

Effect of Low Volume Normal Saline on Polymorphonuclear Cell Population in Repeat Breeder Cows

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ABSTRACT

The present study examines the effect of uterine flushing in repeat breeder cows with sterile normal saline solution 30 ml on day selection (oestrus), 4, 8, and 12. All the experimental groups the uterine flushing was common, the therapeutic protocol differ in each group after the uterine flushing. The percentage of polymorphonuclear cells (PMNs) detected with endometrial cytology as an indicator of subclinical endometritis. It was hypothesized uterine flushing would be a technique to reduce the number of PMNs in the uterus, and hence be beneficial for cows affected by subclinical endometritis. Cytology samples were taken by low-volume flushing from 72 repeat breeder cows. In this concluded that normal saline uterine flushing in all experimental groups revealed decreased PMN cells in repeat breeder cows than at time of selection, therefore, uterine flushing technique was a useful and practical method to decrease the number of PMNs in the uterus of cattle.

Keywords: Repeat breeder, Normal saline, uterine flushing and cow.

I. INTRODUCTION

The success of the dairy farm lies in ensuring proper and optimal reproductive rhythm of each individual cow in the herd within the normal physiological limits (Dhaliwal, 2005). Repeat breeding syndrome accounts to an annual culling of 3 - 10 per cent of dairy animals and is a perennial problem seen all over the world (Kantharaj, 2015). Conception failure needs to be given importance for rendering optimal production and profit in the farm. The incidence of repeat breeding is in the range of 10.1-24 per cent (Gustafsson and Emanuelson, 2002 and Yusuf *et al.*, 2010). The potential causes of the repeat breeding syndrome mainly include nutritional deficiency, age of the dam, improper oestrus detection, endocrine dysfunction and subclinical uterine infection (Ahmed and Elsheikh, 2014). Among these, subclinical uterine infection is the major contributor to the repeat breeder syndrome of bovines (Sheldon *et al.*, 2009 and Noakes *et al.*, 2001).

In animals without signs of clinical endometritis, SCE is diagnosed by measuring the proportion of neutrophils present in a sample collected by a small-volume lavage

of the uterine lumen or by means of a cytobrush (Gilbert *et al.*, 2005). This uterine inflammation normally decreases with time in healthy cows. The proportion of cows with uterine inflammation diagnosed by cytology decreased from 100 per cent at 2 weeks postpartum to 89 per cent, 58 per cent, and 41 per cent at 4, 6, and 8 weeks postpartum, respectively (Gilbert *et al.*, 2005).

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.

II. MATERIALS AND METHODS

A total of 72 pluriparous, crossbred cows which failed to conceive after three or more consecutive artificial inseminations with good quality semen were selected during oestrus for the study. The selected cows were between 2nd and 5th parity. All the selected experimental cows were randomly divided into six groups viz., Group I (Control group), II, III, IV, V and VI (Treatment groups), each group consisting of 12 cows.

The cytology samples were collected from the all experimental animal on day 0 (selection), day 4, 8 and day 12 post-oestrus. The uterine flushing was common treatment for all repeat breeder cows. The treatment protocols differ among the group after uterine flushing.

III. UTERINE FLUSHING PROCEDURE

In all the selected cows, after induction of epidural anesthesia, the sterile Rusch catheter (18'') was inserted into the body of the uterus and the cuff was inflated with 10 - 12 ml of air.

Sterile normal saline (30 ml) solution was infused into the uterus by using a 50 ml disposable syringe. After 3-5 min, the uterine fluid was recovered by gentle massage and back racking (Singh *et al.*, 2000). After flushing the uterine body the catheter was removed by deflating the air. The collected flushing samples were kept in sterile tubes and stored in refrigerator for cytological examination.

IV. PMN CELL EXAMINATION

All the samples were centrifuged at 1000 rpm for 5 min. A drop of sediment was placed on a clean slide and smear was prepared. It was fixed in methanol and stained with giemsa (Barlund *et al.*, 2008).

V. RESULTS AND DISCUSSION

Repeat breeding syndrome was mainly caused due to the frequent invasion of uterus by specific and nonspecific infectious agents (Javed and Khan, 1991), these infections alter the uterine environment resulting in impairment of sperm transport, sperm death and hostile environment to the subsequent development and maintenance of conceptus, leading to their death, there by affecting conception rate (Azawi, 2008).

In the present study the mean (\pm SE) PMN cell concentration on the day of selection (0 day) was ranged between 5.60 ± 3.82 and 12.50 ± 2.96 per cent. The results of the present study was concurred with the results of Barlund *et al.*, (2008) (8 per cent) and Kasimanickam *et al.*, (2004) (10 per cent) repeat breeder cows affected with subclinical endometritis. Kantharaj (2015) reported that the PMN cell concentration ranged between 5-10

per cent indicated subclinical endometritis in repeat breeder cows. The results of the present study also revealed the presence of subclinical endometritis in the repeat breeder cows.

The mean (\pm SE) PMN cells concentration on the 4th day has increased (table-1) in all the experimental and control groups and thereafter, the PMN cells were reduced marginally on day 8 and 12. This finding was corroborated with the study of Singh *et al.*, (2003) and Palanisamy (2012) in the endometritis affected cows.

The mean (\pm SE) PMN cells concentration on the 4th day has ranged between 7.50 ± 2.33 to 20.29 ± 6.48 per cent. This increase in the neutrophils concentration might be due to the uterine flushing carried out on the day of selection (0 day). Lavage of the uterus would have triggered the irritation of the endometrium and induced the migration of neutrophils into uterine lumen or stimulation of serum opsonins. This replacement of non-functional neutrophils with active neutrophils could be considered as a helpful phenomenon for killing and removing of bacteria located in the uterus (Dini *et al.*, 2015)

The mean (\pm SE) PMN cells concentration on the day 8 and 12 was ranged between 4.00 ± 1.78 to 11.86 ± 6.89 and 1.00 ± 0.08 to 7.00 ± 2.92 per cent. Inflammation of the uterus (SCE) led to adverse effects on reproductive performance, and also interferes with proper fertility (Gilbert, 2012). Dini *et al.*, (2015) also reported that the cytological study after 10 days of uterine lavage revealed the reduced PMN cell concentration in the uterus. Wiebold (1988) reported that most of the embryonic mortality occurred before day 5 in cows, which was associated with a uterine environment which significantly differed that of cows with normal embryos. The uterine lavage exerted beneficial effect on the uterus by stimulating the uterine contraction and expulsion of debris from the uterus (Brinsko *et al.*, 1990) and the removal of exudates from the uterine lumen and reduced bacterial population would be the reason for the reduction in the PMN cell concentration on 8 and 12th day (Dini *et al.*, 2015) and increased conception rate. The uterine flushing technique used in the study might have eliminated the uterine pathogens thereby reduction in lymphocyte concentration (Fig.1).

TABLE.1

PMN CELL POPULATION ON DIFFERENT DAYS OF UTERINE FLUSHING IN REPEAT COWS

Cells (per cent)	Uterine flushing	Group I	Group II	Group III	Group IV	Group V	Group IV
PMN	I (D 0)	12.50b±2.96	9.40b±5.45	5.60b±3.82	6.00 c±2.96	8.50b±2.36	9.40bc±1.29
	II (D 4)	20.29c±6.48	9.70b±4.01	7.50bc±2.33	11.90d±4.09	9.00b±2.98	11.60d±3.01
	III (D 8)	11.86b±6.89	8.10 a±1.96	5.20b±2.38	4.00b±1.78	8.40 b±3.68	8.00b±4.06
	IV(D 12)	4.20a±1.89	7.00a±2.92	3.00a±1.56	1.00a±0.08	1.80a±1.21	4.40a±1.73

Mean values bearing different superscript (a, b, c, d) between columns differed significantly ($p < 0.05$). Group I - control, Group II -UF + PGF2 α , Group III- UF + PGF2 α GnRH at the time of AI, Group IV- UF + PGF2 α GnRH at the time of AI + FM on day 5 and 12 PAI), Group V- UF + PGF2 α GnRH at the time of AI + AO on day 5 and 12 PAI and Group VI - UF + PGF2 α GnRH at the time of AI + FM+AO on day 5 and 12 PAI.

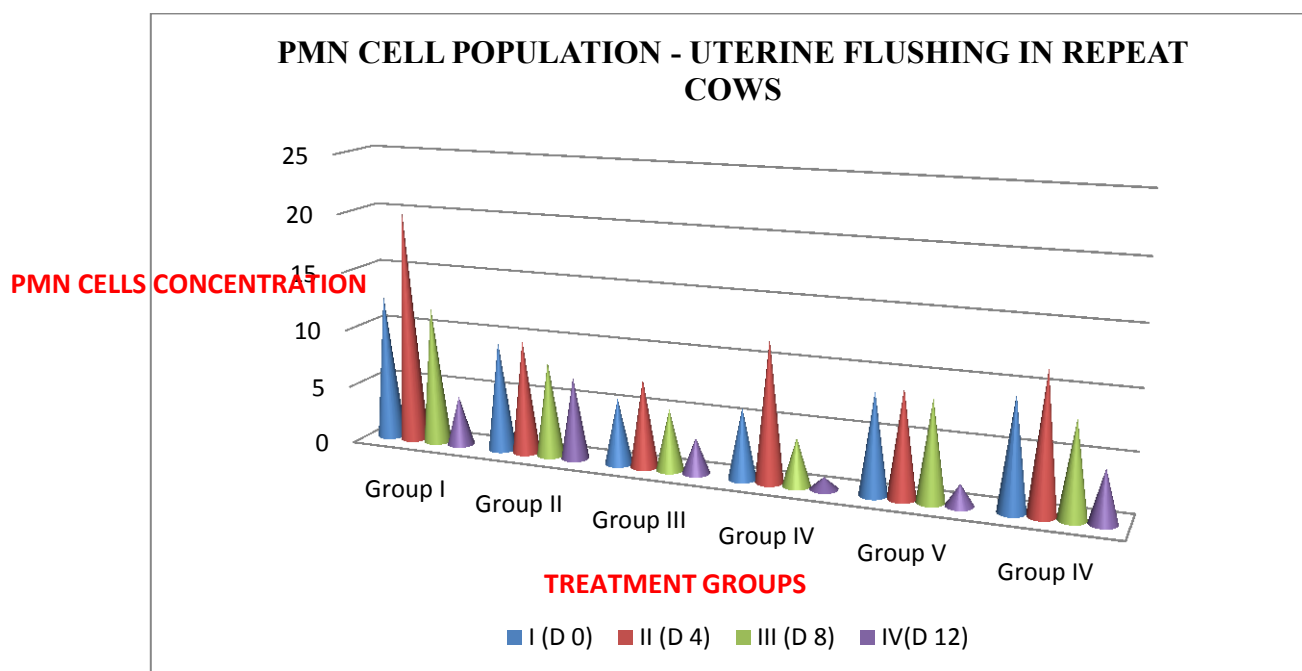


Figure 1

VI. REFERENCES

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