RESEARCH ARTICLE

Effect of Uterine Lavage, Levamisole, PGF2α and its Combinations on Haematological Indices and Bacterial Load in Estrual Mucus of Endometritic Buffaloes

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Abstract

The objective of this study was to compare uterine lavage, Levamisol S/C, $PGF_2\alpha$ i/m and its combinations on haematological indices, bacterial load in cervico-vaginal mucus (CVM) and therapeutic response in endometritic buffaloes. Thirty six repeat breeder buffaloes were randomly assigned into six equal groups. Six animals were kept as positive control (no treatment, only sexual rest, T0), and the rests were assigned to five treatment group, *i.e.,* T1 (uterine lavage), T2 (Levamisol S/C), T3 ($PGF_2\alpha$ IM), T4 (uterine lavage plus Levamisol s/c) and T5 (uterine lavage plus $PGF_2\alpha$ IM). Significant ($p<0.05$) decline in bacterial load and TLC values were recorded after treatment over pretreatment in all five treatment groups. Furthermore, Hb, TEC and PCV increased significantly after treatment as compared to control. Significant drop in neutrophils and rise in lymphocytes was recorded post-treatment in all treatment groups. The eosinophil, basophil and monocytes did not differ significantly within and amongst the groups. The therapeutic response was 33.33, 50.00, 66.67, 66.67, 83.33 and 83.33% in T0, T1, T2, T3, T4 and T5, respectively. The corresponding conception rates were 16.67, 33.33, 50.00, 50.00, 66.67 and 66.67 %. The results conclude that, uterine lavage along with Levamisol or $PGF_2\alpha$ can be used effectively to manage endometrtitic buffaloes.

Key words: Buffalo, Endometritis, Levamisole, $PGF_2\alpha$, Uterine lavage.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.2.06

Introduction

Repeat breeder buffaloes commonly have subclinical endometritis and non-specific infections (Samatha and Ramesh, 2013). Subclinical endometritis is an important cause of infertility and it often goes undiagnosed (Dutt *et al.,* 2017). It not only causes infertility but also results in subfertility even after successful resolution of the disease. To diagnose the disease, transrectal palpation and ultrasonography of the reproductive tract are commonly undertaken under field conditions (Kasimanickam *et al.,* 2004; Barlund *et al.,* 2008), while evaluation of vaginal mucus to define an inflammatory discharge through vaginoscopy, the Metricheck tool or vaginal discharge score (VDS grading system) has also been used (Le Blanc *et al.,* 2002; Wang *et al.,* 2021). Furthermore, uterine cytology (cytobrush, lavage, or biopsy) (Kasimanickam *et al.,* 2004; Lee *et al.,* 2018), may be a more accurate method of diagnosis, as it permits determination of the proportion of endometrial cells and PMNs, and differentiate endometritis from vaginitis, cervicitis or other reproductive tract diseases, thus reducing the chances of false positive diagnoses (Westermann *et al.,* 2010).

Endometritis is often self-limiting with recovery occurring after subsequent estrous cycles. If uterine defense mechanism (UDM) is compromised, bacteria may colonize the uterus and lead to the development of uterine infection and endometritis. Thus, the ideal therapy for uterine infections is to eliminate bacteria from the uterus without compromising UDM and no adulteration of milk or meat. Various drugs, *viz.,* antibiotics (Kumar *et al.,* 2008; Makki *et al*., 2017), non-antibiotics (Ahmadi *et al*., 2019), hormones (Sheldon *et al*., 2004; Butani *et al.,* 2009; Kumar *et al*., 2010) and immunomodulators (Singh *et al*., 2018) have been used to treat uterine infections with varying success. Levamisol is effective in decreasing severity and/or resolving the endometritis and may be used as alternative or adjunct to antibiotic therapy (Singh *et al.,* 2017). Uterine lavage, an important therapeutic tool for the treatment of uterine

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How to cite this article: Singh, N., Singh, B., & Kumar, R. (2023). Effect of Uterine Lavage, Levamisole, PGF2α and its Combinations on Haematological Indices and Bacterial Load in Estrual Mucus of Endometritic Buffaloes. Ind J Vet Sci and Biotech. 19(2), 28-33.

Source of support: Nil

Conflict of interest: None

Submitted: 27/11/2022 **Accepted:** 20/01/2023 **Published:** 10/03/2023

inflammation, eliminates unwanted neutrophils, debris, and other inflammatory products and causes uterine contractions which aid in a physical clearance of uterine contents (Brinsko *et al.,* 2011). The hypothesis of present study was that uterine lavage along with parenteral administration of Levamisol or $PGF_2\alpha$ would ameliorate subclinical endometritis and augment reproductive outcomes of such buffaloes. Therefore, the present study was aimed to evaluate these approaches on haematological indices, bacterial load in CVM and therapeutic response in endometritic buffaloes.

Materials and Methods

The study was carried out on repeat breeder buffaloes following approval from Institutional Animal Ethics Committee (No. IAEC/CVSc/2019/P-02). The cases presented at Veterinary Clinical Complex, College of Veterinary Science, ANDUAT, Kumarganj, Ayodhya and State Veterinary Hospitals in adjoining areas of Kumarganj (India) were included in the study. Thirty six repeat breeder buffaloes were selected on the basis of history and breeding records, per-rectal examination and physico-chemical characteristics of cervico-vaginal mucus at estrus (appearance and consistency, white side test, pH), and randomly divided in to 6 equal groups, *viz*., Group T0 (positive control, *i.e.*, untreated RB buffaloes); T1 (uterine lavage with 50 mL NSS as slow IU infusion, repeated six times on the day of estrus), T2 (Levamisole @ 2.5 mg/kg b.wt, S/C, at 0, 7^{th} and 14^{th} day of the cycle), Group T3 (Cloprostenol @ 250 µg, IM , on 10^{th} day of the cycle), Group T4 (uterine lavage as above, plus Levamisole @ 2.5 mg/kg, S/C, at 0, 7^{th} and 14^{th} day of the cycle), and Group T5 (uterine lavage as above plus Cloprostenol @ 250 µg, IM, on 10^{th} day of the cycle).

Approximately 10 mL blood was collected aseptically in clean sterile EDTA vials by jugular vein puncture. Each animal was sampled twice, at start of treatment and at subsequent estrus after treatment and haematology was done immediately. Similarly, estrual CVM was collected twice using pipette and recto-vaginal technique and bacterial load was estimated. At subsequent standing estrus following treatment all the buffaloes were inseminated twice, 12 h apart. If returned to estrus, they were inseminated again at second and third subsequent estrus. Pregnancy was confirmed per-rectally at 45-60 days after last insemination.

Data were presented as mean ± SE and analyzed by using completely randomized design and Duncan's MRT for effect of groups and periods at significance of $p<0.05$ (Snedecor and Cochran, 1994).

Results and Discussion

Bacterial Load ($x10^6$/ml) in Cervico-Vaginal Mucus (CVM)

The mean bacterial load declined significantly in CVM of treated buffaloes (T1 to T5) as compared to their pretreatment values, whereas in control group no significant change was noted (Table 1). It seems that uterine lavage has additive effect with Levamisol or $PGF_2\alpha$ as evidenced by significantly ($p<0.05$) lower bacterial load in T4 and T5 groups as compared to anyone alone. The reduction in bacterial load of CVM after treatment with uterine lavage might be due to dilution of the infection and removal of the inflammatory contents from the uterus. Uterine lavage also provokes endometrial stimulation with subsequent rise of neutrophil migration to the uterine lumen. Uterine lavage even with normal saline is an important therapeutic tool for the treatment of uterine inflammation in equine medicine (Liu and Troedsson, 2008). Uterine lavage removes non-functional neutrophils and other inflammatory products and causes uterine contractions,

Table 1: Effect of different treatment on bacterial load in CVM and blood parameters of endometritic buffaloes

Groups (n=6)	Bacterial load ($x10^6$/mL) in CVM		TLC ($x10^3$/µL)		Hb (g/dL)		TEC ($x10^6$/ µL)		PCV (%)	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
T0 (Positive Control)	42.67± 1.74	39.17± 1.70^{A}	11.48± 0.04^{A}	11.41± 0.04^{A}	10.00± 0.06	10.05± 0.07^{C}	5.51± 0.08	5.59± 0.07^{C}	31.08± 0.24^{B}	31.50± 0.26^{C}
T1 (Uterine Lavage)	44.17± 1.56^{a}	6.00± 0.30^{Bb}	11.33± 0.05^{Ba}	9.98± 0.03^{Bb}	9.90± 0.13^{a}	10.68± 0.12^{Bb}	5.55± 0.04^{a}	6.21± 0.04^{Bb}	29.83± 0.21^{Ca}	31.67± 0.17^{Cb}
T2 (Levamisol)	43.00± 1.39^{a}	3.17± 0.30^{Bb}	11.43± 0.04^{Aa}	9.71± 0.03^{Db}	10.07± 0.09^{a}	11.10± 0.09^{Ab}	5.51± 0.03^{a}	6.34± 0.04^{ABb}	32.08± 0.20^{Aa}	34.17± 0.21^{Ab}
T3 ($PGF_2\alpha$)	43.33± 1.47^{a}	4.17± 0.22^{Bb}	11.42± 0.03^{Aa}	9.82± 0.02^{Cb}	10.10± 0.05^{a}	11.06± 0.05^{Ab}	5.53± 0.04^{a}	6.28± 0.04^{Bb}	31.92± 0.27^{Aa}	33.75± 0.31^{ABb}
T4 (Uterine Lavage+ Levamisol)	44.33± 1.70^{a}	1.92± 0.15^{Bb}	11.46± 0.03^{Aa}	9.51± 0.03^{Eb}	10.01± 0.07^{a}	11.18± 0.08^{Ab}	5.48± 0.05^{a}	6.46± 0.06^{Ab}	31.08± 0.47^{Ba}	34.92± 0.24^{Ab}
T5 (Uterine Lavage+ $PGF_2\alpha$)	43.00± 1.61^{a}	2.67± 0.10^{Bb}	11.45 ± 0.03^{Aa}	9.60± 0.03^{Eb}	10.03± 0.06^{a}	11.12± 0.07^{Ab}	5.51± 0.05^{a}	6.40± 0.04^{ABb}	31.67± 0.28^{ABa}	34.42± 0.20^{ABb}

Means with different superscripts within group (a, b) and between groups (A, B, C) differ significantly ($p<0.05$)

Pre-T (Pre-treatment); Post-T (Post-treatment)

results in the physical clearance of uterine contents (Brinsko *et al.,* 2011), which might be the reason of higher percentage recovered (50.00 *vs* 33.33%; p<0.05) and conceived (33.33 *vs* 16.67%; p<0.05) buffaloes with uterine lavage as compared to untreated group. Perusal of literature however revealed no information regarding effect of uterine lavage on bacterial load of CVM and fertility in endometritic buffaloes.

Levamisole stimulates cell-mediated immune reactivity by potentiating the rate of T-lymphocyte differentiation, the sensibility to antigens and mitogens and activity of phagocytes, which in turn clears the infection. The reduction in the bacterial load of CVM after $PGF_2\alpha$ administration might be due to removal of suppressive effect of progesterone on the uterine defense mechanism. $PGF_2\alpha$ stimulated the myometrial contraction that expels debris and microorganisms from the contaminated uterine lumen. Furthermore, $PGF_2\alpha$ may stimulate the phagocytic activity of uterine PMN cells as well. In present study, reduced bacterial load of CVM after treatment with Levamisole and $PGF_2\alpha$ might be responsible for higher conception rates. To date, reports on bacterial load in CVM and conception rate in endometritic buffaloes treated with Levamisole or $PGF_2\alpha$ are not available, however, reduced bacterial load (Biswal *et al*., 2014) and improved conception rate (Singh *et al.,* 2017) has been observed after Levamisol treatment in endometritic cows. Reduction in bacterial load of CVM in untreated control group may be due to natural uterine defense mechanism. All treatment protocols reduced uterine infection significantly as evidenced by reduced bacterial load in CVM post therapy, which in turns improve conception rate in treated buffaloes as compared to untreated ones.

Erythrocytic Indices during Pre- and Post-Treatment Period

The mean haemoglobin (Hb) concentrations (gm/dL) in different groups (T0 to T5) did not differ significantly at pretreatment stage, but post-treatment Hb levels were significantly (p<0.05) higher as compared to corresponding pretreatment values in group T1 to T5, whereas no change was noted in control group (Table 1). Post-treatment Hb concentrations at subsequent estrus, were significantly higher in T2 to T5 than T0, and T1. This might be due to dilution of uterine microorganisms, debris and inflammatory contents with improved UDM of the uterus in treated groups. Sabasthin *et al.* (2012), Perumal *et al.* (2013) and Sultana *et al.* (2022) reported significantly (p<0.05) lower level of Hb in repeat breeding cows and buffaloes as compared to normal cyclic fertile ones.

The mean TEC values ($\times 10^6$/mL) did not differ significantly before the treatment amongst the group (T0 to T5), but significant (p<0.05) increase in post-treatment values were noted as compared to corresponding pretreatment values in T1 to T5; whereas no change was registered in T0 (Table 1). At subsequent estrus, post-treatment TEC values did not differ significantly between T4 and T5, but both were significantly (p<0.05) higher than T1, T2 and T3. The findings indicate that among different treatment protocols, uterine lavage plus Levamisole and uterine lavage plus $PGF_2\alpha$ are equally effective in improving TEC values followed by any one alone. Our findings are consistent with Patil (2010), who also reported lower values of TEC in endometritic buffaloes. Reddy *et al.* (2012) also recorded a non-significant increase in the values of TEC in endometritic cows following treatment with uterine lavage.

The mean PCV values (%) of different groups (T0 to T5) did not differ significantly at pretreatment stage; however the post-treatment values were significantly (p<0.05) higher among treatment groups (T1 to T5) than their corresponding pretreatment values, whereas no significant change was noted in T0 (Table 1). At subsequent estrus, post-treatment PCV values of T2 to T5 groups were significantly (p<0.05) higher than T1, suggesting that these therapeutic protocols were equally effective in improving packed cell volume of endometritic buffaloes. Similar observations were also recorded in endometritic cattle (Heidarpour *et al*., 2014) and buffaloes (Sabasthin *et al.,* 2012; Patil *et al.,* 2015). In inflammatory disease, erythropoietin is reduced because of inflammatory cytokines leading to lowered erythropoiesis and ultimately lowered blood cell volume (Thrall, 2004). Significantly improved PCV post-uterine lavage in our study is to some extent supported by similar study in cattle (Reddy *et al.,* 2012). To the best of our knowledge, no citation is available regarding effect of these approaches on haemoglobin concentration and PCV in endometritic buffaloes.

Leukocyte Indices during Pre- and Post-Treatment Period

The total leukocyte count ($\times 10^3$/ml) in blood samples of different groups (T0 to T5) did not differ significantly at pretreatment stage, but dropped significantly (p<0.05) post-treatment in all groups, except control, than their pretreatment values (Table 1). It seems that among different treatment protocols, uterine lavage plus Levamisole and uterine lavage plus $PGF_2\alpha$ are more effective in ameliorating uterine infections by improving UDM of endometritic buffaloes. However, perusal of literature did not reveal any information regarding present approaches on values of TLC in endometritic buffaloes.

The leukocytes play an essential role in immune defense and include different subpopulations, *viz.,* neurophil, eosinophil, basophil, monocytes and lymphocytes. The increased values of TLC in endometritic buffaloes observed in the present study might be due to infection of the genital tract (Kekan *et al.,* 2005). Significantly (p<0.05) higher level of TLC has been reported in repeat breeding buffaloes in comparison to normal cyclic fertile buffaloes (Sabasthin et al., 2012) Sabasthin *et al.* (2012). However, Perumal *et al.* (2013) reported lower levels of TLC in repeat breeding cows than normal cyclic fertile cows. All treatment protocols eliminated uterine infection variably, and significantly (p<0.05) declined

Table 2: Effect of different treatment on differential leukocyte count of endometritic buffaloes

Groups (n=6)	Eosinophil		Basophil		Neutrophil		Lymphocyte		Monocyte	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
T0	4.00± 0.26	5.00± 0.36	0.67± 0.21	0.50± 0.22	48.83± 0.31	48.17± 0.40^{A}	43.67± 0.49	44.17± 0.48^{C}	2.83± 0.17	2.00± 0.26
T1	4.33± 0.21	5.83± 0.31	1.33± 0.21	0.67± 0.21	47.33± 0.49^{a}	43.67± 0.33^{Bb}	43.50± 0.85^{a}	47.17± 0.40^{Bb}	3.50± 0.43	2.67± 0.33
T2	4.33± 0.21	6.00± 0.26	1.33± 0.21	0.83± 0.17	47.83± 0.31^{a}	42.67± 0.33^{Bb}	42.67± 0.56^{a}	47.67± 0.42^{Bb}	3.83± 0.31	2.83± 0.17
T3	4.50± 0.21	6.00± 0.21	1.50± 0.22	0.67± 0.21	47.83± 0.31^{a}	43.67± 0.42^{Bb}	42.83± 0.54^{a}	47.50± 0.34^{Bb}	3.50± 0.34	2.17± .0.98
T4	4.33± 0.21	6.67± 0.21	1.17± 0.31	0.50± 0.22	48.17± 0.31^{a}	40.33± 0.33^{Cb}	43.17± 0.54^{a}	50.83± 0.54^{Ab}	3.17± 0.48	1.67± 0.33
T5	4.67± 0.21	6.83± 0.17	1.33± 0.21	0.50± 0.22	47.33± 0.33^{a}	40.50± 0.50^{Cb}	43.17± 0.40^{a}	49.83± 0.65^{Ab}	3.50± 0.34	2.00± 0.26

Means with different superscripts within group (a, b) and between groups (A, B, C) differ significantly ($p<0.05$); Pre-T (Pre-treatment); Post-T (Post-treatment)

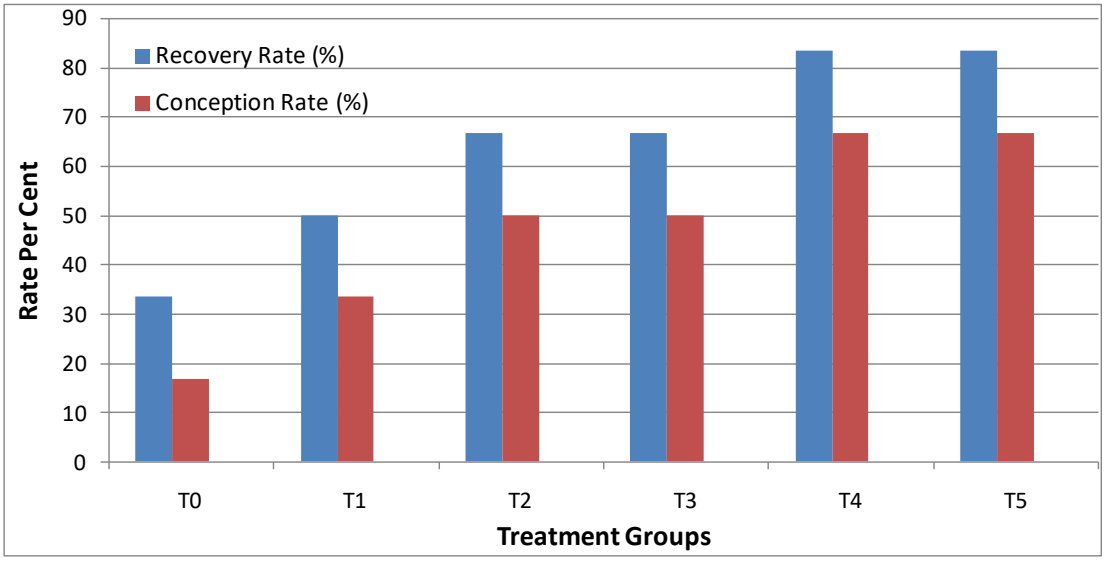

Fig. 1: Per cent recovery and conception rate in different treatment groups of repeat breeding buffaloes

TLC at subsequent estrus. Somewhat similar TLC findings as in T1 were also recorded in endometritic cows by Reddy *et al.* (2012).

The mean eosinophil, basophil and monocyte counts (%) did not differ significantly within and amongst the groups before and after therapy (Table 2). In normal cyclic buffaloes, the eosinophil, basophil and monocyte count ranges from 0 to 20.0 %, 0 to 2.0 %, and 2.0 to 7.0 % (Feldman *et al.,* 2000). The present pretreatment values of eosinophil, basophil and monocyte counts are comparable with many previous reports in repeat breeder cattle and buffaloes (Patil, 2010; Reddy *et al.,* 2012; Sabasthin *et al.*, 2012; Perumal *et al.*, 2013; Dash *et al.*; 2019). Furthermore, to the best of our knowledge, no literature is available regarding effect of various approaches we tried on eosinophil, basophil and monocyte count of endometritic buffaloes.

The mean pretreatment neutrophils and lymphocytes counts (%) did not differ significantly amongst different groups (T0 to T5), but at subsequent estrus following all five therapies neutrophil count declined and lymphocyte count got elevated significantly ($p<0.05$) , however no such change was noticed in control group (Table 2). All the treatment regimens in general, and uterine lavage plus Levamisole and uterine lavage plus $PGF_2\alpha$ in particular, effectively ameliorated uterine infection, which might be responsible for declined neutrophil count and elevated lymphocyte count at subsequent estrus. The increased neutrophil count of endometritic buffaloes observed in the present study might be due to infection of the genital tract (Kekan *et al.,* 2005). The significant drop in neutrophil count was noticed with all treatment regimens. Similar declined neutrophil count and increment in lymphocyte count was recorded in Surti buffalo

treated with IU infusion of normal saline (Patil, 2010) and uterine lavage in cows (Reddy *et al.,* 2012; Dash *et al.,* 2019).

The clinical response rates and conception rates in different groups are depicted in Fig. 1. The overall recovery rates in uterine lavage plus Levamisole and lavage plus $PGF_2\alpha$ treated groups recorded were same (83.33%) and in Levamisole, $PGF_2\alpha$ and uterine lavage alone treated groups these were 66.67 %, 66.67 % and 50.00 %, respectively. The conception rate was also recorded as 66.67 % each in combined treatment groups 50.00% each with Levamisole and $PGF_2\alpha$ alone, and 33.33 % for uterine lavage (Fig 1). The recovery rate was highest in uterine lavage plus Levamisole and uterine lavage plus $PGF_2\alpha$ treated groups than Levamisole, $PGF_2\alpha$ and uterine lavage alone. Higher conception rates were also recorded earlier in endometritic cows subjected with uterine lavage (Swain *et al.,* 2011; Reddy *et al.,* 2012), Levamisole (Swain *et al.,* 2011; Biswal *et al.,* 2014; Singh *et al.,* 2017) and $PGF_2\alpha$ (Biswal *et al.,* 2014; Palanisamy *et al.,* 2015).

Conclusion

On the basis of findings in present study it can be inferred that a combination of uterine lavage plus Levamisole and uterine lavage plus $PGF_2\alpha$ therapy are more effective than the Levamisole, $PGF_2\alpha$ and uterine lavage alone in treatment of repeat breeder buffaloes with respect to bacterial load of CVM, haematological indices, clinical recovery rate and conception rate, and hence may be practiced under field conditions.

Acknowledgements

We gratefully acknowledge the support and cooperation extended by various departments, and Dean of the College, ANDUAT, Kumarganj, Ayodhya for this research work.

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