RESEARCH ARTICLE

Comparative Evaluation of Whiteside Test, Endometrial Cytology and Uterine Lavage Optical Density for Diagnosis of Endometritis in Postpartum Crossbred Cows

Kunal Devlekar[1], Sandeep Gaikwad*[1], Nilesh Dagli[1], Ravindra Chaudhari[1], Nidhi Shingatgeri[1], Rajesh Pharande[2]

Abstract

The present study was conducted to evaluate Whiteside test (WST), endometrial cytology and uterine lavage sample optical density (ULSOD) for diagnosis of endometritis in postpartum crossbred cows. A total of 34 cows between 30-45 days in milk were screened under field conditions. Endometrial cytology samples collected using cytobrush technique were examined for polymorphonuclear neutrophil (PMN) cells, and samples with ≥10% PMNs were considered positive. Whiteside test was performed using cervico-vaginal mucus and 5% sodium hydroxide solution, while uterine lavage samples were analysed spectrophotometrically at 620 nm. Optical density (OD) values ≥0.052 were considered positive for uterine inflammation. The incidence of endometritis recorded by cytology, WST and ULSOD was 73.53, 73.53 and 58.82%, respectively. Based on WST, 23.53, 35.29 and 14.71% cows showed mild, moderate and strong positive reactions, respectively. Sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and kappa value for ULSOD were 72, 77, 90, 50, 73.52% and 0.46, respectively, while corresponding values for WST were 92, 77.8, 92, 77.8, 88.23% and 0.72. The findings indicated that endometrial cytology remains a reliable diagnostic method, while WST serves as a rapid and economical field screening test and ULSOD provides an objective quantitative assessment of uterine inflammation.

Keywords: Cytology, Endometritis, Postpartum cows, ULSOD, Whiteside test.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.5.06

Introduction

Maintenance of optimal reproductive efficiency in dairy animals is essential for sustaining dairy productivity and profitability. Postpartum uterine disorders, particularly metritis and endometritis, are among the major reproductive problems affecting dairy cattle worldwide. These disorders adversely affect fertility, prolong calving-to-conception interval and increase economic losses due to repeat breeding and reduced milk production (LeBlanc, 2008; Galvão, 2018). During the postpartum period, bacterial contamination of the uterus is common; however, the normal uterine defence mechanisms generally eliminate invading microorganisms. Failure of uterine clearance results in persistent inflammation of the endometrium leading to clinical or subclinical endometritis (Sheldon *et al.,* 2006). Endometritis interferes with ovarian cyclicity and reproductive performance through the action of inflammatory mediators and bacterial toxins, which impair luteinizing hormone secretion, follicular development and steroidogenesis (Sheldon *et al.,* 2002). Early diagnosis of uterine inflammation is therefore essential for timely therapeutic intervention and improvement of reproductive efficiency in dairy herds.

Several diagnostic techniques have been employed for detection of endometritis in cattle. Whiteside test (WST) is a rapid, inexpensive and practical cow-side diagnostic method based on colour reaction produced by interaction between sodium hydroxide and cellular components present in cervico-vaginal mucus. The test has been successfully used for detection of nonspecific uterine infections and subclinical endometritis under field conditions (Gupta *et al.,* 2011; Bhat *et al.,* 2014). Endometrial cytology is considered one of the most reliable methods for diagnosis of subclinical endometritis based on the proportion of polymorphonuclear neutrophils (PMNs) present in endometrial samples, and is widely used in both research and field practice (Kasimanickam *et al.,* 2004; Dubuc *et al.,* 2010; de Boer *et al.,* 2015). However, cytology

[1]Department of Animal Reproduction, Gynaecology and Obstetrics, Mumbai Veterinary College, Parel, Mumbai-400012, MAFSU, Maharashtra, India

[2]Department of Veterinary Microbiology, Mumbai Veterinary College, Parel, Mumbai-400012, MAFSU, Maharashtra, India

Corresponding Author: Dr. Sandeep Gaikwad, Department of Animal Reproduction, Gynaecology and Obstetrics, Mumbai Veterinary College, Parel, Mumbai-400012, MAFSU, Maharashtra, India. e-mail: sandeepgaikwad@mafsu.ac.in

How to cite this article: Devlekar, K., Gaikwad, S., Dagli, N., Chaudhari, R., Shingatgeri, N., & Pharande, R. (2026). Comparative Evaluation of Whiteside Test, Endometrial Cytology and Uterine Lavage Optical Density for Diagnosis of Endometritis in Postpartum Crossbred Cows. Ind J Vet Sci and Biotech, 22(5), 28-31.

Source of support: Nil

Conflict of interest: None

Submitted 20/05/2026 **Accepted** 29/06/2026 **Published** 10/09/2026

© The Author(s). 2026 Open Access This work is licensed under a Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International License.

requires sample preparation, staining and microscopic evaluation, which may limit its field applicability. Recently, uterine lavage sample optical density (ULSOD) has emerged as a quantitative and objective diagnostic approach for assessment of uterine inflammation. Machado *et al.* (2012) demonstrated that optical density of uterine lavage fluid measured at 620 nm was significantly higher in cows with endometritis and showed good diagnostic sensitivity and specificity. Considering the importance of early diagnosis of postpartum uterine inflammation and the need for practical field-level diagnostic methods, the present study was undertaken to comparatively evaluate Whiteside test, endometrial cytology and uterine lavage sample optical density for diagnosis of endometritis in postpartum crossbred cows.

Material and Methods

Experimental Animals

The present study was conducted on 34 postpartum crossbred cows maintained under field conditions. Cows between 30 and 45 days in milk (DIM) with history of normal calving and without any systemic illness were included in the investigation. Animals were screened irrespective of visible clinical signs in order to include both apparently healthy and suspected cases of endometritis.

Whiteside Test

Cervico-vaginal mucus samples were collected aseptically and equal volumes of mucus and 5% sodium hydroxide (NaOH) solution were mixed in a clean test tube. The mixture was heated until boiling and subsequently allowed to cool. Development of yellow to dark brown colour was considered indicative of uterine inflammation. Depending upon intensity of colour change, reactions were categorized as mild, moderate or strong positive (Bhat *et al.,* 2014).

Endometrial Cytology

Endometrial samples were collected aseptically using cytobrush technique. The collected material was rolled onto clean glass slides, air dried and stained using Leishman's stain. Stained smears were examined microscopically under oil immersion objective and at least 100 cells were counted for determination of polymorphonuclear neutrophil (PMN) percentage. Samples containing ≥10% PMNs were considered positive for endometritis as described by Kasimanickam *et al.* (2004).

Uterine Lavage Sample Optical Density

Uterine lavage samples were collected by low-volume uterine flushing technique using approximately 20 mL sterile normal saline. The recovered lavage fluid was processed and optical density was measured spectrophotometrically at 620 nm. Samples with OD values ≥0.052 were considered positive for uterine inflammation as per Machado *et al.* (2012).

Statistical Analysis

The prevalence of endometritis was determined based of results of different tests as per cent frequency. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, likelihood ratio and kappa coefficient were calculated for Whiteside test and ULSOD using endometrial cytology as the reference diagnostic method.

Results and Discussion

A total of 25 out of 34 cows (73.53%) showed positive reaction to Whiteside test, indicating uterine inflammation. Based on colour intensity, 23.53, 35.29 and 14.71% cows exhibited mild, moderate and strong positive reactions, respectively, while 26.47% animals showed negative reaction (Table 1, Fig. 1). The colour reaction observed during WST is attributed to interaction of sodium hydroxide with leukocytic cellular components present in cervico-vaginal mucus, reflecting inflammatory exudation. Previous workers including Raja *et al.* (2012), Purohit *et al.* (2013) and Bhat *et al.* (2014) reported incidence of endometritis ranging from 62.8 to 72.4% using WST in cattle, which corroborates the present findings. Lower incidences reported by Zarekar (2014) and Guruvandana *et al.* (2025) may be attributed to differences in postpartum stage, herd management, diagnostic interpretation and selection criteria. In the present investigation, moderate to strong WST reactions corresponded more closely with clinically significant uterine inflammation.

Table 1: Endometritis based on Whiteside test in postpartum crossbred cows

Whiteside Test Reaction	No. of cows	Per cent
Negative (No colour change)	9	26.47
Mild (Light yellow colour)	8	23.53
Moderate (Distinct yellow colour)	12	35.29
Strong (Deep yellow to brown colour)	5	14.71
Total	**34**	**100**

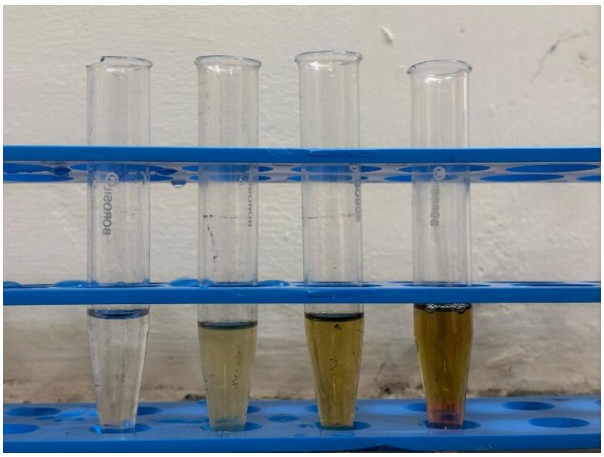


Fig. 1: Whiteside test reactions in postpartum cows (1 – No colour change, 2 – Mild colour change, 3 – Moderate colour change and 4 – Strong colour change)

Endometrial cytology revealed that 25 out of 34 cows (73.53%) had ≥10% PMN cells and were considered positive for cytological endometritis (Fig. 2). The PMN threshold of ≥10% was selected based on the recommendations of Kasimanickam *et al.* (2004), who demonstrated its association with impaired reproductive performance in postpartum cows. The present findings were comparable with those of Gilbert *et al.* (2005), Oral *et al.* (2009), and Singh *et al.* (2016), who reported prevalence of subclinical endometritis ranging from 51.2 to 80.64% using cytological evaluation. However, lower incidences have been reported by Dubuc *et al.* (2010), Madoz *et al.* (2014), Barrio *et al.* (2015) and Pascottini (2016). Such variation among studies may be attributed to differences in postpartum period at examination, herd management, environmental conditions, sampling technique and PMN cut-off values. Cytology remains one of the most dependable diagnostic methods because it provides direct evidence of inflammatory cell infiltration within the uterine endometrium. In the present study, Papanicolaou staining showed superior nuclear clarity and cellular differentiation compared to Leishman staining, particularly in borderline inflammatory cases.

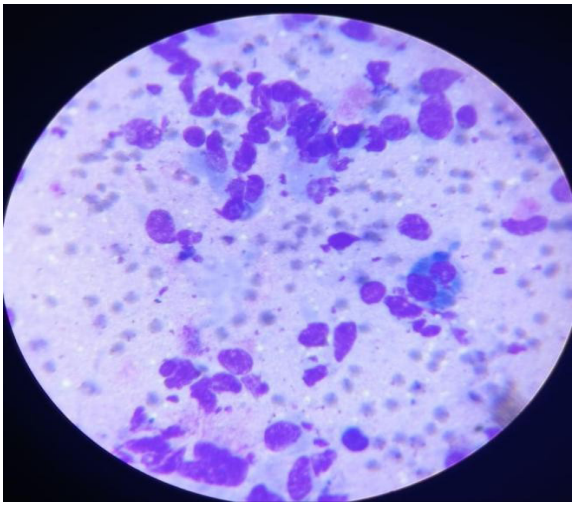


Fig. 2: Cytological evaluation of uterine samples collected using cytobrush technique.

Uterine lavage sample optical density measured at 620 nm identified 20 out of 34 cows (58.82%) as positive for endometritis using an OD cut-off value of 0.052 with sensitivity, specificity and Youden index of 0.72, 0.77 & 0.49, respectively (Table 2). Increased optical density reflects greater turbidity caused by inflammatory exudate, cellular debris, leukocytes and bacterial contamination present within the uterine lumen. The cut-off value of 0.052 was selected because it yielded the highest Youden Index, indicating the best balance between sensitivity and specificity. This value was also biologically relevant as it closely corresponded with the threshold reported by Machado *et al.* (2012), who demonstrated that ULSOD measured at 620 nm had superior diagnostic accuracy compared to other wavelengths. The wavelength of 620 nm was selected because Machado *et al.* (2012) observed the highest area under the ROC curve at this wavelength, suggesting greater discriminatory ability for detection of uterine inflammation. Increased absorbance at 620 nm likely reflects elevated concentrations of inflammatory cells, proteins and bacterial products in uterine lavage fluid.

Table 2: Sensitivity and specificity of different optical density cut-off values

OD Cut-off	Sensitivity	Specificity	Youden Index
0.051	0.88	0.44	0.32
0.052	0.72	0.77	0.49
0.054	0.68	0.77	0.45
0.060	0.52	0.88	0.40
0.070	0.44	0.88	0.32

When compared with cytology as the reference diagnostic test, ULSOD demonstrated sensitivity of 72%, specificity of 77%, positive predictive value of 90%, negative predictive value of 50% and diagnostic accuracy of 73.52%. The kappa value of 0.46 indicated moderate agreement between ULSOD and cytology (Table 3).

Whiteside test exhibited higher sensitivity (92%) and substantial agreement with cytology (κ=0.72), indicating that it can serve as an effective field-level screening tool for detection of endometritis (Table 3). Similar observations were made by Habibzadeh *et al.* (2016) and Parikh *et al.* (2022). The high sensitivity observed in WST suggests its usefulness in identifying positive animals rapidly under field conditions, whereas ULSOD provides a more objective and quantitative assessment of uterine inflammation.

A progressive increase in mean optical density values was observed with increasing intensity of Whiteside test reaction, indicating greater inflammatory activity with increasing severity of uterine infection, which concurred with Habibzadeh *et al.* (2016). All samples showing negative WST reaction exhibited OD values below the diagnostic threshold, demonstrating good agreement between the two methods in identifying healthy animals.

Table 3: Diagnostic performance of ULSOD and Whiteside test (WST) against cytobrush cytology

Diagnostic technique	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic efficacy	Kappa (κ)	Likelihood ratio	
							LR+	LR-
ULSOD	72.0%	77.0%	90.0%	50.0%	73.52%	0.46	3.13	0.36
WST	92.0%	77.8%	92.0%	77.8%	88.23%	0.72	4.14	0.10

Conclusion

The present study demonstrated that postpartum endometritis was highly prevalent in crossbred cows between 30 and 45 DIM. Endometrial cytology remained the most reliable diagnostic method for detection of uterine inflammation. Whiteside test exhibited high sensitivity and substantial agreement with cytology, indicating its suitability as a rapid and economical field-level screening test. Uterine lavage sample optical density measured at 620 nm provided a quantitative and objective assessment of uterine inflammation with moderate agreement to cytological findings. The combined use of Whiteside test, cytology and ULSOD may improve early diagnosis and management of postpartum endometritis, thereby enhancing reproductive efficiency in dairy cattle.

Acknowledgement

The authors are thankful to the Department of Animal Reproduction, Gynaecology and Obstetrics, Mumbai Veterinary College for providing necessary facilities and support to carry out the present investigation.

References

Barrio, M., Vigo, M., Quintela, L.A., Becerra, J.J., García-Herradón, P.J., Martínez-Bello, D., Sánchez, F., Prieto, A., Cainzos, J., & Peña, A.I. (2015). Influence of subclinical endometritis on the reproductive performance of dairy cows. *Spanish Journal of Agricultural Research, 13*(4), e05SC01.

Bhat, F.A., Bhattacharyya, H.K., & Hussain, S.A. (2014). White side test: A simple and rapid test for evaluation of nonspecific bacterial genital infections of repeat breeding cattle. *Veterinary Research Forum*, *5*(3), 177-180.

de Boer, M., Buddle, B.M., Heuer, C., Hussein, H., Zheng, T., LeBlanc, S.J., & McDougall, S. (2015). Associations between intrauterine bacterial infection, reproductive tract inflammation and reproductive performance in pasture-based dairy cows. *Theriogenology*, *83*(9), 1514-1524.

Dubuc, J., Duffield, T.F., Leslie, K.E., Walton, J.S., & LeBlanc, S.J. (2010). Definitions and diagnosis of postpartum endometritis in dairy cows. *Journal of Dairy Science*, *93*(11), 5225-5233.

Galvão, K.N. (2018). Uterine diseases in dairy cows: understanding the causes and seeking solutions. *Animal Reproduction*, *10*(3), 228-238.

Gilbert, R.O., Shin, S.T., Guard, C.L., Erb, H.N., & Frajblat, M. (2005). Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, *64*(9), 1879-1888.

Gupta, J.P., Shyma, K.P., Mohandas, A.C., & Sneharaj, R.K. (2011). Use of Whiteside test to study subclinical endometritis in crossbred cattle. *Bhartiya Krishi Anusandhan Patrika*, *26*(3-4), 123-125.

Guruvandana, P., Abdul Azeez, C.P., Chacko, L., Lekshmi Bhai, K., Pramod, S. and Prasad, A. (2025). Occurrence of subclinical endometritis and oestrus characteristics in repeat breeder cows. *Journal of Veterinary & Animal Sciences, 56*(1), 60-67.

Habibzadeh, F., Habibzadeh, P., & Yadollahie, M. (2016). On determining the most appropriate test cut-off value: The case of tests with continuous results. *Biochemia Medica*, *26*(3), 297-307.

Kasimanickam, R., Duffield, T.F., Foster, R.A., Gartley, C.J., Leslie, K.E., Walton, J.S., & Johnson, W.H. (2004). Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, *62*(1-2), 9-23.

LeBlanc, S.J. (2008). Postpartum uterine disease and dairy herd reproductive performance: A review. *Veterinary Journal*, *176*(1), 102-114.

Machado, V.S., Knauer, W.A., Bicalho, M.L.S., Oikonomou, G., Gilbert, R.O., & Bicalho, R.C. (2012). A novel diagnostic technique to determine uterine health of Holstein cows at 35 days postpartum. *Journal of Dairy Science*, *95*(3), 1349-1357.

Madoz, L.V., Giuliodori, M.J., Migliorisi, A.L., Jaureguiberry, M., & de la Sota, R.L. (2014). Endometrial cytology, biopsy and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science*, *97*(1), 195-201.

Oral, H., Sozmen, M., Serin, G., & Kaya, S. (2009). Comparison of the cytobrush technique, vaginoscopy and transrectal ultrasonography methods for the diagnosis of postpartum endometritis in cows. *Journal of Animal and Veterinary Advances*, *8*(7), 1252-1255.

Parikh, S.S., Kavani, F.S., Parmar, K.H., Patbandha, T.K., Singh, V.K., Ahlawat, A.R., & Kumar, R. (2022). Diagnostic and therapeutic management of subclinical endometritis in dairy bovine: A review. *Animal Reproduction Update, 2*(2), 1-11.

Pascottini, O.B. (2016). Subclinical endometritis in dairy cattle: A practical approach. *Doctoral Dissertation,* Ghent University, Belgium.

Purohit, G.N., Dholpuria, S., Yadav, S., Barolia, Y., & Kumar, P. (2013). Diagnosis and treatment of clinical and sub-clinical endometritis: A clinical study in 30 cows. *Intas Polivet*, *14*(1), 31-33

Raja, S., Devanathan, T.G., Kulasekar, K., Pazhanivel, N., & Balachandran, C. (2012). Whiteside test and endometrial biopsy for diagnosis of endometritis in repeat breeding cows. *Indian Journal of Animal Reproduction*, *33*(1), 56-58.

Sheldon, I.M., Noakes, D.E., Rycroft, A.N., Pfeiffer, D.U., & Dobson, H. (2002). Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction*, *123*(6), 837-845.

Sheldon, I.M., Lewis, G.S., LeBlanc, S., & Gilbert, R.O. (2006). Defining postpartum uterine disease in dairy cattle. *Theriogenology*, *65*(8), 1516-1530.

Singh, J., Honparkhe, M., Chandra, M., Kumar, A., Ghuman, S.P.S., & Dhindsa, S.S. (2016). Diagnostic efficacy of uterine cytobrush technique for subclinical endometritis in crossbred dairy cattle. *Indian Veterinary Journal*, *93*(11), 11-13.

Zarekar, A.U. (2014). Diagnosis and treatment of repeat breeder crossbred cows with modified approaches. *Master's Thesis*. Maharashtra Animal and Fishery Sciences University, Nagpur, India.