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Research Article

PHYSICAL AND ANALYTICAL CHARACTERISTICS OF UTERINE DISCHARGE IN SUBCLINICAL AND CLINICAL ENDOMETRITIC BUFFALOES

THANGAMANI. A*, MANDA SRINIVAS[1], M. MUTHA RAO[2], MAKKENA SREENU[3] and K. ASWANI KUMAR[4]
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tirunelveli-627358 (Tamil Nadu), [1]Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Salem-636112, TANVASU, India, [2]Livestock Research Station, Guntur-522018 (A.P.)
[3]Department of Veterinary Surgery and Radiology, College of Veterinary Science, Garividi, Vizianagarem (A.P.)
[4]Department of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram-521102 (A.P.), India

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ABSTRACT

The present study was undertaken to estimate the physical (Turbidity, Specific Gravity and Electric Resistance), analytical characteristics (Sodium, Potassium and Chloride) and enzyme assay (Leucocyte esterase, catalase and SOD) of uterine discharge in subclinical (SCE) and clinical endometritic (CE) in comparison with normal buffaloes without endometritis. Physical parameters revealed that mean concentrations of turbidity, specific gravity (SG) and electrical resistance (ER) was significantly ($p<0.05$) higher in CE as compared to SCE and normal buffaloes. Analytical parameters revealed that mean electrolyte concentrations (potassium and chloride) were significantly higher (p) in CE buffaloes as compared to SCE affected and normal healthy buffaloes. Also, Enzymes like leucocyte esterase and catalase showed increasing trend however SOD showed decreasing trend with the severity of infection in uterine discharge of Normal, SCE and CE buffaloes. In conclusion, estimation of physical and analytical parameters of uterine discharge could be a novel non-invasive method for diagnosis of degree of endometritis in affected buffaloes.

Keywords: Buffalo, Electrical resistance, Enzymes, Endometritis, Specific gravity, Turbidity, uterine discharge

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The key for optimum fertility in dairy cow and buffalo herds is the healthy or ambient uterine environment since any disturbances of endometrium affected the normal reproductive functions and culminated to infertility or subfertility in terms of repeat breeding (Jabbour *et al.*, 2009; Sheldon *et al.*, 2009). Postpartum uterine infection has been associated with the delayed uterine involution followed with increased service period and in turn increased calving intervals culminating in economic losses (Gahlot, 2016). Subclinical endometritis is an important cause of repeat breeding and usually remains undiagnosed (Dutt *et al.*, 2017). Endometrial cytology is considered as a reference method for cytological diagnosis of subclinical endometritis, because of quality of sample obtained by either low volume uterine flushing or cytobrush with great repeatability of the test (Kasimanickam *et al.*, 2005). Uterine discharge molecules could be used to diagnose the SCE in buffaloes (Gahlot *et al.*, 2017). However, no published data was available on physical (turbidity, specific gravity and electrical resistance) and analytical parameters like electrolyte concentration (sodium, potassium, chloride) and enzymes (leucocyte esterase, catalase and superoxide dismutase) pertaining to subclinical and clinical endometritis in buffaloes. In this context, the present study was designed to estimate the changes in physicaland analytical parameters of uterine discharge of SCE, CE affected in comparison with normal healthy buffaloes without endometritis.

*Corresponding author: thangamtamil19@gmail.com

MATERIALS AND METHODS

The lactating postpartum buffaloes with history of failure of conception even after three consecutive artificial inseminations (repeat breeder) presented to the Gynaecology Unit, NTR College of Veterinary Science, Gannavaram and buffaloes maintained at informal small dairy units located in and around Gannavaram, Krishna District, Andhra Pradesh were screened. The uterine discharge was collected by uterine flushing technique from all the selected buffaloes at the time of estrus as described by Nehru *et al.* (2019). Clear uterine discharge with less than 5% of PMN cells and clear uterine discharge with more than 5 percentage PMN on cytology considered as normal and SCE affected buffaloes, respectively. However, uterine discharges with flakes of pusand abundant PMN cells on cytology were considered as CE as described by Sheldon *et al.* (2009) and Gahlot *et al.* (2016). An attempt was made to estimate the physical (Turbidity, Specific gravity and Electrical resistance) and analytical characteristics (electrolytes, enzymes) of uterine discharge of normal (n=10) and endometritis (subclinical (n=10) and clinical (n=10)) affected buffaloes on the day of estrus when presented for treatment.

Physical characteristics of uterine discharge:

Turbidity: Turbidity of uterine discharge sample was measured by using digital Nephelo-Turbidity meter and expressed in Nephelometric turbidity unit (NTU).

Specific gravity (SG): Specific gravity of uterine discharge sample was measured by using digital specific gravity meter and expressed in g/cm^3.

Electrical resistance: Electrical resistance of uterine discharge sample was measured by using electrical conductivity meter as per procedure described by Ahmed *et al.* (2017) and expressed in Ohm (Ω).

Analytical characteristics of uterine discharge:

Electrolytes concentration: Electrolytes concentration of uterine discharge sample were measured by using Elyte kit (Tulip diagnostics Pvt. Ltd.,) and expressed in mmol/l.

Enzymes concentration:

Leucocyte esterase enzyme: Leucocyte esterase enzyme concentration of uterine discharge sample was assessed by using Leucocyte esterase ELISA Kit and expressed in ng/ml.

Catalase enzyme: Catalase enzyme concentration of uterine discharge sample was assessed as procedure described by Aebi (1983) and enzyme activity was calculated as mM H_2O_2 utilized/min/mg protein, using a molar extinction coefficient of 43.6 M-1 cm-1.

Superoxide dismutase (SOD) enzyme: SOD enzyme concentration was estimated as procedure described by Madesh and Balasubramanian (1998).

RESULTS AND DISCUSSION

The mean level turbidity was significantly (P≤0.01) higher in CE affected buffaloes (0.55±0.19) than SCE (0.14±0.31) affected and normal healthy buffaloes (0.05±0.00). However, the mean levels of turbidity were non-significantly (P>0.05) higher in the uterine discharge of SCE buffaloes (0.14±0.31) than normal (0.05±0.00) healthy buffaloes (Table 1). The mean levels of turbidity were significantly higher in CE affected buffaloes might be due to the presence of higher bacterial load and excessive levels of metabolites in the uterine lumen of buffaloes affected with different grades of endometritis (Bondurant, 1999 and Miller *et al.*, 2007). It was inferred that turbidity level in uterine discharge was increased in accordance with degree of uterine infection.

The mean specific gravity (SG) was significantly (P≤0.05) higher in CE affected buffaloes (1.10±0.00) than SCE (1.06±0.00) affected and normal healthy buffaloes (1.03±0.00). However, the mean SG was non-significantly (P>0.05) higher in the uterine discharge of SCE buffaloes (1.06±0.00) than normal (1.03±0.00) healthy buffaloes (Table 1). The mean levels of SG were significantly higher in clinical endometritis affected buffaloes correlated with presence of extra substances in the uterine discharge *viz.*, debris, cellular components, inflammatory exudates, bacterial metabolites and probably flakes of pus or purulent material (Machado *et al.*, 2012). It was inferred that SG could help the clinician to differentiate between SCE and CE when used in combination with turbidity assessment of uterine discharge sample.

The mean electrical resistance (ER) was significantly (P≤0.01) higher in CE affected buffaloes (180.09±7.71) than SCE (148.15±8.43 Ù) affected and normal healthy buffaloes (132.06±7.80). However, the mean ER was non-significantly (P>0.05) higher in the uterine discharge of SCE buffaloes (148.15±8.43) than normal (132.06±7.80) healthy buffaloes (Table 1). The mean electrical resistance(ER) was significantly higher in CE affected buffaloes might be due to the presence excessive inflammatory products in the uterine discharge.

Collectively, the mean concentrations of turbidity, SG and ER was significantly (P<0.05) higher in CE affected buffaloes as compared to SCE affected and normal healthy buffaloes. However, mean concentrations of turbidity, SG and ER were non-significantly (P>0.05) higher in the uterine discharge of SCE buffaloes than normal healthy buffaloes.

The mean concentrations of sodium was significantly (P≤0.05) lower in CE affected buffaloes (82.22±5.04) than SCE (104.19±3.94) affected and normal healthy buffaloes (118.19±6.27). However, mean concentrations of sodium was non-significantly (P>0.05) lower in the uterine discharge of SCE buffaloes (104.19±3.94) as compared to normal (118.19±6.27) healthy buffaloes. The mean concentrations of potassium was significantly (P≤0.05) higher in CE affected buffaloes (11.59±1.11) than normal healthy buffaloes (7.82±0.56), while the potassium concentration was moderately higher in SCE (9.10±1.05) affected buffaloes as compared to normal healthy buffaloes (7.82±0.56) and moderately lower as compared to CE affected (11.59±1.11) buffaloes. The mean concentrations of chloride was significantly (P≤0.05) higher in CE affected buffaloes (36.18±3.10) as compared to SCE (27.82±1.36) affected and normal healthy buffaloes (24.06±1.69). However, the mean concentrations of chloride was non-significantly (P>0.05) higher in the uterine discharge of SCE buffaloes (27.82±1.36) than normal (24.06±1.69) healthy buffaloes (Table 2). Higher concentrations of chloride and potassium in the uterine discharge of the present study reflected the increased concentration of electrolytes present in the lumen of the uterus in relation to degree of uterine infections as a result of endometrial insult in cyclical animal as stated by Igarashi (1954) and Heap (1962).

Table 1. The physical characteristics of uterine discharge (Mean±SE) in normal, subclinical and clinical endometritic buffaloes

Physical characteristics	Normal (n=10)	Subclinical endometritis (n=10)	Clinical endometritis (n=10)	F-value
Turbidity (NTU)	0.05±0.00^{b}	0.14±0.31^{b}	0.55±0.19^{a}	5.667
Specific gravity (g/cm^{3})	1.03±0.00^{b}	1.06±0.00^{b}	1.10±0.00^{a}	28.141
Electrical resistance (Ω)	132.06±7.80^{b}	148.15±8.43^{b}	180.09±7.71^{a}	9.357

Means bearing different superscripts (ab) differ significantly (P≤0.01 or P≤0.05) within a row

Table 2. The electrolyte concentration (Mean±SE) in uterine discharge of normal, subclinical and clinical endometritic buffaloes

Electrolyte concentration	Normal (n=10)	Subclinical endometritis (n=10)	Clinical endometritis (n=10)	F-value
Sodium(mmol/l)	118.19±6.27^{a}	104.19±3.94^{a}	82.22±5.04^{b}	12.259
Potassium(mmol/l)	7.82±0.56^{b}	9.10±1.05^{ab}	11.59±1.11^{a}	4.145
Chloride(mmol/l)	24.06±1.69^{b}	27.82±1.36^{b}	36.18±3.10^{a}	8.044

Means bearing different superscripts (ab) differ significantly (P≤0.01 or P≤0.05) within a row

Table 3. The enzymes concentration (Mean±SE) in uterine discharge of normal, subclinical and clinical endometritic buffaloes

Electrolyte concentration	Normal (n=10)	Subclinical endometritis (n=10)	Clinical endometritis (n=10)	F-value
Leucocyte esterase (ng/ml)	0.17±0.01^{b}	0.24±0.05^{ab}	0.28±0.07^{a}	5.951
Catalase (µmol of H_2O_2 Consumed/min/mg of protein)	52.94±3.74^{b}	63.17±4.88^{ab}	68.89±3.94^{a}	3.662
Superoxide dismutase (Units/min/mgof protein)	4.65±0.21^{b}	4.10±0.28^{b}	2.61±0.30^{a}	15.032

Means bearing different superscripts (ab) differ significantly ≤0.01 or P≤0.05) within a row

On the whole, the mean electrolyte concentrations (potassium and chloride) were significantly altered in CE affected buffaloes as compared to SCE affected and normal healthy buffaloes. However, the mean concentrations of potassium and chloride (mmol/l) were not much altered in the uterine discharge of SCE buffaloes than normal buffaloes.

The mean concentrations of leucocyte esterase enzyme (LE) were significantly (P≤0.05) higher in CE affected buffaloes than SCE affected and normal healthy buffaloes, (Table 3). The mean concentrations of catalase enzyme were significantly (P≤0.05) higher in CE affected buffaloes than SCE affected and normal buffaloes (Table 3).

The mean concentrations of SOD were significantly (P≤0.05) lower in CE affected buffaloes than normal and SCE affected buffaloes. However, mean concentrations of SOD were not much altered in the uterine discharge of subclinical endometritic buffaloes and normal healthy buffaloes (Table 3). Enzyme concentrations in uterine discharges of normal, SCE and CE affected buffaloes were increased in concentration especially that of LE (ng/ml) and catalase (µmol) enzymes, which increased with severity of uterine infection. While, SOD activity was decreased with increase in severity of uterine infection.

The results of present study were comparable with the reports of Gahlot *et al.* (2017) who observed LE concentrations (IU/L) in uterine fluid were non-significantly differed between CE buffaloes and normal buffaloes, similarly between SCE and normal buffaloes. Variations in the concentration of LE in the present study might be due to the activity of LE, an enzyme produced from neutrophils and was elevated in inflammatory exudate (Cheong *et al.*, 2012, Couto *et al.*, 2013 and Nazhat *et al.*, 2018). Increased concentration of catalase enzyme in CE culminated to the generation of free radicals, which in turn aggravated the inflammatory reaction resulting in reduction of uterine muscle activity and reduced the uterine muscle tone causing build-up of inflammatory products in the lumen of the uterus, thus

increased the severity of uterine inflammation (Li *et al.*, 2010). Further, these changes adversely affected the life of spermatozoa during its transport through the uterus eventually resulting in fertilization failures as opined by Zhong and Zhou (2013). SOD activity decreased with increase in severity of uterine infection in the present study was in conformity with the investigation of Kaya *et al.* (2017) who reported that total antioxidant capacity decreased in endometritis affected animals and inferred that stress related enzymes could be used as biochemical markers for assessment of the severity of endometritis in bovines.

CONCLUSION

Estimation of physical and analytical parameters of uterine discharge could be novel non-invasive method for diagnosis of degree of endometritis in buffaloes as Turbidity, SG, ER, LE and catalase enzymes concentrations increases however SOD activity decreases with the severity of uterine infection.

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