RESEARCH ARTICLE

Comparative Evaluation of Cytobrush, Whiteside Test and Antimicrobial Sensitivity in Subclinical Endometritis of Repeat Breeding Gir Cows

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Abstract

The present study was undertaken to evaluate the diagnostic efficacy of cytobrush technique and Whiteside test for detection of subclinical endometritis (SCE) in repeat breeding cows, along with bacterial isolation and antimicrobial sensitivity pattern. A total of 98 repeat breeding cows were screened. Cytobrush examination revealed an incidence of 30.61% SCE, whereas Whiteside test detected 24.49% cases. Considering cytobrush as gold standard, Whiteside test showed sensitivity of 80.00% and specificity of 100%. Mean PMN count was significantly higher in SCE affected cows compared to healthy animals. Bacteriological examination revealed *Staphylococcus* spp. (36.67%) as predominant organism followed by *Escherichia coli* (26.67%), *Streptococcus* spp. (16.67%), *Corynebacterium* spp. (6.67%), and *Bacillus* spp. (6.67%). Antibiotic sensitivity testing showed maximum sensitivity to ciprofloxacin followed by gentamicin and levofloxacin. The study concluded that cytobrush technique is more reliable diagnostic tool, whereas Whiteside test can be used as field screening method. Antibiotic therapy should be guided by antimicrobial sensitivity testing to ensure effective treatment and minimize resistance.

Key words: Antibiotic sensitivity, Cytobrush, Repeat breeding, Subclinical endometritis, Whiteside test.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.4.20

Introduction

Repeat breeding syndrome is a major cause of economic loss in dairy animals due to decreased fertility and increased calving interval. One of the hidden causes of this syndrome, apart from endocrine, nutritional and breeding management strategies, is subclinical uterine infection, jeopardising the survival of gametes and/or zygote. Subclinical endometritis (SCE) is one of the most important hidden causes of infertility, characterized by elevated polymorphonuclear (PMN) cell count in the endometrium without obvious clinical signs. Accurate diagnosis is essential for proper management. The Cytobrush technique is widely accepted as a reliable method for diagnosis of SCE based on PMN cell percentage. On the other hand, Whiteside test is a simple, rapid and economical field diagnostic method based on detection of leukocytes in cervical mucus. However, bacterial infection plays a crucial role in SCE and inappropriate use of antibiotics without sensitivity testing contributes to antimicrobial resistance (Bazzazan *et al.,* 2025) and failure of outcomes. Therefore, the present study was designed to compare cytobrush and Whiteside test for diagnosis of SCE and to evaluate bacterial isolates along with their antimicrobial sensitivity pattern in repeat breeding 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, Gujarat (India). The study was conducted on 98 repeat breeding cows maintained under field conditions in and around Junagadh, Gujarat. Ethical approval was obtained prior to conducting the study. Endometrial cytology samples were collected using cytobrush technique. Samples showing ≥5% polymorphonuclear (PMN) cells were considered positive for SCE. Whiteside test was performed by mixing cervical mucus with equal volume of 5% sodium hydroxide and heating the mixture to observe color change (Kumar *et al.,* 2015).

For microbiological examination, 30 samples of SCE affected cows were considered along with 10 healthy animals. The samples were cultured on Brain Heart Infusion (BHI) agar

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How to cite this article: Nandaniya, N. R., Parikh, S. S., Vala, K. B., Ramoliya, U. B., & Parmar, D. J. (2026). Comparative Evaluation of Cytobrush, Whiteside Test and Antimicrobial Sensitivity in Subclinical Endometritis of Repeat Breeding Gir Cows. Ind J Vet Sci and Biotech, 22(4), 108-112.

Source of support: Nil

Conflict of interest: None

Submitted 12/04/2026 **Accepted** 30/04/2026 **Published** 10/07/2026

and isolates were identified using standard bacteriological techniques (Quinn *et al.,* 1994). Antimicrobial sensitivity testing was performed using disc diffusion method on Mueller-Hinton agar, and zone of inhibition was measured following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2014).

Statistical Analysis

The diagnostic techniques employed for the detection of SCE were compared based on their sensitivity and specificity. The data generated during the study were summarized as Mean ± SE using descriptive statistical procedures. The cytobrush technique was considered the reference (gold standard) test for diagnosing SCE due to its high sensitivity (Se) and specificity (Sp). The statistical analysis was performed using the Vassar Stat online statistical software. The difference was considered statistically significant if the p-value was <0.05. Sensitivity, specificity, positive and negative predictive values as well as disease prevalence were expressed as percentages.

Results and Discussion

Incidence of Subclinical Endometritis and Diagnostic Comparison

In the present study, the incidence of SCE diagnosed by cytobrush technique was 30.61%, whereas the Whiteside test detected 24.49% cases (Table 1). The higher detection rate obtained through cytobrush indicates its superior sensitivity, as it directly evaluates endometrial cytology and quantifies polymorphonuclear (PMN) cell infiltration. These findings were in close agreement with recent reports documenting SCE prevalence ranging from 28 to 36% using cytobrush-based diagnosis (Kumar *et al.,* 2023).

The Whiteside test exhibited 80.00% sensitivity and 100% specificity with a high agreement (κ=0.88) when compared with cytobrush technique (Table 2). The Whiteside test detects leukocyte-derived myeloperoxidase and other oxidative enzymes in cervical mucus, which oxidize hydroxide and organic constituents, producing the characteristic yellow to brown discoloration on heating, whereas cytobrush sampling directly contacts the endometrial surface, allowing collection of epithelial cells and infiltrating neutrophils from the site of inflammation rather than from the uterine lumen. The high specificity suggests that the Whiteside test is highly reliable in identifying true negative animals, while its relatively lower sensitivity indicates that a proportion of mild or localized infections may remain undetected. This limitation may be attributed to its indirect mechanism, which relies on leukocyte-induced color change in cervical mucus and is influenced by mucus consistency, contamination, and stage of estrus (Mandhwani *et al.,* 2017). Thus, while cytobrush remains the gold standard for accurate diagnosis, the Whiteside test can serve as a rapid and economical field screening tool for identifying cows requiring further cytological evaluation.

PMN Cell Population and its Role in Infertility

The mean PMN cell percentage was significantly higher in SCE affected cows (8.21±0.51%, n=30) compared to healthy cows (2.34±0.61%, n=68) (p<0.001), confirming active uterine inflammation. The threshold of ≥5% PMNs used in the present study is widely accepted for diagnosing SCE and aligns with several recent studies (Rashid *et al.,* 2025; Pande *et al.,* 2025). The elevated PMN levels reflect activation of innate immune response triggered by bacterial invasion, where neutrophils migrate into the endometrium under the influence of pro-inflammatory cytokines such as IL-1, TNF-α, and chemokines. Although this response is essential for pathogen clearance, persistent PMN infiltration release reactive oxygen species, matrix metalloproteinases, and elastase; sustained exposure damages the epithelial barrier, alters expression of adhesion molecules and growth factors, and impairs endometrial remodeling required for embryo implantation. From a reproductive perspective, this inflammatory environment adversely affects sperm transport, oocyte fertilization, and embryo survival. Increased PMNs also alter uterine secretions and reduce endometrial receptivity, thereby contributing to repeat breeding. Similar associations between elevated PMN levels and reduced fertility have been reported by Rashid *et al.* (2025). Therefore, PMN percentage serves as a reliable and objective indicator of uterine health and fertility status in repeat breeding cows.

Bacterial Isolation and Uterine Microbial Dynamics

Bacteriological examination of the uterine swabs from 30 SCE affected cows revealed *Staphylococcus* spp. (36.67%) as the predominant isolate, followed by *Escherichia coli* (26.67%), *Streptococcus* spp. (16.67%), *Corynebacterium* spp. (6.67%), and *Bacillus* spp. (6.67%), while 2 6.67%) showed

Table 1: Incidence of SCE diagnosed by cytobrush and Whiteside test and their relative efficacies

Sr. No.	Diagnostic techniques	Total number of cows examined	Cows with SCE	Cows with SCE (%)	Relative efficacy (%)
1.	Cytobrush	98	30	30.61	100.00
2.	Whiteside test		24	24.49	80.00

Table 2: Sensitivity and specificity of Whiteside test against cytobrush technique as a gold standard in diagnosis of SCE

Diagnostic technique	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa	Likelihood ratio	
						LR+	LR-
Whiteside test	80.00	100	100	91.89	0.88	∞	0.20

no growth (Table 3). In contrast, healthy cows showed lower bacterial prevalence parallel to SCE cows with 40% of samples exhibiting no growth, indicating effective uterine defence mechanism. The predominance of *Staphylococcus* spp. and *E. coli* in SCE affected cows is consistent with earlier and recent reports identifying these organisms as major opportunistic uterine pathogens (Behera *et al.,* 2015; Nath *et al.,* 2024). These bacteria typically gain entry into the uterus during or after parturition when physical and immunological barriers are compromised. Pathogen-induced inflammation leads to disruption of uterine homeostasis through endotoxin production, biofilm formation, and activation of inflammatory pathways. *E. coli*, in particular, produces lipopolysaccharides that stimulate strong immune responses, while *Staphylococcus* spp. can persist through biofilm formation, making infections chronic and difficult to eliminate (Ozturkler, 2025).

Recent studies have further suggested that SCE is associated with microbial dysbiosis rather than infection by a single pathogen, where imbalance in uterine microbiota leads to persistence of inflammation and impaired uterine recovery (Rashid *et al.,* 2025; Pande *et al.,* 2025). The significantly higher bacterial load observed in SCE cows compared to healthy animals in the present study clearly supports the role of microbial invasion and imbalance in the pathogenesis of subclinical endometritis.

Antimicrobial Sensitivity Pattern and Resistance Trends

The antimicrobial susceptibility pattern of isolates revealed that ciprofloxacin was the most effective antibiotic, showing 85.29% overall sensitivity. However this value is slightly lower than the higher sensitivity ranges (85-95%) reported in earlier studies by Chacko *et al.* (2020), Nath *et al.* (2024), Khalifa *et al.* (2025), and Ozturkler *et al.* (2025), possibly reflecting gradual resistance development due to repeated field use of fluoroquinolones. This was followed by gentamicin (70.59%) and levofloxacin (61.76%), the sensitivity observed in this study falls within a moderate range. It is somewhat lower than the 70-82% reported by Nath *et al.* (2024) and Iancu *et al.* (2025), but higher than the 32-40% reported by Dutt *et al.* (2017) and Manjhi *et al.* (2019). Levofloxacin sensitivity (61.76%), although appreciable, was also lower than the values exceeding 80% reported by Parikh (2021), further suggesting a declining trend in the effectiveness of fluoroquinolones. In contrast, oxytetracycline and ampicillin/sulbactam exhibited the lowest sensitivity (47.06%). The findings revealed that fluoroquinolones and aminoglycosides demonstrated relatively higher effectiveness against uterine pathogens. Ceftizoxime exhibited moderate efficacy (55.88%), followed by chloramphenicol (52.94%) and cefoperazone (50.00%). Importantly, none of the antibiotics tested achieved complete sensitivity across all isolates, indicating a diverse and partially resistant bacterial population within the uterus of repeat breeding Gir cows (Table 4). Among individual isolates, both *Staphylococcus* spp. and *E. coli* showed maximum sensitivity to ciprofloxacin, indicating the continued efficacy of fluoroquinolones against uterine pathogens. These findings are consistent with earlier reports, where ciprofloxacin demonstrated high therapeutic success in endometritis cases (Bhat *et al.,* 2013; Kumar *et al.,* 2014).

Table 3: Different bacteria isolated from healthy and SCE affected Gir cows

Sr. No.	Bacterial Isolates	SCE affected cows (n=30) (%)	Healthy cows (n=10) (%)	Overall (n=40) (%)
1	*Staphylococcus* spp.	11 (36.67)	3 (30.00)	14 (35.00)
2	*E. coli*	8 (26.67)	2 (20.00)	10 (25.00)
3	*Streptococcus* spp.	5 (16.67)	1 (10.00)	6 (15.00)
4	*Corynebacterium* spp.	2 (6.67)	0 (0)	2 (5.00)
5	*Bacillus* spp.	2 (6.67)	0 (0)	2 (5.00)
6	No growth	2 (6.67)	4 (40.00)	6 (15.00)
	Total	**30 (100)**	**10 (100)**	**40 (100)**

Table 4: Overall susceptibility of isolated bacteria from SCE cows to different antibiotics

Bacterial species	Total isolates	No. of isolates sensitive to							
		Oxytetra-cycline	Gen-tamicin	Chloram-phenicol	Levoflox-acin	Cefopera-zone	Ceftizox-ime	Ampicillin + Sulbactam	Ciprofloxa-cin
Staphylococcus	14	7	10	8	9	8	9	7	12
E. coli	10	4	6	5	6	4	5	4	8
Streptococcus	6	3	4	3	4	3	3	3	5
Corynebacterium	2	1	2	1	1	1	1	1	2
Bacillus	2	1	2	1	1	1	1	1	2
Total	**34**	**16**	**24**	**18**	**21**	**17**	**19**	**16**	**29**
% Sensitivity	—	**47.06**	**70.59**	**52.94**	**61.76**	**50.00**	**55.88**	**47.06**	**85.29**

However, the reduced sensitivity compared to earlier studies suggests a gradual decline in antibiotic efficacy, likely due to indiscriminate and repeated use of antimicrobials under field conditions. The moderate sensitivity observed for most antibiotics indicates the emergence of multidrug resistance among uterine pathogens, a trend increasingly reported in recent literature (Iancu *et al.,* 2025). From a mechanistic perspective, antimicrobial resistance develops through genetic mutations, horizontal gene transfer, and biofilm-associated protection, which allow bacteria to survive antibiotic exposure. This not only reduces treatment success but also prolongs uterine inflammation and infertility.

Drug susceptibility testing of individual bacterial isolates (Table 5) indicated that *Staphylococcus* spp., the most frequently isolated organism, exhibited the highest sensitivity to ciprofloxacin (78.57%), followed by gentamicin (71.43%) and levofloxacin (64.29%). A moderate response was observed with ceftizoxime (57.14%), whereas comparatively lower sensitivity was noted for oxytetracycline and ampicillin-sulbactam (42.86% each). These results suggest that *Staphylococcus* associated uterine infections are still largely responsive to fluoroquinolones; however, the occurrence of resistant strains highlights the need for treatment decisions based on antibiogram findings.

In the case of *Escherichia coli*, ciprofloxacin again demonstrated the greatest effectiveness (70.00%), while gentamicin and levofloxacin showed equal sensitivity (60.00% each). The remaining antibiotics ceftizoxime, cefoperazone, chloramphenicol, oxytetracycline, and ampicillin-sulbactam displayed relatively low sensitivity (40.00% each), indicating reduced susceptibility and suggesting the development of multidrug resistance among *E. coli* isolates recovered from the uterus. *Streptococcus* spp. showed the highest sensitivity to ciprofloxacin (83.33%), followed by gentamicin and levofloxacin (66.67% each). Intermediate responses (50.00% each) were recorded for ceftizoxime, cefoperazone, and chloramphenicol, while lower sensitivity was observed with oxytetracycline and ampicillin-sulbactam (33.33% each). In contrast, *Corynebacterium* spp. and *Bacillus* spp. exhibited a consistent but moderate sensitivity pattern (50.00%) across all tested antibiotics, indicating variability and uncertainty in therapeutic outcomes for these opportunistic pathogens (Table 5). These observation concurred well with the previous reports already cited above, suggesting a declining trend in the effectiveness of fluoroquinolones.

Conclusion

The present study confirmed that SCE is an important cause of repeat breeding in Gir cows, with an incidence of 30.61% based on cytobrush technique, a more sensitive and reliable diagnostic method than Whiteside test

Table 5: Antibiotic sensitivity pattern of uterine bacterial isolates

Bacteria → Antibiotics ↓		***Staphylococcus* spp. (14)**	***E. coli* (10)**	***Streptococcus* spp. (6)**	***Corynebacterium* spp. (2)**	***Bacillus* spp. (2)**
Ciprofloxacin	S	11 (78.57%)	7 (70.0%)	5 (83.33%)	1 (50.0%)	1 (50.0%)
	I	1 (7.14%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	2 (14.29%)	2 (20.0%)	1 (16.67%)	1 (50.0%)	1 (50.0%)
Ceftizoxime	S	8 (57.14%)	5 (50.0%)	3 (50.0%)	1 (50.0%)	1 (50.0%)
	I	2 (14.29%)	1 (10.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	4 (28.57%)	4 (40.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)
Gentamicin	S	10 (71.43%)	6 (60.0%)	4 (66.67%)	1 (50.0%)	1 (50.0%)
	I	2 (14.29%)	1 (10.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	2 (14.29%)	3 (30.0%)	1 (16.67%)	1 (50.0%)	1 (50.0%)
Cefoperazone	S	7 (50.0%)	4 (40.0%)	3 (50.0%)	1 (50.0%)	1 (50.0%)
	I	2 (14.29%)	2 (20.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	5 (35.71%)	4 (40.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)
Chloramphenicol	S	7 (50.0%)	4 (40.0%)	3 (50.0%)	1 (50.0%)	1 (50.0%)
	I	2 (14.29%)	2 (20.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	5 (35.71%)	4 (40.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)
Oxytetracycline	S	6 (42.86%)	4 (40.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)
	I	3 (21.43%)	1 (10.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	5 (35.71%)	5 (50.0%)	3 (50.0%)	1 (50.0%)	1 (50.0%)
Ampicillin/ Sulbactam	S	6 (42.86%)	4 (40.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)
	I	3 (21.43%)	2 (20.0%)	1 (16.67%)	0 (0.0%)	0 (0.0%)
	R	5 (35.71%)	4 (40.0%)	3 (50.0%)	1 (50.0%)	1 (50.0%)
Levofloxacin	S	9 (64.29%)	6 (60.0%)	4 (66.67%)	1 (50.0%)	1 (50.0%)
	I	2 (14.29%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	3 (21.43%)	3 (30.0%)	2 (33.33%)	1 (50.0%)	1 (50.0%)

(24.49%). A significantly higher PMN count in SCE-affected cows compared to healthy cows indicates its strong diagnostic value for uterine inflammation and fertility status. *Staphylococcus* spp. and *E. coli* were the predominant bacterial isolates, with higher efficacy of ciprofloxacin, gentamicin, and levofloxacin. Overall, cytobrush-based diagnosis combined with bacteriological examination and sensitivity testing is essential for effective management of SCE and improving reproductive performance in repeat breeding cows, and antibiotic therapy should not be empirical but must be guided by bacteriological culture and antimicrobial sensitivity testing to ensure effective treatment and to minimize the development of resistance.

Acknowledgement

Authors are grateful to the authorities of Kamdhenu University and Cattle Breeding Farm, Junagadh for the support and facilities provided.

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