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Transrectal Doppler Characterization of Uterine Vascular Dynamics In Buffaloes With Clinical Metritis and Subsequent Recovery

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ABSTRACT

The study was conducted at the Referral Veterinary Polyclinic (VGO-OPD), ICAR–Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India during 2023–2024 to determine whether Doppler-based uterine hemodynamic indices can objectively differentiate the vascular alterations associated with clinical metritis in early-postpartum buffaloes and to evaluate their normalization following therapeutic recovery. The study characterized uterine vascular dynamics in pluriparous buffaloes diagnosed with clinical metritis within 21 days postpartum using transrectal color and spectral Doppler ultrasonography. Ten affected animals were evaluated, and Doppler indices of the middle uterine artery viz. resistance index (RI), pulsatility index (PI), time-averaged maximum velocity (TMAX), arterial diameter, and blood flow volume (BFV) were recorded before and after therapeutic intervention consisting of ceftiofur, flunixin meglumine, uterine lavage, and supportive therapy. During the disease phase, RI and PI were significantly reduced (p<0.05), while TMAX, BFV, and arterial diameter were significantly increased (p<0.05), indicating uterine vasodilation and hyper perfusion associated with inflammation. All vascular parameters gradually returned toward baseline values following clinical recovery, reflecting restoration of vascular tone. Correlation analysis revealed a strong positive association between RI and PI (r=0.63, p<0.05) and a negative association between RI and BFV (r=-0.45, p<0.05), consistent with expected hemodynamic responses.

KEYWORDS: Buffalo, clinical metritis, doppler, resistance index, uterine hemodynamics

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Postpartum uterine infections are among the most significant reproductive disorders in dairy animals, particularly in tropical livestock systems where buffaloes form the backbone of milk and draft production. Clinical metritis is a severe, acute uterine inflammation occurring within three weeks of calving, typified by fetid watery discharge, fever, and systemic illness. It leads to reduced fertility, prolonged calving-to-conception intervals, and substantial economic losses (Sheldon et al., 2006). The reported incidence of clinical metritis in buffaloes ranges from 7.2% to 9.8%, consistent with field observations (Nguyen et al., 2025), although lower rates, such as 1.47%, have been documented in specific Egyptian herds (Hamouda et al., 2024). The disease is initiated by the invasion of bacteria such as *Escherichia coli*, *Trueperella pyogenes*, and *Fusobacterium necrophorum* (Noakes et al., 2018). These pathogens stimulate neutrophil influx and the release of inflammatory mediators, including tumor necrosis factor-α, prostaglandins, and nitric oxide (Cui et al., 2022), resulting in vasodilation, endothelial activation, and altered uterine perfusion that can compromise uterine repair and subsequent fertility. Transrectal color and pulsed-wave Doppler ultrasonography now permit non-invasive quantification of uterine hemodynamics in large ruminants (Ginther and Utt, 2004). In healthy cows, estrogen during estrus promotes vasodilation and increased uterine blood flow, whereas progesterone during diestrus increases vascular resistance (Hassan et al., 2017; Chen et al., 2004; Ford and Christenson, 1979). Inflammatory mediators such as histamine, bradykinin, nitric oxide, and prostaglandins reduce vascular resistance and increase perfusion (Ricciotti and FitzGerald, 2011; Moncada and Higgs, 2006; Marceau and Regoli, 2004). These changes appear as reduced resistance (RI) and pulsatility indices (PI), with higher flow velocities and volumes, readily detected by Doppler ultrasonography (Sheldon et al., 2009; Ginther and Utt, 2004). Doppler ultrasonography has been applied to monitor estrous cycling, pregnancy, uterine involution, uterine torsion, incomplete cervical dilatation, and subclinical endometritis (Sahu, 2025; Chaudhari et al., 2023; Hussein, 2013; Heppelmann et al., 2013; Honnens et al., 2008; Bollwein et al., 1998). In women, pelvic inflammatory disease shows similar Doppler signatures with increased uterine blood flow and reduced RI and PI (Jaiyeoba and Soper, 2011). Experimental studies in cattle confirm that endometritis induction leads to rapid increases in timed-averaged maximum velocity (TMAX) and corresponding PI reduction (Debertolis et al., 2016), while subclinical endometritis is associated with higher uterine blood flow volumes and lower resistance indices early postpartum (Sahu, 2025). Comparable findings in mares and ewes (Still and Greiss, 1978; Bollwein et al., 1998) indicate conserved inflammation-associated uterine hyperemia. Compared to cattle, information on buffalo uterine hemodynamics remains limited. Structural and hormonal differences between species may influence vascular responses. Only a few studies have examined uterine artery flow during physiological states such as pregnancy or puerperium (Gohar et al., 2023; Varughese et al., 2013), and pathological data are scarce. El-Sayed et al. (2024) reported reduced RI and PI and increased TMAX and blood flow volume in buffaloes with endometritis, consistent with inflammatory hyperemia. However, longitudinal Doppler assessments of clinical metritis and recovery in buffaloes are lacking. This study aimed to characterize uterine hemodynamic changes in buffaloes with clinical metritis using transrectal color and spectral Doppler ultrasonography. Doppler indices (RI, PI, TMAX, blood flow volume, and arterial diameter) of the middle uterine artery were evaluated before and after treatment to delineate hemodynamic alterations and their normalization during recovery. We hypothesized that affected buffaloes would exhibit altered uterine blood flow patterns before therapy, which would return to baseline with clinical recovery, providing Doppler-based benchmarks for non-invasive monitoring of uterine inflammation and therapeutic response.

2. MATERIALS AND METHODS

2.1. Study site

The study was conducted at the Referral Veterinary Polyclinic (VGO-OPD), ICAR–Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India (28°22' N, 79°24' E; 564 m above sea level) during 2023–2024. The region has a tropical-humid climate, with mean summer temperatures of 30.7°C (range: 15.1–40.9°C) and average relative humidity of 53.2%. This study aimed to characterize uterine vascular dynamics in buffaloes affected with clinical metritis using transrectal color and spectral Doppler ultrasonography

2.2. Animals and experimental design

Farmer-owned pluriparous buffaloes (*Bubalus bubalis*) of varying breeds, ages, and parities were enrolled. The parity range was 3–6, mean age 7.2±1.4 years, and average body weight 496.3±12.8 kg. A total of ten buffaloes diagnosed with clinical metritis were included in the study.

2.3. Inclusion and exclusion criteria

Puerperal metritis was diagnosed based on an abnormally enlarged uterus, fetid watery red–brown uterine discharge, and systemic illness with fever (>103°F) within 21 days in milk (DIM). Buffaloes with uterine enlargement and fetid discharge within 21 DIM but without systemic signs were classified as having clinical metritis (Galvao et al., 2011).

Clinical endometritis was defined by purulent uterine discharge (>50%) after 21 DIM or mucopurulent discharge (≈50% pus, 50% mucus) after 26 DIM (Sheldon et al., 2006). Only animals meeting the definition of clinical metritis (n=10) were included; others were excluded.

2.4. Treatment of clinical metritis

Affected buffaloes were treated with ceftiofur (1.1 mg kg^{-1}, intramuscular) and flunixin meglumine (1.1 mg kg^{-1}, intramuscular). Uterine lavage with 2% povidone–iodine solution was performed, followed by oral ecbolic administration (100 mL day^{-1}). Supportive therapy included multivitamins and fluids. Treatment duration varied from 3 to 10 days (mean ≈ 5 days), depending on disease severity. Doppler ultrasonography was performed before therapy and throughout the treatment period to monitor hemodynamic changes.

2.5. Spectral doppler measurements

Uterine perfusion was assessed using transrectal color and spectral Doppler ultrasonography. A 7.5 MHz linear transducer (Exago ECM, France) was introduced transrectally to identify the middle uterine artery (MUA). The abdominal aorta was traced caudally to the bifurcation of the internal and external iliac arteries, and the MUA was visualized within the mesometrium using color Doppler.

Spectral Doppler interrogation was performed by positioning the sample gate centrally within the arterial lumen, with gate length adjusted to match the vessel diameter. The insonation angle was maintained at ≤60° (mean ≈ 50°). Color gain was set at approximately 20 dB. Pulse repetition frequency (PRF) was set between 3000 and 5000 Hz (typically 4000 Hz) to minimize aliasing. If aliasing occurred, the PRF or velocity scale was adjusted and the insonation angle reconfirmed.

For each MUA, Doppler recordings were accepted only when at least three consecutive, well-defined cardiac cycles were obtained. Three representative waveforms were frozen and analyzed, and their mean value was used for each side. All examinations were conducted by a single experienced operator to minimize inter-operator variability. Each scanning session lasted approximately 30–45 minutes per animal (Sahu, 2025; Chandra et al., 2023; Bollwein et al., 2000).

2.6. Doppler indices and calculations

Resistance index (RI) and pulsatility index (PI) were automatically calculated by the machine's software using the following equations:

RI=(PSV–EDV)/PSV (Gosling and King, 1974)

PI=(PSV–EDV)/mean velocity (Gosling and King, 1974)

where PSV is peak systolic velocity and EDV is end-diastolic velocity.

Timed-averaged maximum velocity (TMAX) was used to calculate blood flow volume (BFV) using the formula:

$BFV=TMAX\times\varpi\times(D/2)^2\times60$ (Varughese et al., 2013)

where BFV is in mL min^{-1}, TMAX is in cm s^{-1}, and vessel diameter (D) is in cm.

2.7. Measurement of arterial diameter

Immediately after spectral Doppler assessment, the probe was rotated to obtain a transverse B-mode image of the MUA at the same location. Internal vessel diameter (intima-to-intima) was measured on three frozen frames during similar cardiac cycles, and the mean was recorded for analysis (Figure 1).

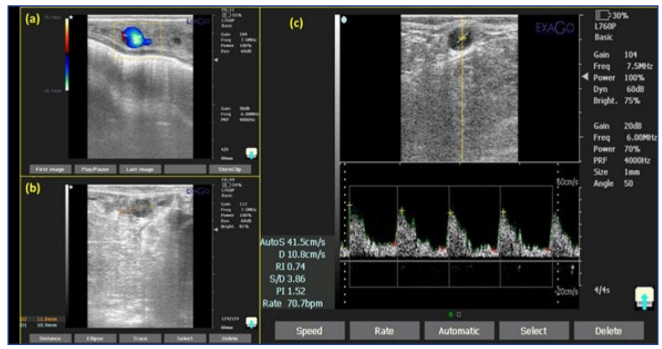

Figure 1: Doppler ultrasonography tracing and measurement of blood flow and diameter of the middle uterine artery of buffaloes with metritis

2.8. Statistical analysis

Data were analyzed using GraphPad Prism 10.6.0 (GraphPad Software, LLC, San Diego, USA). Normality was tested using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Mean Doppler indices (RI, PI), TMAX, arterial diameter, and BFV were compared between diseased and recovered states using independent t-tests. Pearson's correlation coefficients were calculated to examine relationships among Doppler parameters. Data are presented as mean±standard error of the mean (SEM). Statistical significance was considered at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Uterine hemodynamic alterations during clinical metritis

Doppler sonographic evaluation of the middle uterine artery (MUA) in buffaloes affected with clinical metritis and the same animals following recovery revealed significant alterations in uterine hemodynamics. During the diseased phase, both resistance index (RI) and pulsatility index (PI) were significantly reduced ($p<0.05$), indicating a marked decline in vascular resistance within the uterine artery. These indices exhibited a significant increase during the recovery period (Figure 2a, 2b). This decline in vascular resistance during inflammation reflects the physiological effects of

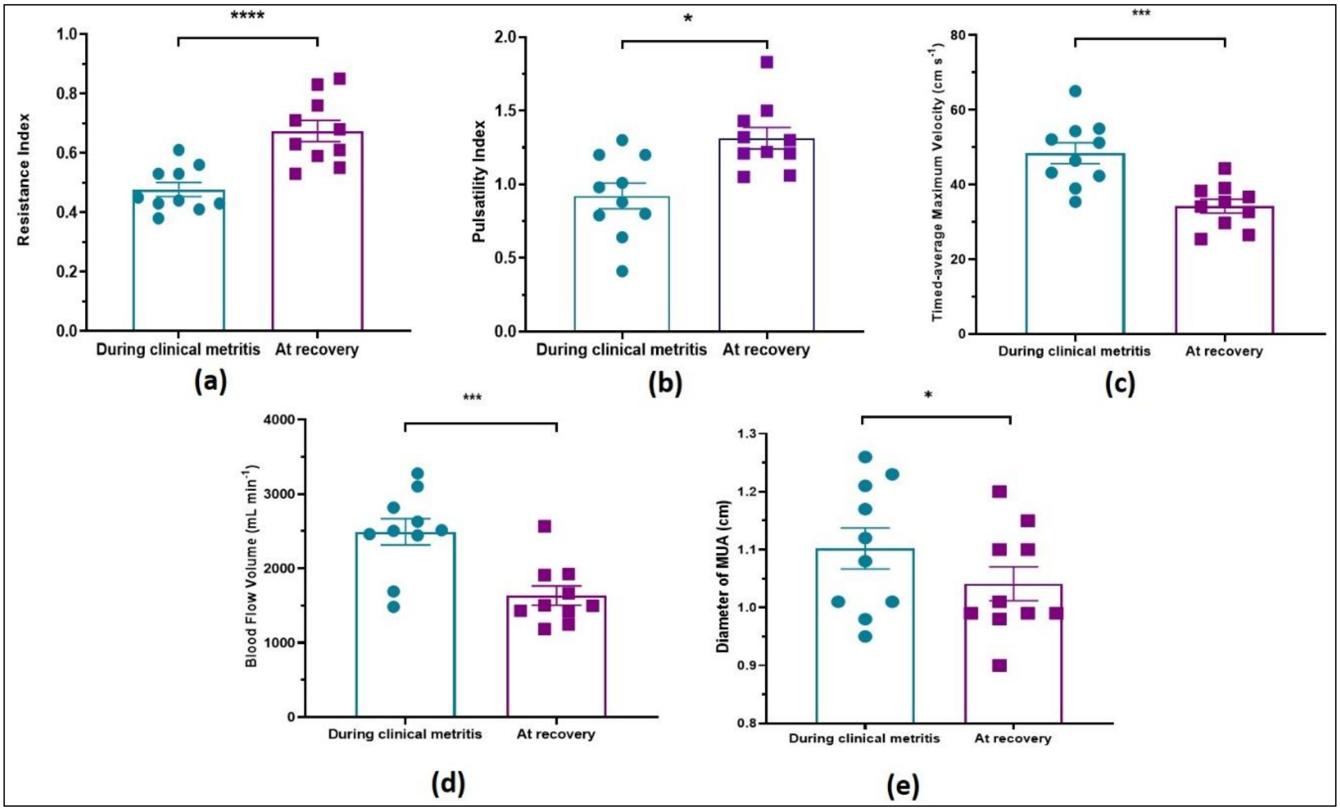

Figure 2: Doppler sonographic findings of the middle uterine artery of buffaloes during and at recovery from clinical metritis

mediators such as prostaglandins, nitric oxide, histamine, and bradykinin, which promote vasodilation and increase uterine perfusion (Moncada and Higgs, 2006; Ricciotti and FitzGerald, 2011). Similar reductions in RI and PI have been reported in cows with clinical and subclinical endometritis (Debertolis et al., 2016; Sharma et al., 2021; Sheldon et al., 2009), indicating conserved inflammatory vascular responses across ruminants. The subsequent normalization of these indices following therapy suggests restoration of vascular tone as inflammation subsides.

3.2. *Flow velocity and perfusion patterns*

Timed-averaged maximum velocity (TMAX) and blood flow volume (BFV) were significantly elevated during metritis compared to the recovered state (p<0.05) (Figure 2c, 2d). This reflects uterine hyperemia associated with inflammatory vasodilation. Comparable elevations in uterine flow parameters have been documented in experimentally induced endometritis in cows (Debertolis et al., 2016) and in clinical endometritis in buffaloes (El-Sayed et al., 2024). These findings indicate that Doppler ultrasonography can reliably capture functional vascular changes during uterine inflammation in buffaloes, as previously shown in bovine and equine studies (Bollwein et al., 1998; Ginther and Utt, 2004).

3.3. *Arterial diameter changes*

The arterial diameter of the MUA was significantly greater during metritis and decreased after clinical recovery (p<0.05) (Figure 2e), consistent with vasodilation during disease. Interestingly, correlation analysis showed no significant relationship between RI and arterial diameter (p >0.05), implying that microvascular adjustments rather than large-vessel dilation may primarily govern changes in uterine perfusion during metritis. This aligns with previous observations in cattle and buffaloes, where microvascular alterations contributed more to perfusion dynamics than conduit vessel changes (Hassan et al., 2017; Sahu, 2025).

3.4. *Correlation analysis of doppler indices*

Correlation analysis demonstrated a strong positive relationship between RI and PI (r=0.63, p<0.05) and a negative correlation between RI and BFV (r=−0.45, p<0.05) (Figure 3a, 3b). These patterns align with fundamental hemodynamic principles: as vascular resistance declines, pulsatility decreases and blood flow volume increases (Bollwein et al., 2000; Hassan et al., 2017). This physiological consistency reinforces the validity of Doppler measurements as objective indicators of uterine vascular changes.

3.5. *Integration with clinical recovery*

The gradual return of RI, PI, TMAX, BFV, and arterial diameter toward baseline values after treatment mirrors the resolution of inflammation and restoration of uterine

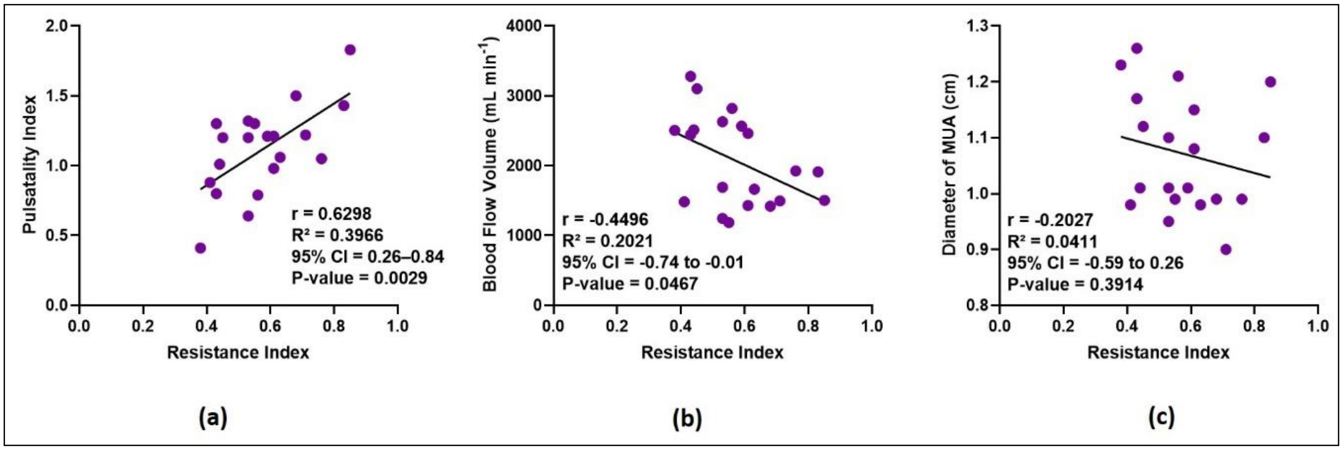

Figure 3: Correlation among middle uterine artery Doppler attributes of buffaloes affected with clinical metritis

vascular homeostasis. Similar patterns have been observed in cattle recovering from uterine infections (Sharma et al., 2021). These Doppler indices provide a real-time, non-invasive means to monitor therapeutic response. Moreover, integrating Doppler evaluation with clinical and molecular assessments could enable early detection and treatment monitoring of uterine disease in buffaloes, which is critical for improving reproductive performance in tropical dairy systems (Bruinje et al., 2024; El-Sayed et al., 2024).

4. CONCLUSION

Transrectal doppler ultrasonography proved useful in detecting altered uterine blood flow patterns during clinical metritis in buffaloes and in tracking their improvement after treatment. The observed decrease in RI and PI, along with increased TMAX and BFV, reflected inflammation-associated vasodilation, which gradually normalized as animals recovered.

5. ACKNOWLEDGMENT

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