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Cytological Indices of Postpartum Normally Calved Dairy Cows

S. Alagar[*], M. Selvaraju and R. Ezakial Napolean

Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research institute, Namakkal – 2, Tamil Nadu, India
*Corresponding author

A B S T R A C T

A rapid postpartum uterine involution process followed by the early onset of cyclic ovarian activity is importance for achieving a continuous intensive reproduction in cows. Normally calved 36 pluriparous HF crossbred cows were divided into 4 experimental groups viz., groups I, II, III and IV (control) and on day 2 PP, cows treated with an intramuscular injection of 25 mg $PGF_2\alpha$, 50 IU oxytocin, 5 mg methylergometrine maleate and 5 ml normal saline, respectively. All the all experimental groups were inserted with Controlled Internal Drug Release device on day 45-55 postpartum intra-vaginally and left *in situ* for 9 days along with an intramuscular injection of 25 mg of $PGF_2\alpha$ 24 hours before the withdrawal of CIDR. At 48 and 72 hours of CIDR withdrawal, timed artificial insemination (TAI) using frozen thawed semen was performed to all the cows. There was a reduction in percentages of PMN and increase in lymphocytes in groups I, II and III from day 2 to day 30 postpartum. These changes were predominant in group I followed by group III and group II cows than control group. It concluded that uterine ecbolic drugs promote early uterine involution therefore decrease uterine infection and increased conception rate postpartum cows.

Introduction

Fertility after parturition of dairy cows is considered as the principal economic factor of milk producing farms. Alteration or extension of postpartum period with failure or delay in conception results in progressive economic loss to the poor farming community (Patel *et al.,* 2006).Therefore, the puerperal period is a critical phase in the reproductive cycle of dairy cows (Frazer, 2005).Puerperal uterine soundness is essential for the re-establishment of postpartum ovarian cyclicity and next pregnancy (Tiwari *et al.,* 2004).

Four main events during puerperium in bovines are contraction of myometrium and expulsion of lochia, elimination of bacterial contamination in genital system, anatomical and histological restoration of uterus and return of ovarian cyclicity (Nakao *et al.,* 1992andPeter *et al.,* 2009). Involution of the genital tract after parturition aids the resolution of uterine infection and conversely may be delayed by uterine disease. Delay in the completion of uterine involution and resumption of ovarian activity decreases the reproductive efficiency in cows.

A complex relationship exists between factors influencing uterine health and disease in the postpartum cow (Nebel, 1999). 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). Puerperal uterine diseases may prolong the anovulatory interval by up to 10 days (Zain *et al.,* 1995). The rate of conception depends on the time required for involution of uterus and recurrence of oestrus.

Cytology of uterus

For cytological studies of uterus, different approaches viz. direct swab (Chapwanya *et al.,* 2010), cytobrush (Barlund *et al.,* 2008) and lavage techniques (Kasimanickam *et al.,* 2005) were adopted for collecting uterine secretions. The cytological smears have been evaluated after staining with either Giemsa or Wright stains (Kasimanickam *et al.,* 2005; Azawi, 2008). The proportion of polymorphonuclear leucocytes (PMN) in the total number of endometrial cells is indicative for subclinical endometritis (Dolatkhah *et al.,* 2013). Reports on the use of endometrial cytology for the diagnosis of clinical endometritis, however, are limited to one recent study that described endometrial cytology as the most reliable method of diagnosing endometritis in cattle (Barlund *et al.,* 2008). The accumulation of intrauterine fluid has been shown to be associated with bacterial growth and delayed uterine involution (Mateus *et al.,* 2002).

Among the differential leucocytic count, PMN cells were found to be 12.33 (Singh *et al.,* 2000) and 1.99 ± 0.43 per cent (Promod *et al.,* 2002) in uterine flushings of cows affected with endometritis. While other cells such as lymphocyte and epithelial cell per cent in uterine flushings of follicular phase were recorded to be 14.09 ± 3.01 and 85.56 ± 3.11 and in endometritis affected uteri of buffalos, they were 14.43 ± 1.94 and 83.58 ± 1.99 per cent, respectively (Promod *et al.,*

2002). In this present study uterine flushing techniques with sterile normal saline solution used to harvest the endometrial cytology in repeat breeding cows, to evaluate the clearance or reduce the PMN cell population in the uterine lumen to increase the conception rate in repeat breeder cows.

Materials and Methods

Normally calved 36 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 4 experimental groups viz., group I, II, III and IV (control). Therefore each group consisted of 9 cows. On day 2 postpartum, cows of group I,II,III and IV were treated with an intramuscular injection of 25 mg $PGF_2\alpha$ (Dinoprost Tromethamine, 5ml, Lutalyse®, Pfizer Animal Health India Ltd, India), 50 IU oxytocin (10 ml, Syntophar®, Interphar Healthcare Pvt. Ltd., Chandigarh, India), 5 mg methylergometrine maleate (5 ml, Utrasafe®, Vet Mankind, New Delhi, India) and 5 ml normal saline (Parental drugs (India) limited, Indore, Madhya Pradesh, India), respectively.

Cytological studies of lochia / uterine fluid

In all the cows, cytological studies of uterus were carried out on day 2, 10, 20 and 30 postpartum. About 60 ml of 0.9 per cent sodium chloride solution was infused into the uterus using 52 cm disposable plastic infusion sheath fitted with 60 ml syringe containing the solution. Then the uterus was massaged gently and fluid was recovered by negative pressure aspiration into the syringe. The collected fluid was transferred to a 50ml modified polystyrene centrifuge tube without any preservatives. The collected samples were brought to the laboratory within 2 hours and

centrifuged at 1000 rpm for 5 minutes. The supernatant fluid was discarded and a small drop of sediment was streaked on to a clean, grease free glass slide and air dried. All the slides were fixed with methanol and stained with modified Giemsastain for 20 minutes (Singh *et al.,* 2000) and the cells were counted and percentages of differential count were recorded (Schalm *et al.,* 1975).

Results and Discussion

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 (Figs. 1 and 2).

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 groups I, II and III from day 2 to day 30 postpartum. These changes were predominant in group I followed by group III and group II cows indicating the absence of bacterial infection in uterine ecbolics treated cows.

Table.1 Cytology of uterine fluid in cows treated with uterine ecbolics

S. No.			Differential leucocyte count			
			Day 2 postpartum	Day 10 postpartum	Day 20 postpartum	Day 30 postpartum
1.	Group I	PMN	92.56[a]±0.80	77.11[b]±0.79	72.33[c]±1.20	73.44[c]±0.88
		L	5.33[a]±0.24	21.22[b]±0.62	26.33[c]±0.78	25.51[c]±0.67
		M	2.11[a]±0.26	1.67[b]±0.26	1.34[b]±0.35	1.05[b]±0.33
2.	Group II	PMN	83.89[a]±0.65	77.89[b]±0.72	70.56[c]±0.93	75.56[d]±0.84
		L	15.11[a]±0.85	17.22[b]±0.36	26.44[c]±2.21	23.22[d]±1.18
		M	1.00[a]±0.26	4.89[b]±0.22	3.00[c]±0.29	1.22[a]±0.31
3.	Group III	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
4.	Group IV	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.
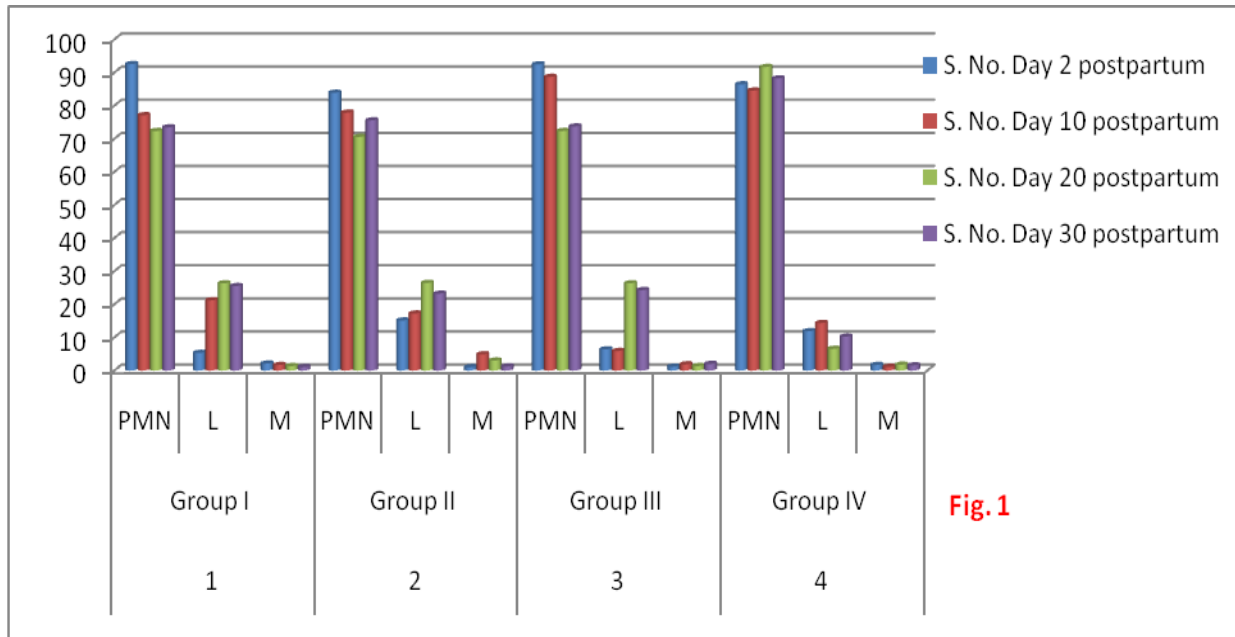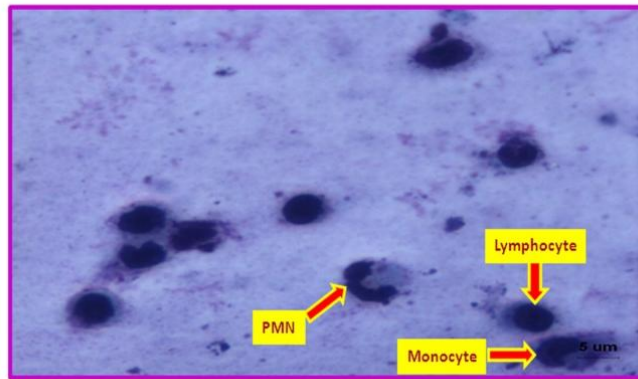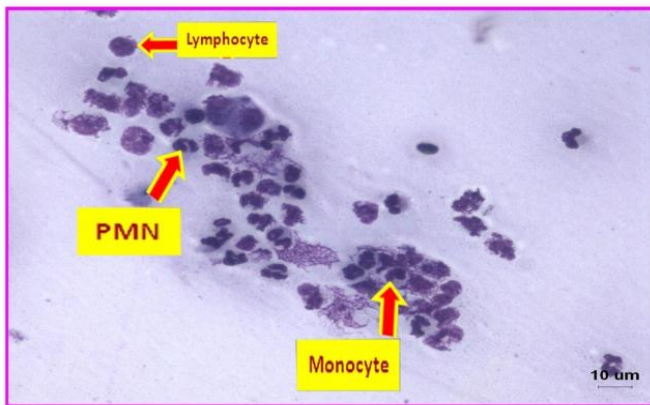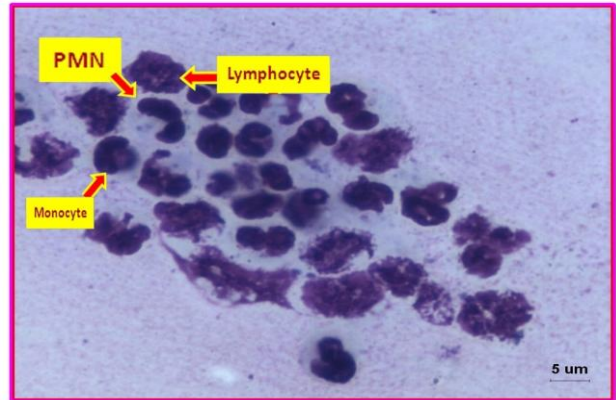*Group I- PGF$_2$α, Group II- Methylergometrine maleate, Group III- Oxytocin, Group IV- Control.*

Fig. 1

Cytology of the postpartum uterus

Giemsa staining Bar=5um

Giemsa staining Bar=10um

Fig. 2

Giemsa staining Bar=5um

In the present investigation, uterine lavage technique was adopted for the cytological studies of the uterus. Various techniques such as direct swab (Chapwanya *et al.,* 2010) cytobrush (Barlund *et al.,* 2008) and lavage techniques (Kasimanickam *et al.,* 2005) were used for this purpose. In this experiment, in all the 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 III than group II and control cows. PMN influx into the uterus got reduced after the administration of these drugs as suggested by Uthai *et al.,* (2013). In these 3 treatment groups, a reduction in mean number of PMN and increased in mean lymphocytes were observed in this study. 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. Among the 3 treatment groups, the mean numbers of lymphocytes were found to be higher in group I followed by group III and II and this pattern concurred with the conception rate of each group in this study.

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