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RESEARCH ARTICLE

Cytological features of uterine flushing in repeat breeder cows

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Abstract : The present study examines the cytological features of uterine flushing in repeat breeder. On different days of interval the uterine flushing was carried out in repeat breeder cows with sterile normal saline solution 30 ml on the day of selection (oestrus), 4, 8, and 12. Uterine flushing was common for all the groups in this experiment and therapeutic protocol changed after uterine flushing day 12. The percentage of polymorphonuclear cells (PMNs) detected with endometrial cytology as an indicator of subclinical endometritis. Cytology samples were taken by low-volume flushing from 72 repeat breeder cows divided into six groups (n=12). In this study concluded that lows volume of uterine flushing by using normal saline at different day's interval might reduce the concentration of PMN. 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.

Key words : Subclinical endometritis, Uterine flushing, Normal saline, PMN

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INTRODUCTION

Subclinical endometritis (SCE) is a postpartum uterine disease characterized by inflammation of the endometrium in the absence of clinical signs of the disease (Sheldon *et al.*, 2006). According to a review (Galvao, 2012), there is general agreement that SCE is highly prevalent affecting approximately 30 per cent of lactating dairy cows with a within herd prevalence ranging from 11 per cent to 70 per cent. 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). Subclinical endometritis is defined by the presence of more than 18 per cent PMNs in an uterine cytology sample collected between 21 and 33 days in milk (DIM) or more than 10 per cent PMNs between 34 and 47 DIM (Sheldon *et al.*, 2006).

Inflammation of the uterus (SCE) leads to adverse effects on reproductive performance, and also interferes with proper fertility (Gilbert, 2012). PMNs and inflammatory mediators such as cytokines, chemokines, eicosanoids, nitric oxide and oxidative stress, are characteristically associated with SCE and are shown to have negative effects on sperm, endometrium and embryos (Gilbert, 2012).

In the recent past the endometrial cytology, which is based on the migration of leucocytes to the site of infection, has been tried elsewhere to rapidly diagnose endometritis. For harvesting leucocytes from uterine secretions lavage technique have been described (Barlund *et al.*,2008).

Removing the inflammatory content from the uterus might be the key for improving later reproductive function and pregnancy outcome. Uterine lavage is an important therapeutic tool for treatment of uterine inflammation in equine medicine (Hurtgen, 2006 ; Liu and Troedsson, 2008). It was proposed that it removes non-functional neutrophils and other inflammatory products and causes uterine contractions which aid in a physical clearance of uterine contents (Brinsko *et al.*, 2011). Although the etiology and pathology of uterine inflammation are different in cattle compared to horses, anecdotal reports of beneficial impacts exist from practitioners who use uterine lavage to improve fertility in cows suspected to suffer from SCE.

In this present study uterine flushing techniques used to harvest the endometrial cytology in repeat breeding cows. Use of low volume uterine flushing using 30 ml sterile saline to evaluate the clearance or reduce the PMN cell in the uterine lumen to increase the conception rate in repeat breeder cows.

RESEARCH METHODOLOGY

Endometrial cytology sample :

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

Treatment:

In all the selected cows, after induction of epidural anesthesia, the uterine flushing was done just before the start of treatment day 0 (oestrus), and day 4, 8 and day 12 post oestrus. 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.

Cytological 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). The leucocytic cells were counted and percentages of differential count were recorded (Schalm *et al*., 1975).

RESULTS AND DISCUSSION

In the present study the mean (±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 Gilbert *et al*. (2005) and Santos *et al*. (2009) (5%), Barlund *et al*. (2008) (8%) and Kasimanickam *et al*. (2004) (10

%) 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 (±SE) PMN cells concentration on the 4th day has increased 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 (±SE) PMN cells concentration on the 4th day has ranged between 7.50±2.33 to 20.29±6.48 per cent (Table 1). 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 (±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). PMNs and inflammatory mediators such as cytokines, chemokines, eicosanoids, nitric oxide and oxidative stress, are characteristically associated with SCE and are shown to have negative effects on sperm, endometrium and embryos (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 (Fig. 1). Repeat breeding syndrome was mainly caused due to the frequent invasion of uterus by specific and nonspecific infectious agents (Javed and Khan, 1991) which led to changes in haematological values and conception rates (Larson *et al.*, 1980). 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 their fertility (Azawi, 2008). 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

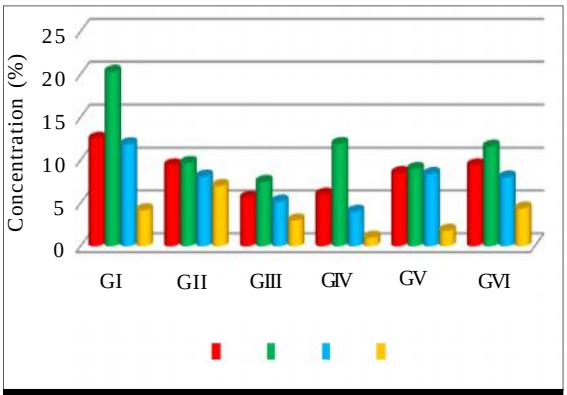

Fig. 1: PMN cells

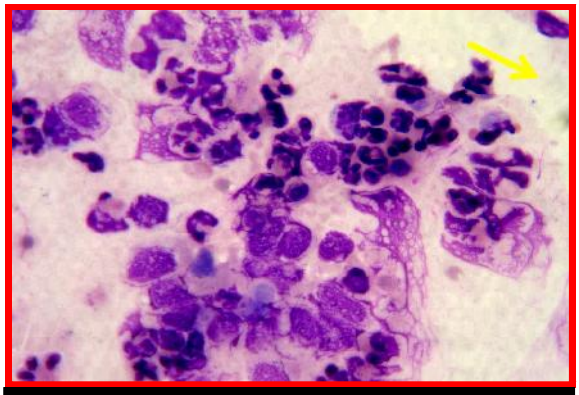

Fig. 2: PMN cells- In uterine flushing fluids

Table 1 : PMN concentration- uterine flushing in repeat breeder cows

Cells (%)	Uterine flushing	Therapeutic groups					
		Group I (UF)	Group II (UF+ $PGF_2\alpha$)	Group III (UF+$PGF_2\alpha$+ GnRH+AI)	Group IV (UF+$PGF_2\alpha$+Gn RH+AI+FM)	Group V (UF+$PF_2\alpha$+Gn RH+AI +AO)	Group VI (UF+AI+AO+FM)
PMN	I (D 0)	12.50^{b}±2.96	9.40^{b}±5.45	5.60^{b}±3.82	6.00^{c}±2.96	8.50^{b}±2.36	9.40^{bc}±1.29
	II (D 4)	20.29^{c}±6.48	9.70^{b}±4.01	7.50^{bc}±2.33	11.90^{d}±4.09	9.00^{b}±2.98	11.60^{d}±3.01
	III (D 8)	11.86^{b}±6.89	8.10^{a}±1.96	5.20^{b}±2.38	4.00^{b}±1.78	8.40^{b}±3.68	8.00^{b}±4.06
	IV (D 12)	4.20^{a}±1.89	7.00^{a}±2.92	3.00^{a}±1.56	1.00^{a}±0.08	1.80^{a}±1.21	4.40^{a}±1.73

Mean values bearing different superscript (a, b, c, d) between columns differed significantly ($p<0.05$)
FM- Flunixin meglumine, AO- Antioxidant, UF- Uterine flushing, AI- Artificial insemination

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 (Fig.1).

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