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EFFECT OF UTERINE LAVAGE, LEVAMISOLE, $PGF_2\alpha$ AND ITS COMBINATIONS ON PHYSICO-CHEMICAL PROPERTIES OF CERVICO-VAGINAL MUCUS AND RECOVERY RATE IN THE ENDOMETRIC BUFFALOES

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ABSTRACT

This investigation aimed to assess the therapeutic efficacy of uterine lavage, levamisole, $PGF_2\alpha$, and their combinations in managing 36 subclinical endometritic repeat breeder buffaloes at the Veterinary Clinical Complex, College of Veterinary and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, and the State Veterinary Hospital in the surrounding regions of Kumarganj. Thirty-six repeat breeder buffaloes were randomly and evenly allocated into six Groups A (positive control), B (uterine lavage), C (Levamisole), D ($PGF_2\alpha$), E (uterine lavage plus Levamisole), and F (uterine lavage plus $PGF_2\alpha$). The recovery rate in descending orders was noted as 83.33, 83.33, 66.67, 66.67, 50, and 33.33% in Groups F, E, D, C, B, and A, respectively. Moreover, the corresponding conception rates were 66.67, 66.67, 50.00, 50.00, 33.33, and 16.67% Groups F, E, D, C, B, and A, respectively. The recovery rate was significantly higher in Groups E and F; lower in Group B, and intermediate in Groups C and D, as compared to the control. Similar trends were also observed in conception rate. In conclusion, the uterine lavage, along with levamisol or $PGF_2\alpha$, is equally effective in the treatment of endometritis in buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, endometritis, levamisole, uterine lavage, $PGF_2\alpha$

INTRODUCTION

Repeat breeding syndrome, being multifactorial in origin, is one of the major infertility problems in buffaloes (Butani *et al.*, 2008). Several postpartum uterine infections, like endometritis, negatively affect fertility, causing an increase in the number of services per conception and in the length of the calving-conception interval. It leads to a drastic reduction in the farm's economy and causes heavy economic losses in the livestock sector in India.

Clinical endometritis is defined as purulent uterine discharge detectable in the vagina of cattle

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21 days or more postpartum or a mucopurulent discharge detectable in the vagina after 26 days postpartum (Scheldon *et al*., 2006). There are no clinical manifestations of the disease in subclinical endometritis (Scheldon *et al*., 2006). Endometritis delays ovarian rebound and uterine involution, increases days open, and accordingly extends the calving interval. It not only induces infertility but also leads to subfertility, even following satisfactory clinical treatment of the condition. Bacterial endometritis is a significant contributor to infertility in bovines, and the condition can be diagnosed through physico-chemical properties (such as appearance, consistency, pH, and white side test) and microbial characteristics (microbial load) of cervico-vaginal mucus, endometrial biopsy, ultrasonography, and cytological examination of the uterus (Parikh *et al*., 2022).

Cervico-vaginal mucus (CVM) consists of a combination of uterine, cervical, and vaginal secretions, comprising 92 to 95% water and ions, with 5 to 8% solid constituents (Tsiligianni *et al*., 2001). The solid fraction comprises mucin glycoproteins, proteoglycans, and lipids. Additionally, mucus has defensive proteins like secretory immunoglobulin A (IgA), lactoferrin, and lysozyme (Tsiligianni *et al*., 2003). The components of mucus are highly autonomous and proportionately regulated; any modification in these components can impair the physical qualities of mucus (Lai *et al*., 2009). CVM safeguards the reproductive system by ensuring continuous lubrication and hydration of epithelial surfaces. The mucus layer serves as a barrier intended to inhibit microbial adhesion and epithelial invasion while facilitating bacterial elimination (Sheehan *et al*., 2006).

Endometritis is frequently self-resolving, with healing taking place during future estrous cycles. When the uterine defense mechanism (UDM) is impaired, germs can colonize the uterus, resulting in uterine infection and endometritis. Consequently, the optimal treatment for uterine infections is the eradication of germs from the uterus but should not inhibit normal UDM and should not cause further adulteration of milk or meat for human consumption. A battery of therapeutic regimens has been tried and tested to manage infertility problems in cattle and buffalo (Butani *et al*., 2009; Kumar *et al*., 2009; Kumar *et al*., 2010; Kumar *et al*., 2020). A variety of bacteria have been isolated from vaginal mucus in cattle and buffaloes (Kumar *et al*., 2008), and various substances, ranging from antibiotics to hormones, have been used earlier to treat uterine infections in buffaloes (Kumar *et al*., 2010; Butani *et al*., 2016). The immunomodulation of animals with drugs such as levamisole, $PGF_2\alpha$, *E. coli* LPS, bacterial modulins, bacterial free filtrate, autologous plasma, autologous serum, hyperimmune serum, oyster glycogen, leukotriene B, granulocyte-macrophage colony stimulating factor, PMN cell extracts (Deori, 2002; Sahadev *et al*., 2007). Most of these immunomodulators act as a chemoattractant and cause an influx of neutrophils and proteins into the uterine lumen after intrauterine infusion. Uterine lavage is a crucial therapeutic method for addressing uterine inflammation in equine medicine (Liu and Troedsson, 2008). Furthermore, uterine lavage removes undesirable PMN cells, debris, and other inflammatory substances, inducing uterine contractions that facilitate the physical expulsion of uterine contents (Brinsko *et al*., 2011). Despite the differing etiopathology of uterine inflammation in buffaloes compared to horses, practitioners report positive outcomes from utilizing uterine lavage to enhance the fertility of buffaloes suffering from subclinical endometritis.

The present study hypothesized that uterine lavage, together with parenteral treatment of levamisole or $PGF_2\alpha$, would improve subclinical endometritis and enhance reproductive results in buffaloes. The current study aimed to assess the impact of uterine lavage, subcutaneous levamisole, intramuscular $PGF_2\alpha$, the combination of uterine lavage and subcutaneous levamisole, and the combination of uterine lavage and intramuscular $PGF_2\alpha$ on the consistency and pH of estrual cervico-vaginal mucus, as well as the conception rate in subclinical endometritic buffaloes.

MATERIALS AND METHODS

This investigation was conducted on repeat breeder buffaloes with consent from the Institutional Animal Ethics Committee (reference number IAEC/CVSc/2019/P-02). The cases were examined at the Veterinary Clinical Complex, College of Veterinary and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, along with State Veterinary Hospitals in the surrounding regions of Kumarganj, which were incorporated into the study. The thirty-six repeat breeder buffaloes were selected based on 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 and equally divided into 6 groups, *viz*. Group A (positive control); B (uterine lavage on 7th day of the cycle; 300 ml normal saline solution (NSS) in divided doses as slow intrauterine infusion), C (levamisole 2.5 mg/kg body weight, s/c, at 0, 7th and 14th day of the cycle), Group D (Cloprostenol sodium) 250 µg, i/m, on 10th day of the cycle), Group E (uterine lavage on 7th day of the cycle; 300 ml normal saline solution (NSS) in divided doses as slow intrauterine infusion plus levamisole 2.5 mg/kg, s/c, at 0, 7th and 14th day of the cycle), and Group F (uterine lavage on 7th day of the cycle; 300 ml normal saline solution (NSS) in divided doses as slow intrauterine infusion plus Cloprostenol sodium 250 µg, i/m, on 10th day of the cycle).

Estrual CVM was obtained twice via the recto-vaginal procedure (Dabas and Maurya, 1988), first before treatment during estrus and again at the subsequent estrus post-treatment; the CVM was visually assessed for appearance (clear/turbid) and consistency (thin/thick). The pH of CVM was measured promptly after collection using pH paper strips with a range of 6.5 to 9.0, and the CVM underwent the white side test (Pateria and Rawal, 1990). During the ensuing standing estrus after treatment, all the buffaloes were inseminated twice, with a 12-hour interval between inseminations. Buffaloes that returned to estrus following the initial insemination were subsequently inseminated during the second and third estrus after treatment. Pregnancy was established via rectal examination 45 to 60 days post-insemination.

The data obtained was analyzed statistically by using a suitable standard method (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSIONS

In the current study, the higher percentage of repeat breeder buffaloes showed thick CVM at the commencement of treatment, and a lower number of buffaloes showed thick mucus following treatment (Table 1) and the findings were compared well with Selvraj *et al*. (2002) documented a greater

incidence (73.60%) of repeat breeder syndrome in bovines, exhibiting thick cervical mucus discharge. Furthermore, a previous study (Lim *et al*., 2014) reported an association between conception rate and consistency of cervical mucus (P<0.10). There is a greater pregnancy rate (74.1 vs 25.9%) in dairy cattle with thin CVM as compared to thick CVM (Lim *et al*., 2014). The thick CVM might impede sperm motility and cause fertilization failure. The transparent and thin estrual CVM discharge post-uterine lavage treatment may result from the dilution of the infection and the expulsion of inflammatory materials from the uterus. Uterine lavage eliminates non-functional neutrophils and other inflammatory byproducts, inducing uterine contractions that facilitate the physical expulsion of uterine contents (Brinsko *et al*., 2011).

The transparent and thin estrual CVM following levamisole treatment may be attributed to levamisole's ability to enhance cell-mediated immune responses by accelerating T-lymphocyte differentiation, increasing sensitivity to antigens and mitogens, and augmenting phagocytic activity (Brunner and Muscoplat, 1980), which may have facilitated the eradication of the infection, thus leading to a clear and thin estrual CVM. The decrease in the proportion of buffaloes exhibiting turbid and thick CVM following PGF2α treatment may be attributed to the alleviation of progesterone's inhibitory influence on the uterine defense mechanism. PGF2α induced myometrial contractions that remove debris and germs from the polluted uterine lumen. The heightened proportion of buffaloes exhibiting reduced estrual CVM following treatment with uterine lavage combined with levamisole or uterine lavage combined with PGF2α may be attributed to the synergistic effect of uterine lavage with either levamisole or PGF2α.

Before treatment, the average pH of CVM across all buffalo groups was alkaline, indicating the presence of a uterine infection. In the ensuing estrus after treatment, the pH considerably decreased (P<0.05) in all treatment groups compared to the control group (Table 2). The findings imply a decrease in alkaline pH towards neutrality. The decrease in pH of estrual CVM during uterine lavage may result from the dilution of infection and the elimination of inflammatory substances from the uterus. Currently, there is no research concerning the impact of uterine lavage on the pH of cervical vaginal mucus in endometritic buffaloes. Consequently, our results could not be juxtaposed. The decrease in the pH of CVM post-treatment may be associated with diminished levels of bacterial metabolites and inflammatory exudates (Salphale *et al*., 1993). Upon eradication of the virus from the uterus, the pH approaches neutrality (Markusfeld, 1984).

In normal healthy buffaloes, the pH value is slightly alkaline to nearly neutral, and the pH is the function of the biophysics and biochemistry of CVM, which in turn is regulated by hormonal changes during the estrous cycle (Ruttland *et al*., 2005). Like our observations, previous workers also recorded elevated pH of CVM during uterine infection or endometritis (Singh, 2014; Krishnan *et al*., 2015; Sarkar *et al*., 2015; Arjunrao, 2017). Moreover, subclinical endometritic cows have significantly higher pH of CVM (7.80±0.06 vs 7.27±0.03) compared to control (Bedewy and Rahawy, 2019). The excessive alkaline or acidic pH of CVM reduces sperm motility and results in failure of fertilization (Hafeez and Hafeez, 2000).

The levamisole reduced the extent of uterine infection by virtue of its immunomodulatory effect, thus reducing the pH of CVM at subsequent estrus. Reports on the pH of CVM post-levamisole treatment in subclinical endometritic buffaloes are

lacking; however, Biswal *et al*. (2014) documented a significant reduction in bacterial count following levamisole treatment, which is positively connected with pH (Kumar *et al*., 2015). Singh *et al.* (2017) reported that the mean pH of the CVM after levamisole treatment also decreased from 7.67±0.19 to 7.28±0.18 in endometritic cattle.

The $PGF_2\alpha$-induced phagocytic activity of uterine polymorphonuclear cells reduces uterine infection and thus reduces the pH of CVM at subsequent estrus. Furthermore, Palanisamy *et al.* (2014) recorded a significant decrease in pH of CVM (8.53±0.03 to 7.22±0.03) in $PGF_2\alpha$-treated endometritic cows. The reduced pH of CVM following treatment with uterine lavage combined with Levamisole or $PGF_2\alpha$ may be attributed to the synergistic impact of uterine lavage and Levamisole or $PGF_2\alpha$. The role of uterine lavage, Levamisole, and $PGF_2\alpha$ has already been discussed vide supra.

The results of the white side test on cervico-vaginal mucus (CVM) before and after treatment are depicted in Table 2. Before treatment, all the groups (A to F) of buffaloes showed a positive reaction to the white side test. After Treatment, B, C, D, E, and F groups of buffaloes showed significant ($P<0.05$) reduction in the percentage of buffaloes that gave positive color reaction to the white side test against control. A higher percentage of buffaloes became negative to the white side test after treatment with uterine lavage combined either with levamisole or $PGF_2\alpha$ treatment (83.33%), levamisole (66.67%), $PGF_2\alpha$ (66.67%), and uterine lavage alone (50%) compared to the Control group (33.33%).

A positive color reaction to the White Side Test has been noted as a sign of uterine infection, explained by the quantity of leukocytes and cellular debris present in the cervical mucus. Normal cervical mucus includes fewer leukocytes, resulting in no color change; conversely, in cases of clinical or sub-clinical endometritis, the cervical mucus exhibits an elevated leukocyte count, leading to a color reaction (Pateria and Rawal, 1990). This color change of cervico-vaginal mucus might be due to neutrophil infiltration and in the condition metrorrhagia, which occurs during estrogen dominance (Ohtani *et al*., 1993).

The higher number of cows with negative color reaction to the white side test at subsequent estrus post-treatment with uterine lavage may result from the dilution of the infection and the expulsion of inflammatory materials from the uterus. Uterine lavage is a crucial therapeutic method for addressing uterine inflammation in equine medicine (Liu and Troedsson, 2008). Uterine lavage eliminates non-functional neutrophils and other inflammatory substances, induces uterine contractions, and facilitates the physical expulsion of uterine contents (Brinsko *et al*., 2011).

Levamisole enhances cell-mediated immune reactivity by increasing T-lymphocyte differentiation rates, sensitivity to antigens and mitogens, and phagocyte activity (Brunner and Muscoplat, 1980), which may subsequently eliminate the infection, leading to a decreased leukocyte count in CVM. The reduced leukocyte count in CVM accounts for the increased proportion of buffaloes exhibiting a negative response to the white side test. Moreover, Singh *et al.* (2017) reported that a high proportion of endometritic cows (66.67%) were negative to the white side test following levamisole treatment. The reduction in the percentage of positive reaction to white side test after $PGF_2\alpha$ treatment may result from luteolysis, thereby eliminating the inhibitory influence of progesterone on the uterine defense system; heightened myometrial activity contractility, which in turns causes evacuation of

Table 1. Consistency (%) of estrual cervico-vaginal mucus in different groups of buffaloes before and after treatment (n=6).

Consistency	Treatment	Group A (n=6)	Group B (n=6)	Group C (n=6)	Group D (n=6)	Group E (n=6)	Group F (n=6)
Thick	Before	83.33 (5)	66.67 (4)	66.67 (4)	83.33 (5)	66.67 (4)	83.33 (5)
	After	66.67 (4)	50.00 (3)	33.33 (2)	33.33 (2)	16.67 (1)	16.67 (1)
Thin	Before	16.67 (1)	33.33 (2)	33.33 (2)	16.67 (1)	33.33 (2)	16.67 (1)
	After	33.33 (2)	50.00 (3)	66.67 (4)	66.67 (4)	83.33 (5)	83.33 (5)

Table 2. Effect of different treatment regimens on pH (Mean ± SE), white side test of estrual cervico-vaginal mucus, and therapeutic response in different groups of buffaloes.

Groups (n=6)	pH of estrual CVM		Positive white side test		Therapeutic response	
	Pre-treatment	Post-treatment	Pretreatment	Post-treatment	Percent recovery rate	Percent conception rate
Group A	8.25 ± 0.04^{Aa}	8.15 ± 0.04^{Aa}	100(6)	66.67^{A} (4)	33.33^{D}(2)	16.67^{D} (1)
Group B	8.18 ± 0.04^{Aa}	7.25 ± 0.04^{Bb}	100(6)	50.00^{B} (3)	50.00^{C} (3)	33.33^{C} (2)
Group C	8.17 ± 0.05^{Aa}	7.27 ± 0.03^{Bb}	100(6)	33.33^{C} (2)	66.67^{B} (4)	50.00^{B}(3)
Group D	8.12 ± 0.05^{Aa}	7.20 ± 0.04^{Bb}	100(6)	33.33^{C} (2)	66.67^{B} (4)	50.00^{B} (3)
Group E	8.10 ± 0.04^{Aa}	7.25 ± 0.04^{Bb}	100(6)	16.67^{D} (1)	83.33^{A} (5)	66.67^{A} (4)
Group F	8.08 ± 0.03^{Aa}	7.20 ± 0.04^{Bb}	100(6)	16.67^{D} (1)	83.33^{A} (5)	66.67^{A} (4)

Means with different superscripts within group (a, b) and among groups (A, B, C) differ significantly ($p<0.05$); Values in parentheses indicate the number of animals.

debris and microorganisms from the contaminated uterine lumen (Lindell and Kindahl, 1983) and enhanced phagocytic activity of uterine PMN cells (Paisley *et al*., 1986). The elevated proportion of buffaloes exhibiting negative reactions to the white side test following treatment with uterine lavage combined with levamisole and uterine lavage with $PGF_2\alpha$ may be attributed to the synergistic effects of these treatments.

The recovery rate for the uterine lavage combined with levamisole or $PGF_2\alpha$ was documented at 83.33%, while the rates for the groups treated with levamisole, $PGF_2\alpha$, and uterine lavage were reported as 66.67%, 66.67%, and 50%, respectively (Table 2). The conception rates were documented as 66.67% for uterine lavage combined with levamisole or $PGF_2\alpha$, 50% for levamisole and $PGF_2\alpha$, and 33.33% for uterine lavage, as illustrated in Table 2. The recovery rate was superior in the group treated with uterine lavage combined with levamisole or $PGF_2\alpha$ compared to those treated with levamisole, $PGF_2\alpha$, or uterine lavage alone.

The elevated conception rate in the uterine lavage-treated group of buffaloes may be attributed to the dilution of infection and the elimination of inflammatory materials from the uterus. Similarly, elevated conception rates were observed in endometritic cows treated with uterine lavage (Swain *et al*., 2011; Reddy *et al*., 2012), levamisole (Saini *et al*., 1999; Swain *et al*., 2011; Biswal *et al*., 2014; Singh *et al*., 2017), and $PGF_2\alpha$ (Sood *et al*., 2003; Sarkar *et al*., 2006; Biswal *et al*., 2014; Palanisamy *et al*., 2014).

CONCLUSION

The findings in the present study clearly support that mild genital infections prevail in repeat breeder buffaloes and can be cleared effectively with the use of uterine lavage along with levamisol or $PGF_2\alpha$ therapy, thereby improving conception rate and achieving the optimum inter-calving interval.

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