

Diagnostic Accuracy of the White Side Test and Assessment of Oxidative Stress Biomarkers in Cows with Subclinical Endometritis

Pawan Preet Singh^{1*}, Asloob Ahmad Malik¹, Khursheed Ahamd Sofi¹, Mehrajuddin Naikoo¹, Zahoor Ahmad Pampori², Mir Nadeem Hassan³, Syed Mohamad Shah⁴

ABSTRACT

Subclinical endometritis (SCE) in dairy cattle negatively impacts fertility and poses a significant diagnostic challenge in bovine practice. The current study was conducted based on the hypothesis that SCE in cows alters the serum concentrations of nitric oxide (NO) and lipid peroxides (LPO). The study was carried out across different districts of the Kashmir Valley. A total of 54 cows that had calved at least once and were more than 60 days in milk (DIM) at the time of estrus were included. Initial screening was performed by endometrial cytology using cytobrush technique as reference method (polymorphonuclear cells $\geq 4\%$). Out of 54 animals screened, 41 cows were found positive for SCE, while 13 were negative. The sensitivity and specificity of White side test based on ROC curve was 78% and 53.8%, respectively, in comparison to endometrial cytology at optimal cut-off point ≥ 0.5 (mild infection). Cows diagnosed with SCE at estrus showed significantly ($p < 0.05$) elevated serum levels of nitric oxide (NO) ($55.95 \pm 3.03 \mu\text{mol}$) and lipid peroxidation (LPO) ($79.65 \pm 7.42 \text{ nmol/mL}$) compared to healthy animals. In conclusion, the White side test showed good sensitivity despite lower specificity compared to endometrial cytology, therefore it can serve as a practical field diagnostic tool. SCE is associated with increased oxidative stress and these biomarkers may help monitor treatment response in dairy cows.

Key words: Cytobrush, Dairy cows, MDA, Nitric oxide, Subclinical endometritis, White side test.

Ind J Vet Sci and Biotech (2026); 10.48165/ijvsbt.22.4.27

INTRODUCTION

Subclinical endometritis (SCE) is an inflammation of the endometrium that occurs without obvious clinical signs and is identified by an elevated proportion of polymorphonuclear (PMN) cells. This makes its diagnosis more difficult than that of clinical endometritis (Villar *et al.*, 2025). Subclinical endometritis represents a major challenge in the modern dairy industry because of its high prevalence, lack of obvious clinical signs, and considerable detrimental effects on reproductive performance (Sheldon *et al.*, 2006). Various techniques have been employed for the effective detection of SCE in bovines including endometrial cytology (Shivhare *et al.*, 2018). White side test is used for the diagnosis of subclinical uterine infections in repeat-breeding cows (Satheshkumar and Punniamurthy, 2007). The positive samples are categorized as slight, moderate, or severe subclinical infections based on the intensity of the color reaction - light yellow, yellow, and dark yellow, respectively. Oxidative stress occurs when the equilibrium between oxidants and antioxidants is disturbed. In cases of bovine endometritis, elevated oxidative stress has been documented and is linked to increased generation of free radicals and nitric oxide (Kaya *et al.*, 2017). This imbalance can adversely affect the quality of sperm and oocytes, as well as impair embryonic development (Zhong, 2013). The present study was carried

¹Division of Animal Reproduction Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, SKUAST-K, Shuhama-190006, J&K, India

²Division of Veterinary Physiology, Faculty of Veterinary Science and Animal Husbandry, SKUAST-K, Shuhama-190006, J&K, India

³Division of Veterinary Microbiology, Faculty of Veterinary Science and Animal Husbandry, SKUAST-K, Shuhama-190006, J&K, India

⁴Mountain Livestock Research Institute, Manasbal-193504, SKUAST-K, J&K, India

Corresponding Author: Dr. Pawan Preet Singh, PhD Scholar, Division of Animal Reproduction Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, SKUAST-K, Shuhama-190006, J&K, India. e-mail: pawanpreetbali@gmail.com

How to cite this article: Singh, P. P., Malik, A. A., Sofi, K. A., Naikoo, M., Pampori, Z. A., Hassan, M. N., & Shah, S. M. (2026). Diagnostic Accuracy of the White Side Test and Assessment of Oxidative Stress Biomarkers in Cows with Subclinical Endometritis. *Ind J Vet Sci and Biotech*, 22(4), 143-145.

Source of support: Nil

Conflict of interest: None

Submitted 18/02/2026 **Accepted** 30/03/2026 **Published** 10/07/2026

out to evaluate the diagnostic accuracy of the White side test and association of biochemical parameters (Lipid peroxidation and nitric oxide) with subclinical endometritis in dairy cows.

MATERIALS AND METHODS

The study was conducted in various regions/districts of Kashmir valley during October 2024 to October 2025. The cows (n=54) having completed atleast one parity with more than 60 days in milk (DIM) at time of estrus were screened under this study. The animals were initially screened by endometrial cytology using cytobrush technique as reference method. The polymorphonuclear cells (PMN) value of $\geq 4\%$ was taken as cut off value for diagnosis of SCE (Asfar *et al.*, 2021) and those animals found positive on said test were further subjected to biochemical analysis. Blood samples (8 mL) were obtained via jugular venipuncture into 15 mL centrifuge tubes, kept in a slanted position for several hours to allow clot formation, centrifuged at 503 g for 15 min, and serum stored at -20°C until further analysis.

Endometrial Cytology, White Side Test, Serum NO and LPO Estimation

Uterine cytology samples were collected using a modified Cytobrush assembly designed for large animals and subclinical endometritis was diagnosed when polymorphonuclear (PMN) cells exceed 4% (Singh *et al.*, 2016), followed by White side test using cervico-vaginal mucus (Asfar *et al.*, 2021). The intensity of colour changes were studied and graded as negative (no colour), mild infection (light yellow), moderate infection (yellow) and severe infection (dark yellow) (Bhat *et al.*, 2014). Serum nitric oxide (NO) concentration was determined using the modified Griess reaction (Sastry *et al.*, 2002), while Malondialdehyde (MDA) levels, used as an indicator of lipid peroxidation, were determined using TBA reagent and the double-heating method described by Draper and Hadley (1990).

Statistical Analysis

The data was analysed using unpaired 't' test using SPSS 2020. The sensitivity and specificity of White side test was analysed using Receiver Operator Characteristics (ROC) and the Area Under Curve (AUC). The cut-off point with highest sum of sensitivity and specificity was selected as optimal. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Based on endometrial cytology using cytobrush technique, 41 animals were found positive for subclinical endometritis and 13 animals were found negative. The sensitivity and specificity of White side test based on ROC curve was 78% and 53.8%, respectively, in comparison to endometrial cytology at optimal cut-off point > 0 (Negative). AUC was 0.72 and p value was 0.01. Raja *et al.* (2012) reported that 72.4% (21/29) of cows tested positive for subclinical endometritis (SCE) using the White side test (WST). Similarly, Bhat *et al.* (2014) observed 71.25% (57/80) animals were positive by WST. Asfar *et al.* (2021) reported comparable sensitivity of 63.3% and specificity of 55.6% in comparison to endometrial cytology.

The WST has been reported as a diagnostic method for detecting subclinical endometritis (Panta and Mandal, 2024). In normal estrual discharge, the leukocyte count is too low to produce a positive color change in the WST (Panta and Mandal, 2024). Subclinical discharge contains a moderate number of leukocytes, resulting in a moderate color reaction, whereas clinical discharge, with a markedly higher leukocyte count, produces an intense color change.

Cows affected with subclinical endometritis exhibited significantly ($p < 0.05$) higher serum concentrations of nitric oxide and lipid peroxidation compared to non-infected healthy cows (Table 1). The results were in accordance with those of Li *et al.* (2010) and Krishnan *et al.* (2014). El-Sayed *et al.* (2024) also reported significant ($p < 0.05$) increase in the levels of nitric oxide and MDA in subclinical endometritic cows.

Table 1: Nitric Oxide (μmol) and MDA levels (nmol/ml) in SCE cows (Mean $\pm$ SEM)

Group	Nitric Oxide	MDA
Infected (n=41)	55.95 $\pm$ 3.03 ^a	79.65 $\pm$ 7.42 ^a
Control (n=13)	11.73 $\pm$ 3.53 ^b	71.57 $\pm$ 5.03 ^b

Means in column with different superscripts (a, b) differ significantly ($p < 0.05$).

Nitric oxide (NO) is a highly reactive free radical containing a single unpaired electron and has a very short half-life of approximately 2-6 seconds. It is predominantly generated during inflammatory conditions (Biswal *et al.*, 2024), where phagocytic cells produce it as part of the immune response. During infections caused by *E. coli*, excessive release of NO can function as a cytotoxic mediator. In animals affected with metritis, neutrophils produce substantial quantities of NO along with the enzyme myeloperoxidase. Together, these substances may contribute to the formation of nitrotyrosine, which can degrade proteins and exert damaging effects on tissues (Jozwik *et al.*, 2012). Blum *et al.* (2000) reported a positive correlation between intramammary and systemic TNF- α levels and NO production in cases of acute mastitis. Nitric oxide also exhibits antibacterial activity through the formation of peroxynitrite, a reactive nitrogen species produced from the oxidation of NO, and is considered an important component of the primary defence mechanism (Biswal *et al.*, 2024). Elevated peripheral NO concentrations have been associated with uterine infections in postpartum cattle and buffaloes (Li *et al.*, 2010). Furthermore, Baithalu *et al.* (2016) observed that cows which developed uterine infections had higher peripheral NO levels from one week before parturition through the day of calving compared to cows that remained free of uterine infections.

Malondialdehyde (MDA) is widely used as a biomarker of oxidative stress and is generated as a by-product of lipid peroxidation. It is a reactive carbonyl compound that, at elevated concentrations, can exert harmful effects on cells by altering their function, either through the formation of DNA adducts or by damaging cellular proteins (Biswal *et al.*, 2024). MDA levels in biological fluids can be quantified using



various techniques, among which the thiobarbituric acid reactive substances (TBARS) assay (Janero, 1990) is the most commonly employed method. Baithalu *et al.* (2016) reported that cows which developed uterine infections exhibited higher peripheral MDA concentrations from one week before calving to one week after calving, with peak levels observed on the day of calving, compared to cows that did not develop uterine infections.

In brief, the findings of this study indicate that White side test owing to its moderate sensitivity but lower specificity compared to endometrial cytology may be used as cow side test under field conditions. Besides this SCE is associated with increased oxidative stress, and hence the biomarkers NO and MAD may be useful for the diagnosis of SCE or for evaluating treatment response in dairy cows.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Anusandhan National Research Foundation SERB-SURE Government of India, DST for funding the project on Subclinical Endometritis in Dairy Cow (SUR/2022/004354) under which the study was done.

REFERENCES

- Asfar, A., Sofi, K.A., Bhat, M.A., Malik, A.A., & Malik, A.A. (2021). Leukocyte esterase reagent strips: Rapid, economical and reliable cow side test for subclinical endometritis. *Turkey Journal of Veterinary and Animal Science*, 45(3), 540-546.
- Baithalu, R.K., Singh, S.K., Kumaresan, A., Mohanty, A.K., Mohanty, T.K., Kumar, S., & Agarwal, S.K. (2016). Transcriptional abundance of antioxidant enzymes in endometrium and their circulating levels in Zebu cows with and without uterine infection. *Animal Reproduction Science*, 177, 79-87.
- 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.
- Biswal, P., Lathwal, S.S., Baithalu, R.K., & Bisht, D. (2024). Oxidative stress indicators during peripartum uterine infection of buffaloes: A review. *Buffalo Bulletin*, 43(4), 551-564.
- Blum, J.W., Dosogne, H., Hoeben, D., Vangroenweghe, F., Hammon, H.M., Bruckmaier, R.M., & Burvenich, C. (2000). Tumor necrosis factor- α and nitrite/nitrate responses during acute mastitis induced by *Escherichia coli* infection and endotoxin in dairy cows. *Domestic Animal Endocrinology*, 19(4), 223-235.
- Draper, H.H., & Hadley, M. (1990). Malondialdehyde determination as index of lipid Peroxidation. *Methods in Enzymology*, 186, 421-431.
- El-Sayed, A., Refaai, M., & Ateya, A. (2024). Doppler ultrasonographic scan, gene expression and serum profile of immune, APPs and antioxidant markers in Egyptian buffalo-cows with clinical endometritis. *Scientific Reports*, 14(1), 56-98.
- Janero, D.R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine*, 9(6), 515-540.
- Jozwik, A., Strzałkowska, N., Bagnicka, E., Grzybek, W., Krzyżewski, J., Poławska, E., & Horbańczuk, J.O. (2012). Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows. *Czech Journal of Animal Science*, 57(8), 353-360.
- Kaya, S., Ögün, M., Özen, H., Kuru, M., Şahin, L., Kükürt, A., & Kaçar, C. (2017). The impact of endometritis on specific oxidative stress parameters in cows. *Journal of Hellenic Veterinary Medical Society*, 68(2), 231-236.
- Krishnan, B.B., Kumar, H., Mehrotra, S., Singh, S.K., Goswami, T.K., & Narayanan, K. (2014). Subclinical endometritis increases oxidative stress and modulates polymorphonuclear leukocyte functions in crossbred cows. *Indian Journal of Animal Science*, 84, 1192-1195.
- Li, D., Liu, Y., Li, Y., Lv, Y., Pei, X., & Guo, D. (2010). Significance of nitric oxide concentration in plasma and uterine secretates with puerperal endometritis in dairy cows. *Veterinary Research Communication*, 34(4), 315-321.
- Panta, P., & Mandal, U.P. (2024). Study on subclinical endometritis in cattle of Kathmandu Valley by using White side test. *Cluj Veterinary Journal*, 29(3), 15-30.
- Raja, S., Devanathan, T.G., Kulasekar, K., Pazhanivel, N., & Balachandran, C. (2012). White side test and endometrial biopsy for diagnosis of endometritis in repeat breeding cows. *Indian Journal of Animal Reproduction*, 33(1), 56-58.
- Sastry, K.V.H., Moudgal, R.P., Mohan, J., Tyagi, J.S., & Rao, G. (2002). Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. *Analytical Biochemistry*, 306(1), 79-82.
- Satheshkumar, S., & Punnamurthy, N. (2007). Sub-clinical uterine infections in repeat breeder cows. *Indian Veterinary Journal*, 84(6), 654-655.
- Sheldon, I.M., Lewis, G.S., LeBlanc, S., & Gilbert, R.O. (2006). Defining postpartum uterine disease in 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(02), 11-13.
- Villar, S.L., Pérez-Marín, C.C., Álvarez, J., Yáñez, U., Becerra, J.J., Peña, A.I., & Quintela, L.A. (2025). Evaluation of prevalence, risk factors, and therapeutic approach for subclinical endometritis and oviductal occlusion in repeat breeder Holstein cattle. *Research in Veterinary Science*, 183, 105-111.
- Zhong, R.Z. (2013). Oxidative stress and role of natural plant derived antioxidants in animal reproduction. *Journal of Integrative Agriculture*, 12(10), 1826-1838.