RESEARCH ARTICLE

Therapeutic Modulation of Haematological Parameters and Fertility Outcomes in Postpartum Subclinical Endometritis in Jaffarabadi Buffaloes

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Abstract

The present study investigated the effects of intrauterine lysozyme, povidone iodine, and antibiotic therapy on haematological parameters and conception rates in postpartum subclinical endometritic (SCE) Jaffarabadi buffaloes. Thirty animals (24 SCE-affected and 6 healthy controls) were randomly allocated into five groups: Group I (lysozyme 2 mg in 20 mL PBS once), Group II (2% povidone iodine, 50 mL once), Group III (sensitive antibiotic, levofloxacin, 20 mL for 3 days), Group IV (untreated positive control), and Group V (healthy negative control). Haemoglobin, packed cell volume, total leukocyte count, and differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils) were assessed at 0 h, 72 h, and at subsequent estrus. Conception rate was evaluated on the basis of first service conception (FSCR) at subsequent estrus. Groups I and II buffaloes exhibited significant ($p<0.05$) post-treatment increase in haemoglobin and packed cell volume, whereas other haematological parameters remained unaffected. First service conception rate was recorded as 33.33% in Groups I, II, and V, 16.67% in Group III, and 0.00% in Group IV. These findings indicate that intrauterine administration of lysozyme and povidone iodine improves haematological status and reproductive performance in SCE-affected Jaffarabadi buffaloes.

Key words: Conception rate, Haematological parameters, Intrauterine therapy, Jaffarabadi buffalo, Lysozyme, Povidone iodine, Subclinical endometritis.

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Introduction

Reproductive efficiency is a key determinant of productivity and profitability in dairy herds, with disruptions leading to extended calving intervals, prolonged dry periods, reduced milk yield, and increased culling. High-producing dairy cows and buffaloes are particularly vulnerable to postpartum metabolic stress, which predisposes them to reproductive disorders such as metritis and endometritis (Nazhat *et al.,* 2018). Subclinical endometritis (SCE) is an important cause of infertility, characterized by endometrial inflammation without visible clinical signs such as purulent vaginal discharge. Diagnosis relies on cytological evaluation of uterine samples, with >5% polymorphonuclear cells (PMNs) considered indicative of infection (Kasimanickam *et al.,* 2004). SCE affects approximately 30% of lactating animals and is associated with reduced conception rates, prolonged calving intervals, and repeat breeding (Gilbert *et al.,* 2005; Galvao *et al.,* 2011). Accurate detection of SCE remains challenging due to variability in diagnostic methods including palpation, ultrasonography, vaginoscopy, Metricheck evaluation, cytobrush sampling, and biopsy.

Traditional management relies on antibiotics, hormones, and anti-inflammatory agents, but their indiscriminate use has contributed to antimicrobial resistance (Bajaj *et al.,* 2016). Alternative strategies, *viz.,* immunomodulators have shown promise in enhancing uterine immunity and improving reproductive outcomes, though data in buffaloes remain limited (Sarma *et al.,* 2012; Makki *et al.,* 2017). Hence, this study was aimed to evaluate the effects of intrauterine lysozyme, povidone iodine and antibiotics on haematological parameters and reproductive performance in SCE-affected buffaloes, providing insights into effective non-antibiotic strategies to enhance fertility.

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Materials and Methods

The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. KU-JVC-IAEC-SA-109-23). The study was conducted on the Jaffarabadi buffalo herd maintained at the Cattle Breeding Farm, Kamdhenu University, Junagadh (Gujarat), Bull Mother Farm, Kamdhenu University, Amreli (Gujarat), and selected areas in and around Amreli, India.

Experimental Animals and Design

A total of 30 postpartum Jaffarabadi buffaloes 60-90 days in milk (DIM), including 24 SCE-affected and 6 healthy controls, were enrolled in the study. All the animals were maintained under standard feeding and management practices. Rectal palpation of the genital tract was performed to exclude any palpable abnormalities.

Subclinical endometritis (SCE) was diagnosed based on the presence of clear cervico-vaginal discharge along with >5% polymorphonuclear cells (PMNs) in endometrial cytology, as described by Gilbert *et al.* (2005). Animals with <5% PMNs were considered healthy, and served as negative controls.

The 24 SCE-affected buffaloes were randomly assigned to four groups (n=6 each), viz., Group I to IV, and Group V served as healthy control. Animals of Group I received intrauterine lysozyme infusion (2 mg in 20 mL PBS, once), Group II with intrauterine 2% povidone iodine (50 mL, once), Group III with intrauterine infusion of levofloxacin (sensitive antibiotic – 20 mL for three consecutive days), and Group IV was kept as untreated positive control (SCE, self-cure), while healthy negative control Group V received no treatment. All animals were inseminated at subsequent estrus, and pregnancy was confirmed at 60 days post-insemination.

Blood Sampling and Haematological Analysis

Blood samples were collected from each buffalo at three time points: pre-treatment (0 h), post-treatment (72 h), and at subsequent estrus. Samples were collected in heparinized vials and analyzed immediately for haematological parameters, *viz.,* haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils) using an automatic haematology analyzer (Exigo, H400, Sweden).

Statistical Analysis

Data were expressed as mean ± standard error (SE). Two-way analysis of variance was performed to evaluate the effects of treatment and time on haematological parameters (Snedecor and Cochran, 1998). Duncan's multiple range test (MRT) was applied as a *post hoc* test to compare pair wise mean differences between groups, with significance considered at $p < 0.05$.

Results and Discussion

Effect of Treatment on Haematological Profiles

In the present investigation, the haematological profile of SCE-affected buffaloes was evaluated in relation to different therapeutic interventions at the initiation of treatment, after 72 h, and at subsequent estrus following therapy. The comparative trends observed among lysozyme, povidone iodine, and antibiotic-treated animals, as well as untreated positive controls and healthy negative controls, are summarized in Table 1, providing insights into the systemic changes associated with both the disease condition and the therapeutic response.

The haematological profile of SCE-affected buffaloes subjected to different therapeutic regimens revealed distinct changes across time points, reflecting both disease-associated alterations and treatment-mediated recovery. Haemoglobin concentration increased gradually from initiation of therapy to subsequent estrus, in lysozyme- and povidone iodine-treated groups showing significantly higher values ($p<0.05$) at estrus compared to baseline. This improvement indicates enhanced erythropoietic activity and better systemic oxygen-carrying capacity following immunomodulatory and antiseptic interventions. Comparable increases in Hb following therapeutic management of subclinical endometritis have been reported in cattle and buffaloes (Patil *et al.,* 2015; Parikh *et al.,* 2022; Verma *et al.,* 2023; Singh *et al.,* 2023), suggesting restoration of general health status. In contrast, the antibiotic-treated and untreated positive control groups showed only marginal improvements, reflecting limited efficacy in restoring haematological vigor.

Packed cell volume (PCV) exhibited a similar trend to Hb, with significant increases at estrus in lysozyme- and povidone iodine-treated groups, highlighting the beneficial role of non-antibiotic therapies in alleviating anaemia-like tendency associated with subclinical endometritis (Table 1). The initially decreased PCV observed in endometritic buffaloes may be related to anaemic alterations induced by infection and variations in haemoconcentration. Optimal levels of Hb and PCV are crucial for efficient oxygen transport, and their restoration following therapy indicates improved systemic health. According to Thrall (2004), inflammatory cytokines can suppress erythropoietin production, thereby reducing erythropoiesis and lowering blood cell volume.

Total leukocyte count (TLC) exhibited only marginal fluctuations across treatments and time points, remaining within physiological ranges. A moderate elevation at estrus in lysozyme- and povidone iodine-treated groups may reflect improved immunocompetence, though not statistically significant. Neutrophil percentages were initially elevated in SCE-affected groups, especially in antibiotic and positive controls, indicative of underlying uterine inflammation. However, a declining trend toward subsequent estrus, particularly in lysozyme and povidone iodine groups, denotes resolution of active inflammation (Table 1). This observation

Table 1: Mean (±SE) values of haematological parameters in healthy and SCE-affected buffaloes at different time intervals of intrauterine therapy

Parameter	Time point	Groups				
		I (Lysozyme)	II (Povidone iodine)	III (Antibiotic)	IV (Positive control)	V (Negative control)
Hb (g/dL)	0 hr	10.63±0.30	10.40±0.36	10.53±0.19	10.37±0.28	11.40±0.48
	72 hr	11.17±0.50	10.67±0.53	11.52±0.37	10.77±0.37	11.65±0.73
	Estrus	11.81±0.37*	11.63±0.33*	11.07±0.60	10.77±0.81	11.77±0.39
PCV (%)	0 hr	30.08±0.94	30.62±1.22	31.05±0.75	30.55±0.79	33.55±1.57
	72 hr	32.22±1.05	31.71±1.10	32.87±1.64	31.05±1.21	33.73±1.33
	Estrus	34.72±1.18*	33.80±1.15*	33.62±2.02	31.05±2.52	33.87±1.28
TLC ($\times10^3$/µL)	0 hr	8.25±0.22	7.68±0.78	8.03±0.61	7.48±0.71	7.33±0.54
	72 hr	8.43±0.30	7.93±0.66	7.82±0.73	7.46±0.52	7.50±0.33
	Estrus	8.78±0.40	8.40±0.52	7.60±0.88	7.46±0.63	8.50±0.28
Neutrophils (%)	0 hr	34.17±4.18	34.17±1.72	45.83±6.44	46.51±3.59	38.83±3.38
	72 hr	36.67±4.95	36.00±2.57	42.17±3.94	45.00±5.25	37.50±3.66
	Estrus	39.17±5.03	37.50±3.04	40.50±2.57	43.00±6.55	35.50±3.96
Lymphocytes (%)	0 hr	55.33±4.11	54.50±2.85	44.67±6.67	42.83±3.95	51.17±3.96
	72 hr	56.33±2.64	55.67±4.82	47.00±3.79	43.67±5.13	52.33±4.28
	Estrus	59.00±3.14	56.33±5.48	50.67±2.76	44.83±7.01	53.66±4.62
Monocytes (%)	0 hr	6.50±0.62	7.00±0.31	6.33±0.49	6.83±0.65	6.17±0.40
	72 hr	6.33±0.42	6.67±0.42	6.50±0.22	6.50±0.34	5.83±0.31
	Estrus	5.90±0.50	6.33±0.49	6.63±0.31	6.34±0.31	5.67±0.67
Eosinophils (%)	0 hr	3.00±0.68	3.33±0.42	2.17±0.17	2.83±0.31	2.83±0.48
	72 hr	2.78±0.34	2.67±0.21	2.33±0.21	2.83±0.21	2.50±0.34
	Estrus	2.67±0.21	2.33±0.33	2.33±0.21	2.83±0.17	2.17±0.17

*p>0.05 within column for a parameter across time points

was consistent with the findings of Parikh *et al.* (2022), Verma *et al.* (2023) and Singh *et al.* (2023), who associated normalization of neutrophil proportions with recovery from endometrial infections.

Conversely, lymphocyte percentages demonstrated a gradual increase during the therapeutic period, attaining higher values at estrus in treated groups, most prominently in lysozyme therapy. The lymphocytic shift signifies restoration of immune homeostasis, as lymphocytes play a central role in uterine defense and tissue repair. Previous workers have also reported lymphocyte predominance during recovery from endometritis (Bajaj *et al.,* 2023). Monocyte and eosinophil counts remained relatively stable with minor, non-significant variations, suggesting minimal systemic impact of SCE on these leukocyte subsets (Table 1). In contrast to present findings Ahmad *et al.* (2003) and Reddy *et al.* (2012) reported lower eosinophil levels in endometritic cows and buffaloes compared to typical cyclic fertile animals. Conversely, Perumal *et al.* (2013) observed higher eosinophil counts in repeat breeder cows than in normal cyclic cows.

Basophils were absent throughout the study, consistent with normal haematological patterns in bovines. Similarly, Bajaj *et al.* (2017) reported nil basophil counts in both SCE-affected and healthy buffaloes. In agreement with our observations, Verma *et al.* (2023) and Singh *et al.* (2023) also found no significant changes in basophil counts following treatment of endometritic buffaloes. Although basophils participate in inflammatory responses and the development of acute and chronic allergic conditions such as hay fever, asthma, atopic dermatitis, and anaphylaxis, they also possess phagocytic capability and contribute to the release of histamine, serotonin, and the anticoagulant heparin.

Overall, the results emphasize that immunotherapeutic (lysozyme) and antiseptic (povidone iodine) interventions not only facilitated uterine recovery but also contributed to systemic haematological normalization. In contrast, conventional antibiotic therapy exhibited modest improvements, while untreated SCE-affected animals (positive controls) showed persistent deviations. These findings highlight the therapeutic superiority of non-antibiotic approaches in mitigating both local uterine pathology and associated systemic haematological disturbances, thereby supporting their practical utility in herd health management.

Effect of Treatment on FSCR

The first service conception rates (FSCRs %) following different therapeutic interventions are presented in Table 2.

Table 2: First service conception rates (FSCRs %) in healthy and SCE-affected buffaloes treated with different intrauterine therapies

Group	Treatment	No. conceived	FSCR (%)
I	Lysozyme	2/6	33.33
II	Povidone iodine (2%)	2/6	33.33
III	Antibiotic (sensitive)	1/6	16.67
IV	Positive control (untreated SCE)	0/6	0.00
V	Negative control (healthy)	2/6	33.33

Among the treatment groups, lysozyme therapy (Group I) yielded the first service conception rate of 33.33%, with two animals conceiving at the first service. These findings corroborated with the earlier reports of Venkatesh *et al.* (2022), Bajaj *et al.* (2023), and Vishvakarma *et al.* (2023), who also achieved higher conception rate following lysozyme-based therapy in endometritic buffaloes. Povidone iodine therapy (Group II) achieved an FSCR of 33.33%. Similar fertility improvements by enhanced uterine health with povidone iodine have also been reported in bovine (Patel *et al.,* 2014; Butani *et al.,* 2016; Mido *et al.,* 2016; Yoshida *et al.,* 2020; Seena *et al.,* 2021). These findings support its effectiveness in the management of subclinical endometritis in bovines. Antibiotic treatment (Group III) resulted in a lower FSCR of 16.67%, while the untreated positive control group (Group IV) had the poorest reproductive outcome with 00.00% FSCR. Parikh *et al.* (2022) also reported relatively higher conception rates at subsequent estrus with *E. coli* LPS (50.00%) and oyster glycogen (37.50%), at par with a control group (37.50%) than the antibiotic and garlic treated groups (25.00% each), and nil in the positive control group of Gir cows with SCE. Intrauterine antibiotic treatment may result in a lower conception rate because it may not be effective *in vivo* for AMR of pathogens, or the residual antibiotics can disrupt the uterine microflora, irritate or damage the endometrial lining, and impair sperm or embryo viability. The healthy negative control group of buffaloes (Group V) exhibited a 33.33% FSCR, which was comparable to the lysozyme group and povidone iodine group.

Conclusion

The present investigation demonstrated that subclinical endometritis in buffaloes is associated with altered haematological indices, including reduced haemoglobin and packed cell volume, along with shifts in leukocyte profiles. Therapeutic interventions with lysozyme and povidone iodine effectively restored these parameters toward normal values, indicating recovery of systemic health and immune balance. Correspondingly, fertility performance was superior in lysozyme and povidone iodine treated buffaloes, achieving the highest overall conception rate (33.33%), while antibiotic therapy and untreated controls showed comparatively lower outcomes. These findings highlight the advantage of non-antibiotic approaches, particularly lysozyme and povidone iodine, as promising alternatives for improving both uterine health and reproductive efficiency in SCE-affected buffaloes.

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