Original Research Article

Effect of Uterine Flushing on Subclinical Endometritis in Repeat Breeding Cows

S. Alagar*, R. Ezakial Napolean, M. Selvaraju, G.A. Balasubramaniam and P. Selvaraj

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

A B S T R A C T

Subclinical endometritis affects approximately 30% of lactating dairy cows, causing significant economic losses to the dairy industry. Yet, there is no efficient treatment available for this condition. 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.

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 prevalence ranging from 11 per cent to 70 per cent within herd. 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 different methods *viz.* direct swab, cytobrush, aspiration and lavage 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.

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 cows were randomly and equally divided into six experimental groups viz., Group I (Control group), II, III, IV, V and VI (Treatment groups). The experiment was designed with 72 cows, each group consisting of 12 cows.

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 protocols 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

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 failure of uterine defense mechanisms and the presence of highly pathogenic bacteria may determine the persistence of infection and further development into endometritis (Dhaliwal *et al.,* 2001).

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 per cent), 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

(Table 2). 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. 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. a). 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.

Table.1 Cytological features of uterine flushing in repeat

Cells (per cent)	Uterine flushing	Therapeutic Groups					
		Group I	Group II	Group III	Group IV	Group V	Group IV
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
lymphocytes	I (D 0)	16.50[d]±5.56	13.60[b]±4.86	14.20[d]±5.25	12.00[c]±4.85	13.60[d]±5.11	21.0[c]±6.13
	II (D 4)	10.20[c]±5.72	3.00[a]±2.58	10.80[c]±5.12	5.80[b]±3.29	9.60[c]±4.52	8.80[b]±4.50
	III (D 8)	7.80[b]±3.81	3.40[a]±2.02	4.00[b]±0.13	2.20[a]±1.20	2.20[b]±1.80	6.60[a]±3.29
	IV (D 12)	4.30[a]±2.10	3.20[a]±1.02	1.20[a]±0.20	1.20[a]±0.20	1.20[a]±0.20	5.40[a]±2.50

BREEDER COWS

Mean values bearing different superscript (a, b, c, d) between columns differed significantly ($p < 0.05$). Group I - control, Group II -UF + $PGF_2\alpha$, Group III- UF + $PGF_2\alpha$ GnRH at the time of AI, Group IV- UF + $PGF_2\alpha$ GnRH at the time of AI + FM on day 5 and 12 PAI), Group V- UF + $PGF_2\alpha$ GnRH at the time of AI + AO on day 5 and 12 PAI and Group VI - UF + $PGF_2\alpha$ GnRH at the time of AI + FM+AO on day 5 and 12 PAI.

Fig.1(a) PMN concentration in uterine lavage; **(b)** Reduction in lymphocyte concentration using uterine flushing technique
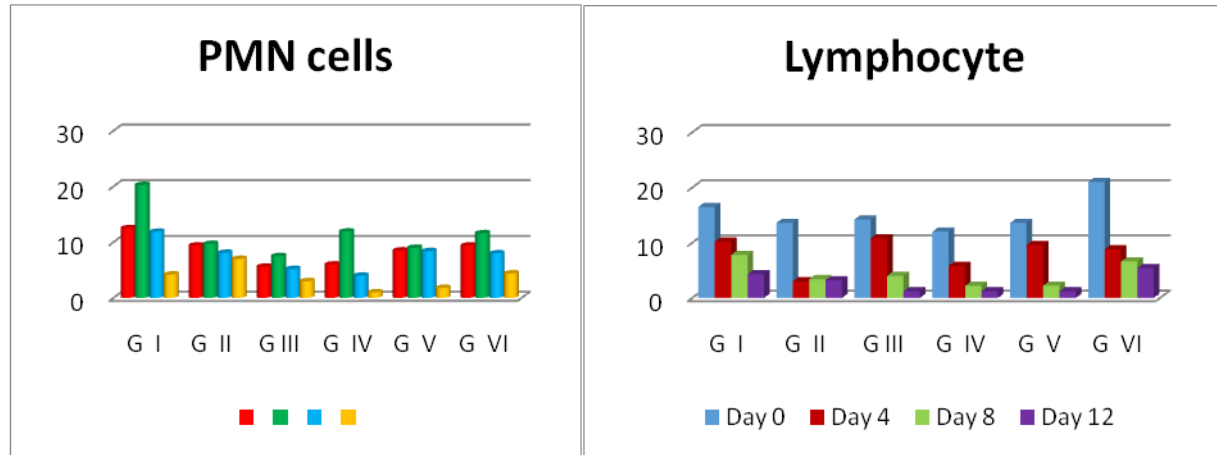
The uterine lavage exerted beneficial effect on the uterus by stimulating the uterine contraction and expulsion of debris from the uterus (Brinsko *et al.,* 1990) and the removal of exudates from the uterine lumen and reduced bacterial population would be the reason for the reduction in the PMN cell concentration on 8 and 12th day (Dini *et al.,* 2015) and increased conception rate.

Lymphocyte

The mean (±SE) lymphocyte count has registered a uniform percentage in the uterine flushing of both experimental and control cows. There was a drastic reduction in the mean (±SE) lymphocyte concentration on the course of uterine flushing from day 0 (selection) to 12th day. Uterus is supplied with ample lymphocytic drainage and contains the full range of lymphohaematopoitic cells and moleculaor regulators required to generate and elicit both humoral and cell mediated immunity. Usually lymphocytes predominate at later stages of infection and in repeaters the chronic inflammatory response would result in elevated lymphocyte concentration in uterine lumen (Babu, 2013). Wiebold (1988) reported that in chronic bacterial infection, the causative bacteria produce certain chemical

factors, which inhibit the stimulation of uterine defense mechanism (UDM). The reason for low numbers of leucocytes in a natural infection could be that the prevalent bacteria were either not conductive to cause a sufficient influx of PMN or those PMN degenerated too quickly (Singh *et al.,* 2000). The uterine flushing technique used in the study might have eliminated the uterine pathogens thereby reduction in lymphocyte concentration (Fig. b).

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