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Therapeutic Outcomes Following Uterine Lavage, Levamisole, $PGF_{2\alpha}$ and their Combinations in Subclinical Endometritic Buffaloes

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ABSTRACT

This study examined the effect of different therapeutic protocols on macro-mineral profile and reproductive performance of endometritic buffaloes. Endometritic buffaloes were assigned in five treatment and one control groups *viz*. uterine lavage (UL), Levamisole (LEV), $PGF_{2\alpha}$ (PG), Uterine lavage+Levamisole (UL+LEV), Uterine lavage+$PGF_{2\alpha}$ (UL+PG) and control (no therapy) consisting of six buffaloes in each group. The Subclinical endometritic buffaloes were diagnosed using White side test. The screened buffaloes were subjected to different treatment regimen as mentioned earlier. It was observed that the post-treatment calcium and phosphorus level was significantly ($P<0.05$) higher in all treatment groups as compared to their corresponding pre-treatment values, whereas no significant ($P>0.05$) change was recorded in control group. The clinical cure rate of endometritis was 33.33, 50.00, 66.67, 66.67, 83.33 and 83.33% in untreated control, UL, LEV, PG, UL+LEV and UL+PG, respectively. The corresponding conception rates were 16.67, 33.33, 50.00, 50.00, 66.67 and 66.67% in untreated control, UL, LEV, PG, UL+LEV and UL+PG, respectively. The results inferred that, uterine lavage along with levamisole or $PGF_{2\alpha}$ can be used effectively to manage endometritic buffaloes.

Key words: Buffalo, Cloprostenol, Endometritis, Levamisole, Uterine lavage.

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INTRODUCTION

Clinical endometritis is defined as purulent uterine discharge detectable in vagina of post-partum cattle or buffaloes 21 days or more postpartum or a mucopurulent discharge detectable in vagina after 26 days postpartum, while, subclinical endometritis is characterized by absence of clinical signs of endometritis (Sheldon *et al*., 2006). Many studies attempted to associate the presence of bacteria with subclinical endometritis (SCE) which is char-

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acterized by absence of purulent vaginal discharge with concurrent endometritis based on >5% polymorphonuclear cells (PMNs)/ neutrophils (Pascottini *et al*., 2020a). Indeed, the role of pathogenic bacteria in genesis of SCE remains to be elucidated (Wagener *et al*., 2017). Moreover, Wang *et al*. (2018) stated that SCE is not associated with uterine pathogens. Furthermore, Pascottini*et al*. (2020b) reported that uterine microbiota is not different between healthy and SCE cows and the disease is a consequence of dysregulation of inflammation rather than changes in uterine microbiota and advocated that regulation of uterine inflammation is worthy of pursuit for prevention and treatment of SCE. SCE is often undiagnosed (Dutt *et al.,* 2017) resulting in subfertility even after cure. 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) which are inefficient methods to diagnose SCE.

The ideal therapy for SCE is to eliminate bacterial infection from uterus without compromising uterine defence mechanism (UDM) and has no milk or meat residues. Various drugs has been used to treat uterine infections with variable success (Sheldon *et al*., 2004; Kumar *et al.,* 2008, 2010; Butani *et al.,* 2009; Makki *et al*., 2017; Singh *et al*., 2018; Ahmadi *et al*., 2019). Levamisole (LEV) 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 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). Prostaglandins are used to synchronize estrus, induce parturition, and to treat retained fetal membrane, luteinized cyst, pyometra, and chronic endometritis (Weems *et al*., 2006). The present study was done with hypothesis that uterine lavage, LEV, PG alone or in combinations would ameliorate SCE by augmenting uterine immune system and thereby improving the reproductive outcomes in buffaloes.

MATERIALS AND METHODS

For this study buffaloes were selected from cases presented at Veterinary Clinical Complex, College of Veterinary Science, Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya and State Veterinary Hospitals in adjoining areas of Kumarganj, Ayodhya, UP (India). This experiment was accomplished under the approval of Institutional Animal Ethics Committee (IAEC), College of Veterinary Science and Animal Husbandry, ANDUAT, Kumarganj, Ayodhya, Uttar Pradesh (India) vide approval number IAEC/CVSc/2019/P-02.

Thirty six buffaloes having problem of repeat breeding with SCE were selected for this study using White side test. Buffaloes were examined 28-35 days in milk (DIM) and selected if they had found positive for white side test (Bhat *et al*., 2014) without visible muco-purulent vulvar discharge with normal uterus on per rectal palpation. Approximately 6 ml of blood was collected aseptically in clean sterile vials via jugular vein puncture. Samples were centrifuged at 1200g for 15 min at 4°C. The plasma was separated and preserved at -20°C until analysis. The plasma was analyzed for estimation of serum calcium and phosphorus using diagnostic kits as per manufacturer's instructions (ARKRAY Healthcare, Pvt Ltd). Each animal was sampled twice, at start of treatment (pre-treatment) and at subsequent estrus after treatment (post-treatment). 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.

At the first examination (28-35 days in milk, DIM) buffaloes with SCE were randomly allocated to six groups: (1) Control group (no treatment); (2) Uterine lavage group (UL): buffaloes were subjected to uterine lavage with 50 mL NSS each time, for six times (total 300mL) as slow intra uterine (IU) infusion at day of estrus; (3) Levamisole group (LEV): endometritic buffaloes were administered with Levamisole (Lemasol-75®, Zydus AH, India) @2.5 mg/kg body weight subcutaneously at 0 (day of estrus), 7th and 14th day of the cycle; (4) $PGF_2\alpha$ group (PG): buffaloes were administered with Cloprostenol (Vetmate®, 2 mL vial, Vetcare, India), @ 250 µg intramuscularly on 10th day of the cycle; (5) Uterine lavage+Levamisole group (UL+LEV): the buffaloes were subjected with uterine lavage (same as Group-II) along with Levamisole (same as LEV group); (6) Uterine lavage + $PGF_2\alpha$ group (UL+PG): the buffaloes were subjected with Uterine lavage (same as Group-II) along with $PGF_2\alpha$ (same as PG group).

Data were represented as mean ± Standard error of mean (SEM) and analyzed by using completely randomized design and Duncan's MRT for effect of groups and periods at significance of P value less than or equal to 0.05 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The results of effect of different treatment on serum calcium of SCE buffaloes have been depicted in Table 1. The

Table 1: Effect of different treatment on calcium and phosphorus status in Endometritic Buffaloes

	Calcium (mg/dL)		Phosphorus (mg/dL)	
Groups	**Pre-treatment**	**Post-treatment**	**Pretreatment**	**Post-treatment**
Control (n=6)	6.51 ± 0.04^{B}	6.59 ± 0.03^{C}	3.57 ± 0.04^{B}	3.66 ± 0.05^{D}
Uterine Lavage (n=6)	6.48 ± 0.03^{Ba}	7.89 ± 0.03^{Bb}	3.70 ± 0.04^{Aa}	4.28 ± 0.05^{Cb}
Levamisole (n=6)	6.61 ± 0.03^{Aa}	8.11 ± 0.02^{Ab}	3.73 ± 0.04^{Aa}	4.49 ± 0.04^{Bb}
$PGF_{2\alpha}$ (n=6)	6.57 ± 0.03^{a}	8.04 ± 0.03^{Ab}	3.65 ± 0.03^{Aa}	4.38 ± 0.03^{Bb}
Uterine Lavage +Levamisole (n=6)	6.51 ± 0.03^{Ba}	8.17 ± 0.33^{Ab}	3.71 ± 0.04^{Aa}	4.62 ± 0.05^{Ab}
Uterine Lavage + $PGF_{2\alpha}$ (n=6)	6.49 ± 0.04^{Ba}	8.13 ± 0.03^{Ab}	3.72 ± 0.04^{Aa}	4.57 ± 0.04^{Ab}

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

mean values elevated significantly (P<0.05) in treated buffaloes after the treatment than their pre-treatment, whereas, in control group, no significant (P>0.05) change was recorded. At subsequent estrus after treatment, the increase in serum calcium level was highest in UL+LEV group (8.17±0.33mg/dL) followed by UL+PG (8.13±0.03mg/dL), LEV (8.11±0.02mg/dL), PG (8.04±0.03mg/dL) and UL group (7.89±0.03mg/dL). It can be concluded that among all the treatment groups, UL+ LEV treatment was more effective in restoring the serum calcium level of endometritic buffaloes at subsequent estrus. Our findings were in agreement with the finding of (Sarwar *et al.*, 2002) and (Sharma, 2018), who also reported lower level of calcium in endometritic buffaloes. Low level of calcium in endometritic cows have been reported by many other workers (Kumar *et al.*, 2009; Magnus and Lali, 2009; Jayaram, 2018). The normal reproductive process is compromised in case of severe calcium deficiency (Youngquist and Thrallfall, 2007) possibly due to lack of tone of uterine muscle, as calcium sensitizes the tubular genital tract for action of hormones (Kumar *et al.*, 2020). Majority of infertility problems in cattle (Kumar *et al.*, 2020) and buffaloes (Butani *et al.*, 2011) are due to nutritional deficiency. Plenty of available reports suggest that anestrus, sub-estrus and repeat breeding in cattle (Kumar *et al.*, 2009, 2011) and buffaloes (Butani *et al.*, 2011) might be due to calcium deficiency or imbalance. Furthermore, (Stojikovic *et al.*, 1989) opined that GnRH (Gonadotrophin releasing hormone) induced LH (Luteinizing hormone) release from pituitary is calcium dependent and sub-threshold of calcium leads to failure of LH release.

The results of effect of different treatment on serum phosphorus of SCE buffaloes have been depicted in Table 1. It was observed that the mean values elevated significantly (P<0.05) in treated buffaloes after the treatment than before treatment, whereas, in control no significant change (P>0.05) was recorded. At subsequent estrus after treatment, the increase in serum phosphorous level was highest in UL+ LEV group (4.62±0.05mg/dL) followed by UL+PG (4.57±0.03mg/dL), LEV (4.49±0.04mg/dL), PG (4.38±0.03mg/dL) and UL group (4.28±0.05mg/dL). It can be concluded that among all the treatment groups, UL+LEV treatment was more effective in restoring the serum phosphorous level of endometritic buffaloes at subsequent estrus. Our findings were in agreement with the finding of previous reports (Jaychandran *et al.*, 2007; Verma, 2015; Sharma, 2018), who also reported lower level of phosphorous in endometritic buffaloes. Moreover, low level of phosphorous in endometritic cows have been reported by many workers (Chandrahar *et al.*, 2003; Kumar *et al.*, 2009; Pandey *et al.*, 2009; Amle *et al.*, 2014; Jayram, 2018). There is no consensus among scientists that hypophosphatemia is a cause of infertility syndrome in bovines (Noakes *et al.*, 2019).

The overall recovery rate in UL+LEV and UL+PG treated group was recorded as 83.33% and in LEV, PG and uterine lavage treated group the cure rate for SCE was reported as 66.67%, 66.67% and 50%, respectively. The conception rate was also recorded as 66.67% for UL+LEV and UL+PG, 50% for LEV and PG, 33.33 % for UL. (Fig 1)

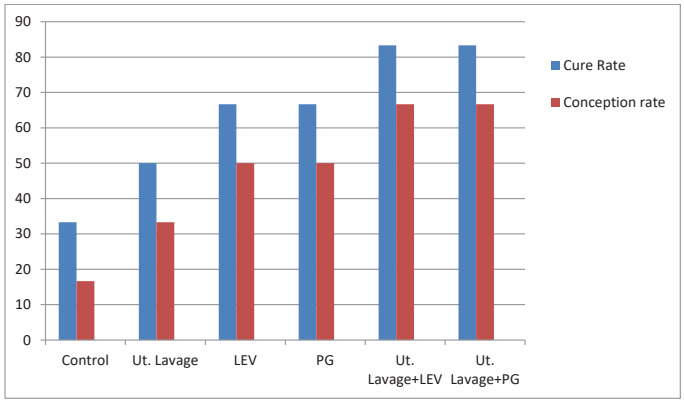

Fig. 1: Effect of different treatment on cure rate and conception rate in Endometritic Buffaloes

Increased conception rate was recorded in all treated buffaloes. Like present findings higher conception rate was also recorded in endometritic cows subjected to uterine lavage (Reddy *et al.*, 2012; Swain *et al.*, 2011), 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). To the best of our knowledge, no citation available regarding the used treatment, so we could not compare our results with buffaloes.

CONCLUSIONS

It could be concluded that low level of calcium and phosphorus was observed in subclinical endometritis affected buffaloes. Also, the therapeutic management of subclinical endometritis buffaloes with uterine lavage along with either levamisole or $PGF_{2\alpha}$ is better approach with more cure rate as well as resultant higher pregnancy rate. It is advised that the results of the current study need more validation in a larger population due to small sample size.

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CONFLICT OF INTEREST

None.

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