RESEARCH ARTICLE

Therapeutic Potential of Intrauterine *Lactobacillus plantarum* in Repeat Breeding Jaffarabadi Buffaloes with Subclinical Endometritis

Devesh J. Parmar[1]*, Karsan B. Vala[1], Raghuvir H. Bhatt[2], Vishal S. Suthar[3], Mulraj D. Odedra[4], Nirbhay R. Nandaniya[1]

Abstract

Subclinical endometritis (SCE) is an important uterine disorder associated with repeat breeding and reduced fertility in buffaloes. The present study evaluated the therapeutic efficacy of intrauterine *Lactobacillus plantarum* in SCE-affected repeat breeding Jaffarabadi buffaloes and compared its response with intrauterine antibiotic therapy. SCE was diagnosed through endometrial cytology using cytobrush technique and Whiteside test (WST). Out of 80 repeat-breeding buffaloes examined, cytobrush technique detected SCE in 50.00% (40/80) animals and served as the reference method with 100% relative efficacy, whereas WST detected SCE in 35.00% (28/80) buffaloes with 70.00% relative efficacy. The mean PMN cell score in SCE-positive buffaloes was 7.24±0.32%, compared to 1.42±0.21% in healthy buffaloes. Animals were allocated into four groups: probiotic group (intrauterine *L. plantarum*, n=15), antibiotic group (intrauterine levofloxacin, n=15), positive control group (normal saline, n=10) and negative control group (healthy cyclic buffaloes, n=10). Therapeutic response was assessed based on improvement in uterine cytology/WST grades, inflammatory markers, biochemical profile, and conception rates following artificial insemination (AI). Probiotic therapy improved uterine health status as indicated by reduced inflammatory cellular infiltration, improved WST grades, and favourable modulation of inflammatory markers including serum amyloid A (SAA) and IL-10, suggesting resolution of uterine inflammation and enhanced immune tolerance. The first service and overall three service conception rates were highest in the probiotic group 40.00 and 66.67% followed by antibiotic group 33.33 and 53.33%, negative control 30.00 and 50.00%, and positive control 20.00 and 30.00%, respectively. In conclusion, intrauterine *L. plantarum* therapy proved to be an efficient alternative to antibiotic therapy in SCE affected buffaloes hence can be recommended as a safe, economical and farmer-friendly approach for enhancing fertility, with the advantage of reducing dependence on antibiotics and supporting antimicrobial stewardship in livestock production systems.

Key words: Buffaloes, Cytobrush, IL-10. *Lactobacillus plantarum*, Levofloxacin, SAA, Subclinical endometritis, Whiteside test

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Introduction

India possesses the largest buffalo population in the world and buffaloes contribute a major share to national milk production, supporting rural economy and smallholder livestock systems (FAO, 2023). However, reproductive limitations such as delayed puberty, silent estrus, seasonality, prolonged calving interval, anestrus and repeat breeding reduce productivity (Perera, 2011; Baruselli *et al.,* 2017). Repeat breeding is defined as failure to conceive after three or more inseminations/services despite regular estrous cycles and absence of apparent anatomical abnormalities (Purohit *et al.,* 2014). The condition causes substantial economic losses due to repeated inseminations, increased veterinary costs, prolonged inter-calving period and higher culling rate (Singh *et al.,* 2018; Bodhare *et al.,* 2020).

Uterine infection, particularly endometritis, is an important cause of repeat breeding. Clinical endometritis is characterized by mucopurulent discharge, whereas subclinical endometritis (SCE) occurs without visible discharge and is diagnosed mainly through cytological evidence of inflammation (Sheldon *et al.,* 2006; LeBlanc,

[1]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India

[2]Department of Veterinary Microbiology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

[3]Division of Animal Biotechnology, Gujarat Biotechnology University, Gandhinagar-382010, Gujarat, India

[4]Cattle Breeding Farm, Kamdhenu University, Junagadh-362001, Gujarat, India

Corresponding Author: Dr. Devesh J. Parmar, MVSc Scholar, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India. e-mail: parmadevesh56@gmail.com, drkkvala@gmail.com

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2008). Increased polymorphonuclear (PMN) leukocytes in endometrial cytology represent uterine inflammation and are linked with reduced conception and early embryonic loss (Kasimanickam *et al.,* 2004; Gilbert *et al.,* 2005). This decline in fertility has been associated with altered uterine pH, impaired immune tolerance, disturbed prostaglandin secretion and reduced endometrial gland function (Barlund *et al.,* 2008; Sheldon and Owens, 2017). Amongst various diagnostic techniques of SCE, cytobrush technique provides representative endometrial cells for PMN estimation with minimal invasiveness (Pothmann *et al.,* 2015). The Whiteside test (WST) is an economical field screening method based on leukocyte-associated colour change in cervico-vaginal mucus and can support cytology under farm conditions (Raja *et al.,* 2012; Bajaj *et al.,* 2016).

Antibiotics are commonly used for uterine infections; however, repeated use raises concerns regarding antimicrobial resistance (AMR) (WHO, 2014). Additional concerns include drug residues in milk/meat and alteration of the normal reproductive tract microbiota (OIE, 2016). Therefore, non-antibiotic alternatives are required for sustainable reproductive management (Sheldon and Owens, 2017). Probiotics, especially *Lactobacillus* spp., inhibit pathogens via production of lactic acid, hydrogen peroxide and bacteriocins and modulate mucosal immune responses (Otero and Nader-Macias, 2006; Lebeer *et al.,* 2010). In bovines, intrauterine or intravaginal probiotic *Lactobacillus plantarum* therapy has shown potential to inhibit uterine pathogens such as *E. coli*, *Staphylococcus* and *Trueperella pyogenes* thus reducing uterine inflammation, supporting restoration of uterine environment and improving fertility (Deng *et al.,* 2015; Suthar *et al.,* 2022; Gohil *et al.,* 2023; Nandaniya *et al.,* 2026). The present study was aimed to evaluate intrauterine *L. plantarum* infusion for treatment of SCE in repeat breeding Jaffarabadi buffaloes and compare it with intrauterine levofloxacin therapy using fertility indices, biochemical profile, progesterone levels and inflammatory markers.

Materials and Methods

The experimental protocol (No. KU-JVC-IAEC-LA-156-25) was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat (India). The investigation was undertaken from January to November 2025 at the Cattle Breeding Farm, Kamdhenu University, Junagadh, and adjoining areas. Out of 80 repeat-breeding buffaloes examined, 40 apparently healthy repeat breeding Jaffarabadi buffaloes, diagnosed with subclinical endometritis and 10 normal Jaffarabadi buffaloes (4-8 years old; >90 days postpartum) were enrolled in the study. The selected animals were kept under standard feeding and management practices practiced at the university farm as well as at farmers' field conditions. Each buffalo was subjected to a comprehensive rectal examination of the reproductive tract to rule out the presence of any congenital, hereditary, or acquired defects.

Diagnosis of SCE and Treatment

Subclinical endometritis (SCE) was diagnosed on the basis of the presence of clear cervico-vaginal discharge along with endometrial cytology obtained using the cytobrush technique, revealing ≥5% polymorphonuclear cells, according to the criteria described by Pothmann *et al.* (2015). The SCE-affected buffaloes were allocated into three treatment groups: Group A (n=15) received probiotic therapy (*Lactobacillus plantarum*, 10^{11} CFU/30 mL saline, intrauterine, single dose), Group B (n=15) received a sensitive antibiotic (Levofloxacin, 30 mL intrauterine, three times at 24 h intervals), and Group C (n=10) served as the positive control (normal saline 30 mL intrauterine, single dose). Additionally, 10 healthy buffaloes (Group D) were maintained as a negative control without any intervention for comparison. The treatments were administered during estrus following sample collection, and the animals were inseminated at the next estrus.

Blood Analysis

Blood samples were obtained from all animals at estrus (day 0), on day 5, and at the subsequent estrus using clot activator vacutainer tubes. Blood glucose levels were determined immediately after collection with a digital glucometer. Serum biochemical parameters, including glucose, urea, calcium, total protein, and progesterone, were analyzed using an automatic biochemical analyzer (Dia-Chem 240 Plus, China) with standard commercial diagnostic kits. The concentrations of the cytokine interleukin-10 and the acute phase protein serum amyloid-A were quantified using ELISA kits.

Statistical Analysis

The data were presented as Mean ± SE. Conception rates at the first, second, and third artificial inseminations (AI), as well as the overall conception rate, were compared using the Chi-square test. Blood parameters were statistically analyzed using one-way analysis of variance to evaluate the effects of treatment and sampling period. Duncan's multiple range test was employed as a post-hoc test for pairwise comparison of means, with the level of significance set at $p < 0.05$.

Results and Discussion

Blood Biochemical Profile

The group-wise findings on different blood biochemical parameters are presented in Table 1, which demonstrated significant variations in blood glucose concentrations between SCE affected and healthy buffaloes during different treatment periods. At estrus (day 0), SCE-affected buffaloes exhibited comparatively lower glucose levels than the healthy control group, suggesting an altered metabolic and inflammatory status associated with uterine infection.

Table 1: Blood biochemical changes before and after treatment in SCE affected Gir cows (Mean ± SE)

Parameters	Treatment groups	Before treatment	After Treatment	
		Day 0	Day 5	Next estrus
Blood glucose (mg/dL)	Gr A	73.40 ± 0.68^{Ab}	68.20 ± 0.55^{Ac}	79.10 ± 1.43^{Aa}
	Gr B	74.10 ± 0.82^{Ab}	69.40 ± 0.38^{Ac}	78.30 ± 2.01^{Aa}
	Gr C	70.60 ± 1.26^{Ab}	64.80 ± 0.44^{Ac}	72.90 ± 0.41^{Aa}
	Gr D	78.90 ± 1.72^{Aa}	76.10 ± 2.05^{Ba}	78.60 ± 2.48^{Aa}
Serum urea (mg/dL)	Gr A	32.10 ± 0.82^{Ba}	30.85 ± 0.74^{Bb}	29.95 ± 0.69^{Ac}
	Gr B	32.85 ± 0.88^{Ba}	31.40 ± 0.79^{Bb}	30.25 ± 0.72^{Ac}
	Gr C	31.95 ± 0.76^{Ba}	31.10 ± 0.73^{Bb}	30.80 ± 0.71^{Ac}
	Gr D	29.60 ± 0.70^{Aa}	29.85 ± 0.68^{Aa}	29.55 ± 0.66^{Aa}
Serum calcium (mg/dL)	Gr A	8.42 ± 0.11^{Ac}	8.86 ± 0.10^{Ab}	9.28 ± 0.09^{Aa}
	Gr B	8.38 ± 0.12^{Ac}	8.79 ± 0.11^{Ab}	9.18 ± 0.10^{Aa}
	Gr C	8.40 ± 0.10^{Ac}	8.44 ± 0.09^{Ab}	8.56 ± 0.08^{Aa}
	Gr D	9.12 ± 0.08^{Ba}	9.18 ± 0.07^{Ba}	9.15 ± 0.07^{Ba}
Serum total protein (g/dL)	Gr A	6.82 ± 0.14^{Ab}	6.95 ± 0.18^{Ab}	7.68 ± 0.16^{Ba}
	Gr B	6.75 ± 0.17^{Ab}	7.02 ± 0.21^{Ab}	7.54 ± 0.19^{Ba}
	Gr C	6.88 ± 0.12^{Aa}	6.80 ± 0.20^{Aa}	6.91 ± 0.15^{Aa}
	Gr D	7.42 ± 0.11^{Ba}	7.48 ± 0.09^{Ba}	7.40 ± 0.18^{Ba}
Serum progesterone (ng/mL)	Gr A	0.72 ± 0.06^{Ac}	1.45 ± 0.08^{Aa}	1.02 ± 0.07^{Ab}
	Gr B	0.75 ± 0.05^{Ac}	1.52 ± 0.09^{Aa}	1.18 ± 0.10^{Ab}
	Gr C	0.78 ± 0.07^{Ac}	1.40 ± 0.08^{Aa}	0.82 ± 0.09^{Ab}
	Gr D	1.10 ± 0.06^{Ba}	2.05 ± 0.11^{Ba}	1.55 ± 0.10^{Ba}
Serum IL-10 (pg/mL)	Gr A	58.42 ± 1.78^{Aa}	49.86 ± 1.42^{Ab}	38.95 ± 1.26^{Ac}
	Gr B	56.91 ± 1.64^{Aa}	48.73 ± 1.55^{Ab}	37.84 ± 1.31^{Ac}
	Gr C	55.68 ± 1.52^{Aa}	54.21 ± 1.83^{Aa}	52.96 ± 1.94^{Aa}
	Gr D	44.12 ± 1.47^{Ba}	43.26 ± 1.39^{Ba}	42.75 ± 1.41^{Ba}
Serum amyloid A (µg/mL)	Gr A	26.80 ± 0.65^{Aa}	23.90 ± 0.50^{Ab}	19.60 ± 0.45^{Ac}
	Gr B	30.90 ± 0.72^{Aa}	28.10 ± 0.60^{Ab}	22.40 ± 0.52^{Ac}
	Gr C	27.95 ± 0.88^{Aa}	27.40 ± 0.55^{Aa}	27.80 ± 0.60^{Aa}
	Gr D	3.40 ± 0.22^{Ba}	3.35 ± 0.20^{Ba}	3.30 ± 0.18^{Ba}

Gr A= Probiotic, Gr B = Sensitive Antibiotic, Gr C= Positive control, Gr D =Negative control; Means with different superscripts within a row (a, b, c) and within a column (A, B) differ significantly (p<0.05).Top of Form

A further decline in glucose concentration was observed on day 5 in the SCE groups, but at the subsequent estrus, a significant ($p<0.05$) increase was recorded in the probiotic and antibiotic treated groups, whereas the positive control group showed only slight improvement, reflecting limited natural recovery in untreated animals. The healthy control group maintained relatively stable glucose values throughout the experimental period. The reduction in blood glucose - hypoglycaemia - during SCE may be attributed to release of pro-inflammatory cytokines, and impaired hepatic gluconeogenesis leading to negative energy balance, which adversely affects follicular growth, ovulation, and luteal function, ultimately predisposing buffaloes to subfertility. The significant post-treatment rise in glucose levels in the therapeutic groups suggests restoration of uterine health, improved metabolic status, and normalization of endocrine function. Similar findings have been reported earlier by Singh *et al.* (2018), Chaudhari *et al.* (2020), and Nandaniya *et al.* (2026).

The group-wise serum urea concentrations (Table 1) showed comparatively higher values in SCE-affected buffaloes of Group A, B and C at day 0, than the healthy control Group D. A gradual decline was observed on day 5 in the treated groups, and at the subsequent estrus a significant ($p<0.05$) reduction occurred in Group A and Group B, whereas Group C showed only a slight decrease and Group D remained stable throughout, indicating better metabolic recovery following treatment. The higher pre-treatment urea levels in SCE-affected buffaloes may be due to increased protein catabolism and metabolic stress associated with uterine inflammation, while the post-treatment decline suggests improved nitrogen utilization, restoration of metabolic balance, and recovery of uterine health. Similar trends of elevated serum urea in endometritic animals and normalization after treatment have been reported by Sheldon *et al.* (2009), Sabasthin *et al.* (2012), Gahlot *et al.* (2018) and Nandaniya *et al.* (2026).

The group-wise serum calcium and total protein concentrations (Table 1) revealed comparatively lower values in SCE-affected buffaloes of Group A, B and C at day 0, than the healthy negative control Group D. A progressive and significant ($p<0.05$) increase in serum calcium and total protein levels was observed on day 5 and at the subsequent estrus in the treated groups, reaching 9.28±0.09 and 7.68±0.16 mg/dL in Group A and 9.18±0.10 and7.54±0.19 mg/dL in Group B, respectively, whereas Group C showed only a marginal rise and Group D remained stable throughout the study period. The lower pre-treatment calcium levels in SCE-affected buffaloes indicate disturbed mineral metabolism and metabolic stress associated with uterine inflammation, while the post-treatment improvement suggests restoration of calcium homeostasis, better uterine contractility, and recovery of immune function. Similar trend pre- and post-treatment of SCE have been reported by Sheldon *et al.* (2009), Sabasthin *et al.* (2012), Martinez *et al.* (2012), Singh *et al.* (2014), and Nandaniya *et al.* (2026) supporting the present findings. The relatively lower pre-treatment total protein in SCE-affected buffaloes may be attributed to increased protein catabolism and diversion of amino acids toward immune and inflammatory processes during chronic uterine infection. The post-treatment increase in total protein in probiotic and antibiotic groups indicates restoration of metabolic balance, improved hepatic protein synthesis, and recovery of uterine health. Similar post-treatment improvement in serum total protein levels in endometritic buffaloes has also been reported by Sharma (2011), Singh *et al.* (2014) and Nandaniya *et al.* (2026).

Hormonal and Immunological Profile

The group-wise serum progesterone concentrations (Table 1) revealed comparatively lower levels in SCE-affected buffaloes at day 0, indicating impaired luteal function and altered ovarian–uterine interactions associated with subclinical uterine inflammation. Following treatment, progesterone levels showed a gradual increase in the probiotic and antibiotic treated groups at day 5 and at the subsequent estrus, whereas the positive control group exhibited only marginal improvement and the negative control group maintained relatively stable values throughout the observation period. The reduced pre-treatment progesterone concentration in SCE-affected buffaloes may be attributed to inflammatory mediators, endotoxins, and increased prostaglandin release from the infected endometrium, which induce premature luteolysis and compromise corpus luteum function. The post-treatment rise in progesterone levels in the therapeutic groups suggests restoration of luteal activity, improved uterine environment, and normalization of reproductive cyclicity. Similar reductions in progesterone levels in endometritic cows and buffaloes and their improvement following effective treatment have been reported by Sheldon *et al.* (2009), Sharma (2011) and Nandaniya *et al.* (2026).

The group-wise interleukin-10 (IL-10) and serum amyloid-A (SAA) concentrations presented in Table 1 showed comparatively lower IL-10 and higher SAA levels in SCE-affected buffaloes at day 0, reflecting an active inflammatory and immunomodulatory response associated with subclinical uterine infection. Following treatment, IL-10 concentrations exhibited a significant ($p<0.05$) increase and SAA showed a significant ($p<0.05$) decline in the probiotic and antibiotic treated groups at day 5 and at the subsequent estrus, whereas the positive control group showed only a slight change and the negative control group maintained relatively stable values throughout the study period. The comparatively lower or dysregulated IL-10 levels before treatment in SCE-affected buffaloes may be attributed to persistent uterine inflammation and impaired anti-inflammatory immune regulation. IL-10 is a key anti-inflammatory cytokine that suppresses excessive inflammatory responses, promotes endometrial repair, and supports restoration of uterine homeostasis. The post-treatment rise in IL-10 levels in the therapeutic groups indicates reduced inflammatory stress, improved immune modulation, and recovery of uterine health. Similar increases in anti-inflammatory cytokines following successful treatment of uterine infections have been reported by Sheldon *et al.* (2009), Ahmed *et al.* (2017), El-Sayed *et al.* (2020) and and Nandaniya *et al.* (2026). The elevated pre-treatment SAA concentration in SCE-affected buffaloes may be attributed to systemic inflammatory stress and hepatic acute-phase protein synthesis triggered by persistent uterine inflammation. Serum amyloid-A is a sensitive biomarker of inflammation and tissue injury, and its increased levels indicate activation of the innate immune response. The marked post-treatment reduction in SAA levels in the therapeutic groups suggests alleviation of inflammatory stress, resolution of uterine infection, and restoration of uterine health. Similar elevations of acute-phase proteins, including SAA, in endometritic buffaloes and their decline following effective treatment have been reported by Sheldon *et al.* (2009), Gahlot *et al.* (2018), El-Sayed *et al.* (2020), and Nandaniya *et al.* (2026) thereby supporting the present findings.

Conception Rates

In the present study, the conception rate of SCE affected buffaloes was evaluated up to the third post-treatment insemination. The first service conception rate was highest in the probiotic-treated group (40.00%, 6/15), followed by the antibiotic-treated group (33.33%, 5/15), negative control (30.00%, 3/10), and positive control group (20.00%, 2/10), with non-significant inter-group differences ($\chi^2=0.60$, $p=0.89$). At the second service, conception rates declined across groups but remained numerically higher in the probiotic group (22.22%, 2/9) compared to antibiotic (20.00%, 2/10), negative control (14.29%, 1/7), and positive control (12.50%, 1/8) groups (χ^2=0.26, p=0.97), indicating gradual fertility recovery in SCE-affected buffaloes. The third service conception rate was comparatively low, with 28.57% (2/7) in the probiotic group, 12.50% (1/8) in the antibiotic group, 16.67% (1/6) in the negative control, and no conception in the positive control group (χ^2=0.20, p=0.57), suggesting limited additional fertility gain beyond subsequent inseminations. The cumulative conception rate up to the third service was highest in the probiotic-treated group (66.67%, 10/15), followed by the antibiotic-treated (53.33%, 8/15), negative control (50.00%, 5/10), and positive control group (30.00%, 3/10), though differences were statistically non-significant (χ^2=3.26, p=0.35). These findings indicate that probiotic therapy resulted in a numerically superior and sustained improvement in conception rate of SCE-affected buffaloes, likely due to enhanced uterine immunity, improved endometrial receptivity, and restoration of uterine microflora, which is in agreement with earlier reports in repeat-breeding bovines (Kumar *et al.,* 2020; Hashem *et al.,* 2024; Nandaniya *et al.,* 2026).

Conclusions

The present study concludes that subclinical endometritis (SCE) in Jaffarabadi buffaloes significantly alters biochemical, hormonal, immunological, and reproductive parameters, resulting in reduced fertility and repeat breeding. SCE-affected buffaloes exhibited lower blood glucose, calcium, total protein, and progesterone levels along with higher serum urea and SAA concentrations, indicating metabolic stress, inflammation, and impaired uterine function. Treatment with probiotic *Lactobacillus plantarum* and sensitive antibiotic improved metabolic balance, immune response, and uterine health, as evidenced by normalization of biochemical parameters, increased IL-10 and progesterone levels, and reduced inflammatory markers. The probiotic (*Lactobacillus plantarum*) treated group showed the highest cumulative conception rate (66.67%) compared to the antibiotic (53.33%), negative control (50.00%), and positive control (30.00%) groups, indicating a better and sustained fertility response. Overall, probiotic *Lactobacillus plantarum* proved to be a safe, sustainable, and effective therapeutic alternative for improving uterine health and conception rate in subclinical endometritis affected repeat-breeding Jaffarabadi buffaloes.

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