocin, Group IV- Control.

Cytology of the postpartum uterus



Giemsa staining Bar=5µm

In this experiment, in treatment and control groups the number of PMN cells were higher on day 2 postpartum than on day 10 postpartum. The cellular defence in uterus against bacterial contamination was provided by uterine leucocytes (Vandeplassche and Bouters, 1983), believed to be migrated from the peripheral circulation. PMNs were the predominant inflammatory cell types found in the uterine fluid (Gilbert *et al.*, 2005). In this investigation, the fully dilated cervix at the time of parturition might have caused entry of bacteria into the uterus and this might have increased the PMNs on day 2 postpartum. Interestingly in this study, the mean number of PMN cells showed drastic decreasing trend from day 2 postpartum to day 30 postpartum in all the treatment groups with the maximum decrease in group I and control cows. Administration of oxytocin caused the uterine contractibility and expelled the lochia along with bacterial contaminants in treated cows. Hence, PMN influx into the uterus got reduced after the administration of these drugs as suggested by Uthai *et al.* (2013). An increase segmented neutrophils (or PMN) in the endometrium was associated with poor reproductive performance whereas the presence of lymphocytes in the endometrium was associated with good fertility (Bonnett *et al.*, 1993). These observations might explain the improved conception rate in treatment groups of this study. The mean number of lymphocytes was found to be higher in group I than group IV and this pattern concurred with the conception rate of treatment group in this study.

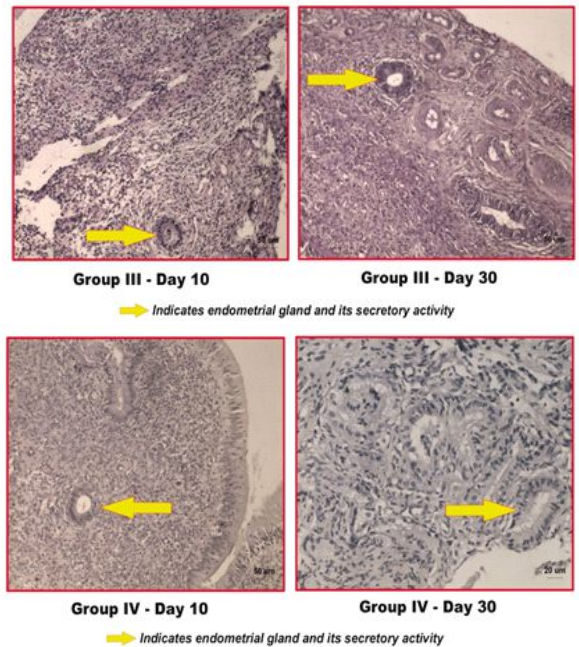
HISTOLOGY OF ENDOMETRIUM

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. 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 (oxytocin) than control group. These proliferative changes were minimal in control group.

On day 30 postpartum, many uterine ecbolics 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 (Fig.1). These regenerative changes were very minimal in group IV (control) (Fig.1) on day 30 postpartum.

FIG. 1 HISTOLOGY OF ENDOMETRIUM IN POSTPARTUM COWS TREATED WITH UTERINE ECBOLIC



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

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