RESEARCH ARTICLE

Diagnostic Efficacy of White Side Test and Endometrial Cytology for Detection of Endometritis in Repeat Breeding Mehsana Buffaloes of Banaskantha District, Gujarat

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Abstract

This study evaluated the diagnostic efficacy of the Whiteside test and endometrial cytology for detecting endometritis in repeat-breeding Mehsana buffaloes. A total of 112 repeat-breeding Mehsana buffaloes from Banaskantha District, Gujarat, were screened for endometritis during estrus. Cervico-vaginal mucus samples were tested using the Whiteside test, and endometrial cytological samples were collected with a cytobrush and stained with Field's stain. Buffaloes with ≥8% polymorphonuclear neutrophils (PMNs) in endometrial cytology were considered positive for endometritis. The Whiteside test detected endometritis in 26 of 112 buffaloes (23.21%), whereas endometrial cytology identified 35 positive cases (31.25%). The relative diagnostic efficacy of the Whiteside test was 74.28% compared with endometrial cytology. Among WST-positive buffaloes, 42.31% exhibited mild, 34.61% moderate, and 23.08% severe endometritis. The mean PMN percentage was significantly higher ($p<0.05$) in buffaloes with endometritis (11.04±2.13%) than in healthy buffaloes (2.50±1.65%). Endometrial cytology detected more subclinical endometritis cases than the Whiteside test. Endometrial cytology technique proved to be a more sensitive and reliable method for detecting subclinical endometritis in repeat breeding Mehsana buffaloes, whereas the White side test served as a rapid, economical, and practical field screening tool. The combined application of both techniques can facilitate early diagnosis and improve reproductive management under field conditions.

Key words: Buffalo, Endometrial cytology, Endometritis, Repeat breeding, Polymorphonuclear neutrophils, White Side Test.

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Introduction

Endometritis is defined as inflammation of the uterine endometrium without systemic illness and is among the most common postpartum uterine disorders affecting fertility in cattle and buffaloes (Sheldon *et al*., 2009; Mohammed, 2024). In buffaloes, the reported incidence ranges from 2.5% to 32% (Kulkarni *et al*., 2002). Based on clinical presentation, endometritis is classified as clinical or subclinical (Sheldon *et al*., 2006). Despite the absence of obvious clinical signs, subclinical endometritis adversely affects reproductive performance by reducing conception rates, increasing the number of services per conception, and prolonging the calving interval (Kasimanickam *et al*., 2004). Endometritis is a major cause of reduced fertility, increased treatment costs, and substantial economic losses in dairy animals (Hanafi *et al*., 2008; Sheldon *et al*., 2009). Therefore, early and accurate diagnosis is essential for timely therapeutic intervention and improved reproductive performance.

Several diagnostic techniques, including rectal palpation, vaginoscopy, ultrasonography, uterine bacteriology, the White side test (WST), and endometrial cytology, have been used to detect uterine inflammation (Kasimanickam *et al*., 2004; Channa *et al*., 2023). Among these, the White side test is a simple, rapid, inexpensive, and field-applicable screening method, whereas endometrial cytology using the cytobrush technique is considered a reliable diagnostic tool for subclinical endometritis by assessing polymorphonuclear neutrophils (PMNs). Although a PMN threshold of >5% during estrus is generally accepted for diagnosing subclinical

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endometritis in buffaloes, threshold values ranging from 4% to 18% have been reported, depending on the study design and stage of the reproductive cycle (Kasimanickam *et al*., 2004; Singh *et al*., 2016; Gahlot *et al*., 2017; Mohammed, 2024). Despite the widespread use of the White side test and endometrial cytology, comparative data on their diagnostic efficacy in repeat breeding Mehsana buffaloes under field conditions remain limited. Therefore, the present study was undertaken to evaluate the diagnostic efficacy of the White side test and endometrial cytology in detecting endometritis in repeat breeding Mehsana buffaloes of Banaskantha District, Gujarat, and to assess their utility as practical diagnostic tools for field application.

Materials and Methods

Animals and Experimental Design

A total of 112 repeat breeding Mehsana buffaloes presented for infertility treatment at the Veterinary Clinical Complex, Deesa; Livestock Research Station, Sardarkrushinagar; and clinical camps conducted in different villages of Banaskantha District, Gujarat, were included in the study. All animals underwent a detailed gynaecological examination. Per-rectal examination was performed to rule out hereditary and acquired abnormalities of the female reproductive tract. Buffaloes free from gross reproductive tract abnormalities were screened for endometritis during estrus using the White side test (WST) and endometrial cytology.

White Side Test (WST)

Cervico-vaginal discharge was collected aseptically from each repeat breeding buffalo into sterile containers and transported immediately to the laboratory. White Side Test (WST) was conducted as per the method of elaborated by Kumar *et al.* (2015). The severity of endometritis was graded according to the intensity of the colour as described by Kumar *et al*. (2015): no color, negative; light yellow color, subclinical endometritis; clear yellow color, moderate endometritis; and dark yellow color, severe endometritis

Endometrial Cytology

Endometrial cytological samples were collected using a sterile cytobrush (AXACTA Pap Smear Kit) mounted on a universal artificial insemination gun following the method of Ghasemi *et al*. (2012). Cytosmears were immediately fixed in 99% methanol for 30 seconds and stained with Field's stain according to the method described by Thangamani *et al*. (2024), with minor modifications. Each smear was examined microscopically under oil immersion, and at least 100 PMN cells were counted. The percentage of PMNs was calculated, and buffaloes with ≥8% PMN were diagnosed as positive for endometritis, whereas those with <8% PMNs were considered negative

Statistical Analysis

Data obtained were statistically analysed using SPSS software (IBM SPSS Statistics version 20.0), and expressed as mean ± standard error (SE) or as percentages. Categorical data on the severity of endometritis were summarized as frequencies and percentages. Differences in PMN cell counts between healthy and endometritis-positive buffaloes were assessed using an independent-samples Student's t-test. Statistical significance was set at $p < 0.05$.

Results and Discussion

White Side Test

The Whiteside test detected endometritis in 26 of 112 buffaloes (23.21%), with a relative diagnostic efficacy of 74.28% compared with endometrial cytology (Table 1). These results indicate that although the Whiteside test is a useful screening tool in the field, the cytobrush technique demonstrated higher sensitivity for detecting endometritis in repeat-breeding buffaloes. While, Whiteside test is simple to perform, requires no specialised equipment, and is a quick, reliable, and accurate method for distinguishing healthy animals from those with endometritis (Kumar *et al.,* 2006).

Zarekar (2014) reported prevalence rates of 20.68% for subclinical endometritis in repeat breeder cows, whereas Parikh (2021) documented an 18.45% prevalence of subclinical endometritis in Gir cows between 40 and 60 days postpartum. In contrast, several researchers have reported a higher incidence of subclinical endometritis in repeat breeding cows ranging from 45.83% to 92.85% (Raja *et al.*, 2012; Singh and Kumar, 2019). Markandeya *et al.* (2010) detected endometritis in 82.76% (24/29) of buffaloes during the first postpartum estrus. Furthermore, Methai *et al.* (2005) and Krishnan *et al.* (2015) reported a 100% incidence of subclinical endometritis in Jersey and crossbred repeat breeding cows, respectively. The variation in the incidence of subclinical endometritis in buffaloes and cattle may be attributed to differences in physiological stage, postpartum interval, parity, management practices, nutritional status, and environmental conditions (Markandeya *et al.,* 2010).

Detection of Severity of Endometritis

Out of 26 buffaloes positive for endometritis with the White side test, 11 buffaloes (42.31%) had mild endometritis, 9 buffaloes (34.61%) had moderate endometritis, and 6 buffaloes (23.08%) had severe endometritis. These findings suggest that mild and moderate endometritis are more prevalent than severe cases among repeat breeding buffaloes diagnosed by the Whiteside test. Similar findings in buffaloes and cattle have been reported by Puro (2016) and Raval *et al.* (2018). In cattle, Lawange *et al.* (2019) reported

Table 1: Detection of endometritis by Whiteside test and endometrial cytology in repeat breeding Mehsana buffaloes

Diagnostic technique	Buffaloes screened (n)	Endometritis positive (n)	Prevalence (%)	Relative diagnostic efficacy (%)
Whiteside test	112	26	23.21	74.28
Endometrial cytology	112	35	31.25	100

19.50% severe endometritis in repeat breeder animals, which closely aligns with the severity distribution observed in the present study. Krishnan *et al.* (2015) recorded 100% mild to moderate positive reactions in repeat breeder crossbred cows.

The comparatively lower proportion of severe endometritis observed in the present study suggests that most cases were identified before progression to advanced uterine pathology (Puro, 2016). This has important clinical implications, as early diagnosis of mild and moderate endometritis facilitates timely therapeutic intervention, thereby improving conception rates and minimising economic losses associated with repeat breeding to the farmer (Channa *et al.,* 2023). The intensity of the color reaction observed in the Whiteside test is directly related to the leukocyte concentration in uterine or cervico-vaginal discharge, reflecting the degree of endometrial inflammation (Mandhwani *et al.*, 2017; Raval *et al.* (2018). The color change may be due of ribonucleic acid in the nuclei of white blood cells, which reacts with a 5% NaOH solution, producing a yellow colour (Gupta *et al.,* 2011). Sometime, a negative result in the Whiteside test indicates that insemination may be possible in animals that have recovered from treatment or are non-infectious and cyclic (Lawange *et al.,* 2019).

Endometrial Cytology

The prevalence of endometritis detected by endometrial cytology cytobrush technique in the present study was 31.25% (35/112; Table 1). The PMNs cell count in cytosmear was significantly higher in endometritis positive buffaloes (11.04 ± 2.13%) as compared to healthy buffaloes (2.50 ± 1.65%) (Table 2). In accordance with the present study, Dubuc *et al.* (2010), and Denis-Robichaud and Dubuc (2015) reported 38%, and 36.20% of subclinical endometritis with ≥5% to ≥6% PMNs as the threshold, respectively. In contrast, some researchers have reported higher endometritis incidence rates using the cytobrush technique, ranging from 51.20% to 80.64% (Oral *et al.,* 2009; Singh *et al.,* 2016; Reddy *et al.*, 2024) at a ≥5% PMN threshold. A slightly lower incidence of endometritis at ≥5% PMN threshold was reported by Parikh (2021). Further, Madoz *et al.* (2013), Gahlot *et al.* (2017), and Mohammed (2024) reported the 17 (PMN ≥5%), 16.67 (PMN >5%) and 16.6 (PMN ≥8%) per cent of endometritis in bovines, respectively. The cytobrush technique directly evaluates endometrial cellular response, particularly polymorphonuclear neutrophil (PMN) infiltration, offering greater sensitivity for detecting low-grade and subclinical uterine infections that might not produce sufficient inflammatory exudate to yield a positive result on the White side test.

Table 2: PMN cell count (%) in cytosmears of healthy and endometritis positive buffaloes

Sr. No.	Group	PMN cell count in smear (%)
1.	Endometritis positive buffaloes (n=35)	11.04 ± 2.13^{B}
2.	Healthy buffaloes (n=77) 't' value = 8.28, p<0.05	2.50 ± 1.65^{A}

Means with different superscripts (A, B) between groups differ significantly (p<0.05).

In the present study, a threshold of ≥8% PMNs was used to distinguish buffaloes with endometritis from healthy animals. To enhance diagnostic precision, several researchers have suggested that this cut-off value requires further refinement (Dubuc *et al.*, 2010). Various studies have reported PMN thresholds of 5%-10% as reliable indicators of endometritis, particularly around 60 days postpartum (Kasimanickam *et al.*, 2004; Baranski *et al.*, 2012). However, the PMN cut-off thresholds used to define endometritis, ranged from 5% to as high as 18% (Kasimanickam *et al.*, 2004; Oral *et al.*, 2009; Reddy *et al.*, 2024; Mohammed, 2024). The variation in endometritis incidence observed in the present study compared with previous reports may be attributable to differences in geographical location, environmental conditions, sample collection techniques, postpartum sampling intervals, and the specific PMN threshold used for diagnosis.

Conclusion

This study evaluated the diagnostic efficacy of the White side test and endometrial cytology for detecting endometritis in repeat breeding Mehsana buffaloes. Endometrial cytology technique proved to be a more sensitive and reliable method for detecting subclinical endometritis in repeat breeding buffaloes, whereas the White side test served as a rapid, economical, and practical field screening tool. The combined application of both techniques can facilitate early

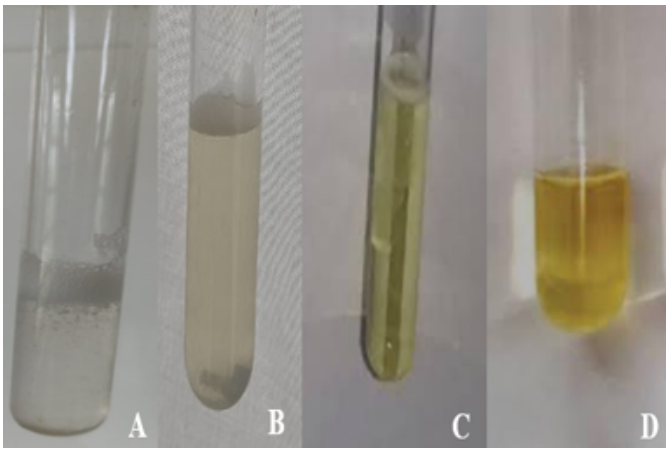


Fig. 1: : Grading of endometritis based on colour intensity in the White Side Test: (A) No color - negative; (B) Light yellow color - subclinical endometritis; (C) Clear yellow color - moderate endometritis; and (D) Dark yellow color - severe endometritis.

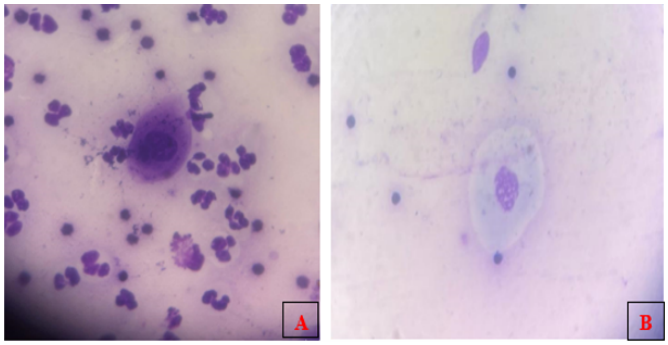


Fig. 2: Representative endometrial cytology smears showing (A) ≥8% polymorphonuclear neutrophils (PMNs), positive for endometritis; and (B) <8% PMNs, negative for endometritis.

diagnosis and improve reproductive management under field conditions.

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References

Baranski, W., Podhalicz-Dziegielewska, M., Zdunczyk, S., & Janowski, T. (2012). The diagnosis and prevalence of subclinical endometritis in cows evaluated by different cytologic thresholds. *Theriogenology, 78*(9), 1939–1947.

Channa, A. A., Yousaf, R., Ullah, N., Khan, R., Ali, M. H., Abbas, G., & Khan, B. (2023). Histopathological changes in the uterus of infertile slaughtered buffaloes. *Pakistan Journal of Science, 75*(1), 125–133.

Denis-Robichaud, J., & Dubuc, J. (2015). Determination of optimal diagnostic criteria for purulent vaginal discharge and cytological endometritis in dairy cows. *Journal of Dairy Science, 98*(10), 6848–6855.

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.

Gahlot, S. C., Kumar, S., Kumaresan, A., Chand, S., Baithalu, R. K., Lathika, S., Patbandha, T. K., Lathwal, S. S., & Mohanty, T. K. (2017). Efficiency of uterine fluid cytology in the diagnosis of subclinical endometritis in the water buffalo (*Bubalus bubalis*). *Reproduction in Domestic Animals, 52*(3), 513–516.

Ghasemi, F., Gonzalez-Cano, P., Griebel, P. J., & Palmer, C. (2012). Proinflammatory cytokine gene expression in endometrial cytobrush samples harvested from cows with and without subclinical endometritis. *Theriogenology, 78*(7), 1538–1547.

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

Hanafi, E. M., Ahmed, W. M., Abd El Moez, S. I., El Khadrawy, H. H., & Abd El Hameed, A. R. (2008). Effect of clinical endometritis on ovarian activity and oxidative stress status in Egyptian buffalo-cows. *American-Eurasian Journal of Agriculture and Environmental Sciences, 4*, 530–536.

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.

Krishnan, B. B., Kumar, H., Mehrotra, S., Singh, S. K., Goswami, T. K., Khan, F. A., Patra, M. K., & Islam, R. (2015). Effect of leukotriene B4 and oyster glycogen in resolving subclinical endometritis in repeat breeding crossbred cows. *Indian Journal of Animal Research, 49*(2), 218–222.

Kulkarni, M. D., Khanvilkar, A. V., & Bansod, R. S. (2002). Incidence of reproductive disorders in indigenous and crossbreds in an organized farm. *Indian Veterinary Journal, 79*(11), 1196–1197.

Kumar, O. R., Prasuna, K., & Rao, T. S. (2006). Study on subclinical metritis in buffaloes using White Side Test. *Intas Polivet, 7*(1), 24–25.

Kumar, S., Bhardwaz, A., Srivastava, A. K., Rao, M., & Kumar, N. (2015). White side test—a field test on the cervical mucus of cows for diagnosis of endometritis. *Intas Polivet, 16*(2), 207–213.

Lawange, S. R., Markandeya, N. M., Kumawat, B. L., Sawale, A. G., Anbhule, R. S., & Kadam, P. D. (2019). Efficacy of garlic extract ± ashwagandha for the treatment of infectious repeat breeding in cattle. *Indian Journal of Animal Reproduction, 40*(1), 31–34.

Madoz, L. V., Giuliodori, M. J., Jaureguiberry, M., Plontzke, J., Drillich, M., & de la Sota, R. L. (2013). The relationship between endometrial cytology during estrous cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science, 96*(7), 4333–4339.

Mandhwani, R., Bhardwaz, A., Kumar, S., Shivhare, M., & Aich, R. (2017). Insights into bovine endometritis with special reference to phytotherapy. *Veterinary World, 10*(12), 1529–1532.

Markandeya, N. M., Patil, A. D., Moregaonkar, S. D., & Bhonsle, A. V. (2010). Efficacy of fluoroquinolone in combination with alpha tocopherol to resolve non-specific infections of reproductive tract of dairy animals. *Intas Polivet, 11*(1), 7–10.

Methai, A., Rajasundaram, R. C., Veerapandian, C., & Ahmad, M.

(2005). Intrauterine plasma treatment for endometritis in Jersey crossbred cows. *Indian Journal of Animal Reproduction, 26*(1), 7–10.

Mohammed, S. N. (2024). Bacteriological finding, vaginal discharges, and endometrial cytology for endometritis detection in postpartum buffaloes. *Diyala Journal for Veterinary Sciences, 2*(3), 31–49.

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. (2021). *Comparative studies on different diagnostic methods and therapeutic approaches for postpartum subclinical endometritis in Gir cows* [Doctoral dissertation, Kamdhenu University, Gandhinagar, Gujarat, India].

Puro, N. A. (2016). *Efficacy of E. coli LPS and Lugol's iodine in endometritis treatment of dairy animals* [M.V.Sc. thesis, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India].

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.

Raval, S. R., Panchal, M. T., Dhami, A. J., & Parmar, S. C. (2018). Endometrial cytology and cervical mucus characteristics of repeat breeding crossbred cows in relation to post-treatment fertility. *Indian Journal of Veterinary Sciences and Biotechnology, 13*(4), 26–31.

Reddy, S. V. B., Shrinivas, M., Chandra Prasad, B., & Sreenu, M. (2024). Therapeutic efficacy of bromelain in treatment of subclinical endometritis in buffaloes. *International Journal of Veterinary Sciences and Animal Husbandry, 9*(2), 1028–1033.

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

Sheldon, I. M., Price, S. B., Cronin, J., Gilbert, R. O., & Gadsby, J. E. (2009). Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reproduction in Domestic Animals, 44*, 1–9.

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*(2), 11–13.

Singh, M., & Kumar, P. (2019). Conception rate based accuracy of different methods to diagnose sub-clinical endometritis in cows. *The Indian Journal of Animal Reproduction, 40*(1), 16–18.

Thangamani, A., Srinivas, M., Rao, M. M., Sreenu, M., & Kumar, K. A. (2024). Differential staining techniques to assess the staining characteristics of uterine epithelial cells from uterine flushing of endometritis affected buffaloes. *The Haryana Veterinarian, 63*(1), 121–124.

Zarekar, A. U. (2014). *Diagnosis and treatment of repeat breeder crossbred cows with modified approaches* [M.V.Sc. thesis, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India].