

Effect of Intrauterine Probiotic Therapy on Fertility, Biochemical and Immunological Parameters in Subclinical Endometritis Affected Repeat Breeding Gir Cows

Nirbhay R. Nandaniya^{1*}, Suryakant S. Parikh¹, Ramesh J. Padodara², Karsan B. Vala¹, Vishal S. Suthar³, Mulraj D. Odedara⁴

ABSTRACT

The present study was undertaken to evaluate the effect of probiotic therapy on fertility performance and associated biochemical and immunological parameters in subclinical endometritis (SCE) affected repeat breeding Gir cows. SCE affected cows (n=10 each) were allotted into probiotic (*Lactobacillus plantarum*, 10¹¹ cfu/30 mL saline, I/U once), antibiotic (ciprofloxacin 30 mL, I/U, thrice at 24 h interval), positive control (normal saline, 30 mL I/U once), and healthy control (no intervention) groups. Fertility response was assessed through conception rate up to three inseminations, while biochemical and immunological changes were evaluated at estrus before treatment, on day 5, and at subsequent estrus. The cumulative conception rate over three inseminations was highest in the probiotic treated group (70.00%), followed by healthy control (60.00%) and ciprofloxacin treated group (50.00%), whereas the untreated SCE group recorded the lowest conception rate (20.00%). Probiotic treated cows showed significant improvement (p<0.05) in blood glucose, total protein, calcium, and progesterone concentrations, along with a significant reduction (p<0.05) in serum urea levels. Immunological evaluation revealed a significant decrease (p<0.05) in serum interleukin-10 and serum amyloid-A concentrations in probiotic and antibiotic treated groups, indicating reduced uterine inflammatory response. The probiotic group consistently exhibited better biochemical recovery and fertility performance compared to untreated SCE cows. The study concludes that intrauterine probiotic therapy using *Lactobacillus plantarum* significantly improves reproductive efficiency, metabolic status, and inflammatory profile in subclinical endometritis affected repeat breeding Gir cows.

Key words: Antibiotic, Biochemical profile, Fertility, Gir cows, Probiotic, Subclinical endometritis.

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INTRODUCTION

The success of the dairy industry largely relies on maintaining the proper and optimal reproductive rhythm for each animal in the herd where repeat breeding causes disruption of rhythm causing financial loss. Repeat breeder cows are those with normal or nearly normal estrus and estrous cycles but fail to conceive after three or more inseminations without any detectable abnormalities in the genital tract (Parkinson, 2019). Among many reasons one is subclinical endometritis (SCE), also known as cytological endometritis, is identified by an elevated percentage of polymorpho-nuclear cells (PMNs) in endometrial cytology samples (Shivhare *et al.*, 2018). Among repeat breeder cows, reported SCE prevalence ranges from 12.70% up to 40.20% (Pothmann *et al.*, 2015). Following parturition, the structural and immune barriers of the reproductive tract weaken, allowing opportunistic pathogens to invade the uterus and establish infections, where current treatments of SCE mostly include antimicrobials and prostaglandin (PGF₂α). However, the reproductive performance was neither improved by antibiotics nor PGF₂α (Dobos *et al.*, 2022). Untargeted antibiotic use, without prior identification of the causative organisms and their susceptibility profiles, frequently leads to

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

²Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India

³Animal Biotechnology Division, Gujarat Biotechnology University, Gandhinagar-382010, Gujarat, India

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

Corresponding Author: Dr. Nirbhay R. Nandaniya, MVSc Scholar, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh-362001, Gujarat, India e-mail: nirbhay1421@gmail.com, drss.parikh@gmail.com

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therapeutic failure and fosters the development of multidrug-resistant strains (Iancu *et al.*, 2025).

The probiotic based approach offers a promising strategy to address the rise in antibiotic resistant bacteria. Probiotics are live microorganisms that, when administered under appropriate conditions, provide benefits to the host where use of different probiotic strains has been proposed to prevent postpartum uterine infections and inflammation (Genis *et al.*, 2017). Based on the known immune-regulatory properties of *Lactobacillus* (LAB) in the animal genital tract, the increased abundance of *Lactobacillus* in cows that were successfully treated with LAB or antibiotics, suggests that *Lactobacillus* may play a key role in immune regulation within the genital tract of healthy cows (Yang *et al.*, 2021). Here the study evaluated effect of probiotic treatment compared with other regimes giving insights on serum biochemical, serum progesterone and immunological profile before and after treatment in SCE affected Gir cows.

MATERIALS AND METHODS

The experimental protocol (No. KU-JVC-IAEC-LA-155-25) was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, KU, Junagadh. The study was carried out over a period of eight months, from February to September, 2025 at the Cattle Breeding Farm, Kamdhenu University, Junagadh, and in nearby areas of Junagadh. A total of 30 apparently healthy repeat breeder cows with SCE and 10 normal Gir cows, aged between 4 and 8 years, more than 90 days postpartum were selected from the farm and surrounding regions for inclusion in the study. The selected animals were managed under the routine feeding and management practices followed either at the University farm or at the farmers' field conditions. Each cow underwent thorough rectal examination of the reproductive tract to rule out any congenital, hereditary, or acquired abnormalities.

Diagnosis of SCE and Treatment

The SCE was diagnosed based on the presence of clear cervicovaginal discharge along with endometrial cytology obtained by cytobrush technique showing $\geq 5\%$ polymorphonuclear cells, as per the criteria described by Pothmann *et al.* (2015). SCE affected cows were divided into three treatment groups, each of 10 animals, *viz.*, Group A: probiotic (*Lactobacillus plantarum*, 10^{11} cfu/30 mL saline, I/Ut, once), Group B: sensitive antibiotic (ciprofloxacin, 30 mL I/Ut, thrice, at 24 h interval), and Group C: positive control (NSS 30 mL I/Ut, once), and one group of 10 healthy cows served as negative control Group D (no intervention) for comparison. These animals were treated during the estrus after sampling, and then inseminated at subsequent estrus.

Blood Analysis

Blood samples were collected from each animal at estrus (day 0), day 5 and at subsequent estrus using clot activator vacutainer tubes. Blood glucose concentration was measured immediately after sample collection using a digital glucometer. Serum biochemical parameters, *viz.*, glucose, urea, calcium, total protein, progesterone, were analyzed using an automatic biochemical analyzer (Dia-Chem 240 Plus, China) with standard commercial diagnostic kits. The cytokine interleukin-10 and acute phases protein serum amyloid-A were estimated using ELISA kits.

Statistical Analysis

The results were expressed as Mean \pm SE. The conception rates at first, second and third AI and overall were compared by using Chi-square test. Statistical evaluation on blood parameters was performed using one-way analysis of variance to assess the effects of treatment and sampling period. Duncan's multiple range test was applied as a *post-hoc* test for pairwise comparison of means at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Blood Biochemical Profile

The group wise findings on different blood biochemical parameters are presented in Table 1, where blood glucose concentrations differed significantly among SCE affected and healthy Gir cows across treatment periods. At day 0, glucose values ranged from 67.33 ± 1.41 mg/dL in Group C to 79.17 ± 1.85 mg/dL in Group D, with SCE-affected groups showing significantly lower concentrations than healthy controls. A significant decline was observed on day 5 in Groups A, B and C, reflecting metabolic stress associated with uterine inflammation. At subsequent estrus, glucose level increased significantly ($p < 0.05$) in Group A to 80.80 ± 1.51 mg/dL and in Group B to 78.20 ± 2.13 mg/dL, whereas Group C showed only a modest rise to 69.66 ± 0.33 mg/dL, indicating limited spontaneous recovery. Group D maintained stable values throughout. The abrupt decline in glucose during active infection indicates negative energy balance caused by reduced feed intake, inflammatory cytokine release, and impaired hepatic gluconeogenesis. Glucose acts as a metabolic signal regulating GnRH and LH secretion; hence, hypoglycaemia directly compromises ovulation and luteal development, predisposing cows to infertility. The marked post-treatment rise in glucose in probiotic and antibiotic groups reflects restoration of uterine health, improved energy partitioning, and normalization of endocrine signaling. Similar associations between low glucose and SCE have been reported by Sarkar *et al.* (2015), Chaudhari *et al.* (2020), Parikh (2021) and Vadher *et al.* (2025) supporting the present findings.



Table 1: Blood biochemical changes before and after treatment in SCE affected Gir cows (Mean±SE, n=10)

Parameters	Treatment groups	Before treatment	After treatment	
		Day 0	Day 5	Next estrus
Blood glucose (mg/dL)	Gr A	70.90±0.67 ^{Ab}	64.10±0.52 ^{Aa}	80.80±1.51 ^{Bc}
	Gr B	71.70±0.87 ^{Ab}	65.50±0.34 ^{Aa}	78.20±2.13 ^{Bc}
	Gr C	67.33±1.41 ^{Ab}	58.50±0.42 ^{Aa}	69.66±0.33 ^{Ab}
	Gr D	79.17±1.85 ^{Ba}	75.17±2.12 ^{Ba}	80.00±3.36 ^{Ba}
Serum urea (mg/dL)	Gr A	36.69±0.67 ^{Bc}	34.60±0.44 ^{Bb}	31.71±0.37 ^{Ba}
	Gr B	37.53±0.47 ^{Bc}	34.71±0.36 ^{Bb}	32.84±0.25 ^{Ba}
	Gr C	36.63±0.21 ^{Ba}	36.32±0.21 ^{Ca}	35.95±0.19 ^{Ca}
	Gr D	29.42±0.22 ^{Aa}	30.20±0.29 ^{Aa}	29.12±0.19 ^{Aa}
Serum calcium (mg/dL)	Gr A	9.34±0.08 ^{Aa}	9.98±0.08 ^{Bb}	10.58±0.07 ^{Ba}
	Gr B	9.43±0.05 ^{Aa}	10.21±0.05 ^{Bb}	11.05±0.04 ^{Cc}
	Gr C	9.26±0.08 ^{Aa}	9.33±0.06 ^{Aa}	9.39±0.06 ^{Aa}
	Gr D	11.01±0.03 ^{Ba}	10.20±0.04 ^{Ba}	11.17±0.05 ^{Ba}
Serum total protein (g/dL)	Gr A	7.31±0.16 ^{Aa}	7.40±0.26 ^{Aa}	8.14±0.18 ^{Bb}
	Gr B	7.21±0.18 ^{Aa}	7.52±0.32 ^{Aa}	8.04±0.15 ^{Bb}
	Gr C	7.38±0.10 ^{Aa}	7.18±0.27 ^{Aa}	7.27±0.06 ^{Aa}
	Gr D	8.00±0.10 ^{Ba}	8.15±0.05 ^{Aa}	7.95±0.22 ^{Ba}
Serum progesterone (ng/mL)	Gr A	0.88±0.21 ^{Aa}	1.71±0.01 ^{Ab}	1.17±0.09 ^{Ac}
	Gr B	0.90±0.03 ^{Aa}	1.70±0.06 ^{Ab}	1.30±0.12 ^{Ab}
	Gr C	0.90±0.08 ^{Aa}	1.60±0.06 ^{Ab}	0.95±0.12 ^{Aa}
	Gr D	1.3±0.01 ^{Ba}	2.47±0.01 ^{Bb}	1.78±0.02 ^{Bc}
Serum IL-10 (pg/mL)	Gr A	65.87±2.16 ^{Aa}	60.90±1.43 ^{Ab}	53.45±1.52 ^{Ac}
	Gr B	63.95±1.65 ^{Aa}	60.24±1.65 ^{Ab}	52.79±1.74 ^{Ac}
	Gr C	63.21±1.33 ^{Aa}	66.03±2.96 ^{Aa}	64.14±3.72 ^{Ba}
	Gr D	54.13±1.52 ^{Ba}	52.11±1.33 ^{Ba}	53.15±1.22 ^{Aa}
Serum amyloid A (µg/mL)	Gr A	27.51±0.63 ^{Ac}	24.89±0.48 ^{Ab}	19.79±0.44 ^{Aa}
	Gr B	31.60±0.76 ^{Ac}	29.01±0.64 ^{Ab}	22.77±0.48 ^{Ba}
	Gr C	28.55±0.92 ^{Aa}	27.88±0.54 ^{Ab}	28.50±0.53 ^{Ca}
	Gr D	18.20±0.68 ^{Ba}	19.05±0.35 ^{Ba}	18.27±0.47 ^{Aa}

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

Serum urea concentrations were significantly higher in SCE affected cows before treatment compared to healthy controls. Following treatment, serum urea declined progressively in Group A (31.71±0.37 mg/dL) and Group B (32.84±0.25 mg/dL) at subsequent estrus, whereas Group C showed minimal change (35.95±0.19 mg/dL)(Table 1). Elevated urea reflects increased muscle proteolysis and inefficient utilization of dietary protein during inflammatory stress. High blood urea negatively alters uterine pH, interferes with sperm viability, oocyte maturation, and early embryo development, thereby reducing fertility. The post-treatment decline indicates restoration of protein metabolism and improved rumen energy-protein synchrony. Similar elevated urea profiles in endometritic cows were reported by Kaya *et al.* (2016) and Chaudhari *et al.* (2020), while variability across studies has also been highlighted by Barrio *et al.* (2015).

Serum calcium levels were significantly lower in SCE affected cows compared with healthy controls on day 0.

At subsequent estrus, it increased significantly in Group A (10.58±0.07 mg/dL) and Group B (11.05±0.04 mg/dL), while Group C remained almost unchanged (9.39±0.06 mg/dL). Calcium is essential for uterine muscle tone, immune cell activation, hormone receptor sensitivity and intracellular signalling. Hypocalcaemia reduces uterine contractility, delays involution, and weakens local defence mechanisms, predisposing cows to persistent uterine infection and infertility. Restoration of calcium following treatment improves neuromuscular excitability, oxytocin sensitivity and endometrial repair. Similar reductions in calcium in SCE cows were reported by Das *et al.* (2012), Chaudhari *et al.* (2020) and Parikh (2021) and Vadher *et al.* (2025) confirming the present observations.

The mean serum total protein concentrations in SCE affected Gir cows before treatment was significantly lower than in healthy cows of Group D (Table 1). At day 5 post-treatment, no significant change was observed in Groups

A, B and C. However, at the subsequent estrus, a significant increase ($p < 0.05$) in serum total protein concentration was recorded in the probiotic treated Group A (8.14 ± 0.18 g/dL) and sensitive antibiotic treated Group B (8.04 ± 0.15 g/dL), which became comparable to the healthy negative control Group D (7.95 ± 0.22 g/dL). In contrast, the positive control Group C failed to show any appreciable improvement and maintained relatively lower values (7.27 ± 0.06 g/dL), indicating persistence of metabolic and inflammatory stress. The lower pre-treatment protein levels in SCE cows may be attributed to altered hepatic protein synthesis, increased utilization of amino acids for immune responses, and enhanced vascular permeability associated with uterine inflammation. Similar observations were reported by Prakash *et al.* (2018), Nischala and Sireesha (2018) and Vallejo-Timaran *et al.* (2020). Therapeutic interventions reduce pro-inflammatory cytokines, improve hepatic protein synthesis, and normalize albumin and globulin fractions, thereby elevating total protein concentration. The persistence of lower protein values in untreated Group C in the present study confirms that subclinical uterine inflammation exerts a continuous negative influence on systemic protein metabolism. Reduced serum total protein compromises immune competence, antibody production, and tissue repair, thereby prolonging uterine pathology and contributing directly to infertility. The significant improvement in probiotic and antibiotic treated groups suggests that correction of uterine inflammation restores metabolic stability, enhances immune recovery, and supports uterine repair mechanisms essential for successful conception.

Hormonal and Immunological Profile

Progesterone concentrations remained consistently low in SCE affected cows on day 0, while Group D recorded a higher value of 1.30 ± 0.01 ng/mL. At day 5, progesterone increased significantly in Group B (1.70 ± 0.06 ng/mL) and Group D (2.47 ± 0.01 ng/mL), but Group C showed limited change. At subsequent estrus, progesterone declined again in Group C (0.95 ± 0.12 ng/mL), whereas treated groups maintained comparatively improved levels (Table 1). Progesterone is essential for luteal maintenance, uterine receptivity and embryo survival. Suppressed progesterone reflects impaired luteal development due to inflammatory mediators affecting ovarian steroidogenesis. Chronic progesterone deficiency leads to poor conception, early embryonic loss and repeat breeding. Similar depressed progesterone in SCE cows has been reported by Perumal *et al.* (2020), Mohames *et al.* (2024) and Nanas *et al.* (2025).

Serum IL-10 concentrations were markedly elevated in SCE affected cows, indicating persistent inflammatory modulation. In Group A, IL-10 declined from 65.87 ± 2.16 pg/mL at day 0 to 53.45 ± 1.52 pg/mL at subsequent estrus. Group B showed a similar reduction from 63.95 ± 1.65 to 52.79 ± 1.74 pg/mL. In contrast, Group C maintained persistently high values (63.21 – 66.03 pg/mL), indicating unresolved inflammation.

IL-10 is an anti-inflammatory cytokine that suppresses excessive immune reactions; however, persistent elevation reflects chronic inflammatory stimulation in SCE. High IL-10 inhibits macrophage and neutrophil activity, delaying uterine clearance of pathogens and prolonging infertility. The decline after treatment indicates normalization of immune regulation and uterine recovery. Similar trends were reported by Biswal *et al.* (2014), Brodzki *et al.* (2015) and Ahmadi *et al.* (2018).

Serum amyloid-A concentrations (SAA) showed a highly sensitive response to treatment compared to pre-treatment values in SCE cows. In Group A, SAA declined significantly from 27.51 ± 0.63 μ g/mL at day 0 to day 5 and further to 19.79 ± 0.44 μ g/mL at the next estrus. Group B followed a similar pattern, conversely, Group C showed no meaningful reduction, with values remaining around 28.50 ± 0.53 μ g/mL across all stages, while Group D maintained consistently low concentrations near 18.20 – 19.05 μ g/mL (Table 1). SAA is an acute phase protein synthesized by hepatocytes and endometrial cells in response to inflammatory cytokines. Its rapid elevation indicates active uterine inflammation, while its decline reflects tissue recovery. Persistently high SAA in untreated cows confirms continuous inflammatory insult to the endometrium, leading to altered uterine micro-environment, impaired embryo survival, and repeat breeding. SAA also modulates cholesterol transport, platelet activation, and immune cell chemotaxis, linking metabolic stress to reproductive failure. Biswal *et al.* (2014), Parikh (2021) and Vadher *et al.* (2025) similarly reported significantly elevated SAA in SCE cows and marked reductions after successful therapy, confirming SAA as a reliable biomarker of uterine inflammation and fertility prognosis.

Conception Rates

The conception rates of Gir cows treated for SCE at the first service showed that the probiotic treated group (Group A) recorded the highest conception rate (40.00%), followed by the negative control (30.00%), antibiotic treated group (20.00%), while no conception occurred in the positive control group. However, the differences among groups were statistically non-significant ($\chi^2 = 3.60$, $p = 0.31$). These findings were comparable with earlier reports in healthy and spontaneously recovered cows, where conception rates ranged between 32–36% (Barlund *et al.*, 2008). During the second service, conception rates remained similar among groups ($\chi^2 = 0.89$, $p = 0.83$), with 20.00% conception in Groups A, B and D, and 10.00% in Group C. At the third service, all groups recorded only 10.00% conception, showing no significant difference ($\chi^2 = 0.41$, $p = 0.94$), indicating limited additional fertility gain beyond the second insemination. The overall conception rate up to the third service was highest in the probiotic group (70.00%), followed by the negative control (60.00%), antibiotic (50.00%) and positive control (20.00%) group, though these differences were statistically non-significant ($\chi^2 = 2.21$, $p = 0.53$). The results of probiotic group closely resembled the conception rates reported by



Suthar *et al.* (2022) and Gohil *et al.* (2023), supporting the beneficial role of probiotics in improving uterine immunity and fertility. The antibiotic group showed lower conception than earlier reports for ciprofloxacin-based therapies (Bhat *et al.*, 2013; Kumar *et al.*, 2014), possibly due to increasing antimicrobial resistance as highlighted by Vadher *et al.* (2025) and Khalifa *et al.* (2025).

The moderate conception rate in the negative control group reflects natural uterine recovery, whereas the poor performance of the positive control group confirms the detrimental effect of untreated SCE on fertility (Parikh, 2021). Overall, both probiotic and antibiotic therapies improved conception performance in SCE affected cows, with probiotics showing a numerically superior advantage. The ability of probiotics to enhance uterine defence without contributing to antimicrobial resistance supports their potential as a sustainable alternative therapy for managing SCE in repeat breeding Gir cows.

CONCLUSIONS

The present study demonstrated that intrauterine probiotic *Lactobacillus plantarum* therapy produced the highest overall conception rate (70.00%) in SCE affected Gir cows, exceeding that of antibiotic therapy (50.00%) and untreated controls (20.00%), and approaching the fertility level of healthy cows (60.00%). Probiotic treatment significantly improved metabolic, endocrine, and immunological profiles, as reflected by increased blood glucose, serum total protein, calcium, and progesterone concentrations, along with marked reductions in serum urea, interleukin-10, and serum amyloid-A levels at subsequent estrus. These changes indicate effective restoration of uterine health, immune balance, and reproductive function. The pronounced decline in serum amyloid-A further confirms its value as a reliable biomarker for monitoring SCE. Overall, probiotic therapy offers a safe, effective, and sustainable alternative to antibiotics for the management of SCE, with the added advantage of avoiding antimicrobial resistance and promoting long-term reproductive efficiency in Gir cows.

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