Indian J Dairy Sci 76(2): 184-187
https://doi.org/10.33785/IJDS.2023.v76i02.013

SHORT COMMUNICATION

Risk association of metabolites and immune response mediator indicators with the occurrence of retained placenta in Murrah buffaloes (*Bubalus bubalis*)

Bhabesh Mili[1] and Sujata Pandita[2]

Received: 03 August 2022 / Accepted: 26 September 2022 / Published online: 20 April 2023

Abstract: This study was designed to investigate the risk association of metabolites and immune response mediator indicators with the occurrence of retained placenta (RP) in Murrah buffaloes (*Bubalus bubalis*). A total of six (n=6) healthy pregnant Murrah buffaloes and four (n=4) buffaloes suffering from RP were selected from the ICAR-NDRI cattle herd. Blood samples were collected from each healthy buffalo on days -56,-48,-42,-35, -28, -21, -14, -7, 0, +7, +14, +21, +28, +35, +35, +42, +48, +56 relative to calving. Blood samples were also collected from the buffaloes on the day of diagnosis of the RP as well as on an alternate day. The result revealed that plasma β-Hydroxybutyric acid (β-HBA) and nitric oxide (NO) levels were significantly ($P< 0.05$) higher in buffaloes suffering from RP compared to normal parturient buffaloes. The level of IgG was significantly ($P<0.05$) lowered in buffaloes suffering from RP (8.84±0.85 mg/mL) compared to normal parturient buffaloes (17.41±1.68 mg/mL). However, plasma Non-esterified fatty acids (NEFA), glucose, Blood Urea Nitrogen (BUN), calcium, Interleukin 6 (IL-6), and Total Antioxidant Activities (TAA) levels were non-significant differences between buffaloes suffering from RP and normal parturient buffaloes. A binary logistic regression assay revealed the positive association of postpartum β-HBA with RP (Odds ratio= 1.85). Overall results suggest that plasma β-HBA, IgG, and NO can be used as screening biomarkers during the transition period for the risk assessment of RP in buffaloes.

Keywords: Buffaloes, β-HBA, IgG, Nitric oxide, Retained placenta

The retained placenta is a common multifactorial postpartum reproductive disease manifesting as failure to expel fetal membranes within 12 hours of calving. RP causes huge financial loss to the dairy industry due to increasing the risk of postpartum infections, infertility, and reduced milk yield and quality (Moretti et al. 2015; Mahnani et al. 2020; Li et al. 2021). The etiology and pathogenesis of RP has been investigated extensively by many researchers to explore early diagnosis in dairy cattle. Many studies have also confirmed that changes in blood metabolites, cytokines, inflammatory factors, immune factors, and hormones are associated with the pathogenesis of RP (Esposito et al. 2014; Moretti et al. 2015; Lu et al. 2020, Mili and Pandita 2021; Li et al. 2021). The changes in metabolism, immune response mediators, and hormonal variables during the transition period are part of homeorhesis in buffaloes (Mili et al. 2014; Mili et al. 2015a; Mili et al. 2015b). The detection of blood biochemical indicators are the most common method for predicting and screening diseases. Hence, the present study aimed to find the risk association of the key metabolites and immune response mediators with the occurrence of RP in buffaloes.

The present experiment was conducted between September 2011 till May 2012 at ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana. The institute is located at an altitude of 250 m above mean sea level, latitude, and longitude positions 29°42N and 79°54E, respectively. The maximum ambient temperature in summer goes up to 45°C, and the minimum temperature in winter comes down to 0°C with a diurnal variation in the order of 15-20°C. The average annual rainfall is 700 mm from early July to mid-September.

A total of six (n=6) numbers of healthy pregnant Murrah buffaloes and retained placenta (n=4) were selected from the institute cattle herd. The buffaloes that did not shed the fetal membrane within 12 hours of parturition were considered cases of RP. All these

[1]Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, CAU (I), Jalukie, Peren-797110, Nagaland, India
E-mail: bhabamili@gmail.com
[2]Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India
E-mail:- sujata.pandita@rediffmail.com

Bhabesh Mili (✉)
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, CAU (I), Jalukie, Peren-797110, Nagaland, India
E-mail: bhabamili@gmail.com

buffaloes were maintained under general managemental practices as followed at the institute.

A blood sample (15ml) was drawn in sterile heparinized vacutainer tubes by jugular venipuncture from each healthy buffalo on days -56, -49,-42,-35,-28, -21, -14, -7, 0, +7, +14, +21, +28, +35,+42, +49, +56 relative to calving. Also, blood sample was collected on the day of diagnosis of the RP and as well as on an alternate day. The heparinized samples were centrifuged at 3000 rpm for 15 minutes, plasma aliquot, and stored at -20°C for further analysis.

NEFA levels were quantified as per the copper soap solvent extraction method modified by Shipe et al. (1980). β-HBA was estimated by "β-Hydroxybutyrate (Ketone Body) Assay Kit -1" of Cayman Chemical Company, Ann Arbor USA as per manufacturer instruction. Glucose, BUN, albumin, and calcium levels were quantified using GOD-POD kits obtained from Span Diagnostics Ltd, respectively as per manufacture instructions.

IgG, IL-6, and TAA levels were estimated by using "Bovine IgG ELISA kit" procured from Koma Biotech Inc., Gangseo-gu Seoul, Korea, bovine interleukin-6 ELISA Kit" procured from Cusabiotech and Antioxidant assay kit purchased from Cayman Chemical Company, Ann Arbor USA respectively as per the instructions provided with the assay kits. The NO levels were quantified using a modified Griess reaction as described by Shoker et al. (1997).

All the values were expressed as mean ± standard error (SEM). The data for healthy buffaloes were analyzed by one-way analysis of variance using a graph prism version 5 to quantify postpartum variations for peripheral levels of NEFA, β-HBA, glucose, calcium, BUN, albumin, IgG, IL-6, NO, and TAA. Since postpartum variations were not statistically significant between days (Already published by Mili et al. 2014 & Mili et al. 2015a), the data for each parameter was clubbed. This served as the reference value for healthy buffaloes for binary logistic regression assay to evaluate the risk association of metabolic variables and immune response mediator indicators with the occurrence of RP in SAS software (7.0 versions). Also, the unpaired student "t" test using graph prism version 5 was applied to compare the data of normally calved buffaloes (day-0) and RP.

The changes in plasma β-HBA, NEFA, glucose, BUN, albumin and calcium levels in buffaloes suffering from RP compared to normal parturient buffaloes is presented in Table 1. Plasma NEFA level was non-significantly very high in buffaloes suffering from RP (418.49±34.00 µmol/L) compared to normal parturient buffaloes (406.69±20.60 µmol/L) with an odds ratio of 0.002. The odd ratio indicated a negative association of plasma NEFA with the occurrences of RP in buffaloes. High concentrations of serum NEFAs have been associated with an increased incidence of periparturient diseases (retained fetal membranes, ketosis, and mastitis), displacement of the abomasum and immune-suppression in dairy cattle (Leblanc et al. 2005). Plasma β-HBA level was significantly high (P<0.05) in RP (591.20±32.74 µmol/L) compared to normal parturient buffaloes (301.61±32.74) with an odds ratio of 1.85. The odds ratio indicated a positive association of plasma β-HBA with the occurrences of RP in buffaloes. Our results were in agreement with previous studies (Seifi et al. 2007; Lazlo et al. 2009; Quiroz-Rocha et al. 2009). Seifi et al. (2007) reported greater concentrations of plasma NEFA and β-HBA in cows with RP. Elevated NEFA and ketone bodies are metabolic indicators of increased risk for RP in cows (Lazlo et al. 2009; Quiroz-Rocha et al. 2009).

Plasma glucose level was no significant association (47.61±2.94 mg/dL in RP buffaloes vs- 47.81±2.32mg/dL normally calving buffaloes) with the occurrence of RP (odds ratio= 0.002) in buffaloes. In contrast to our results, Mandali et al. (2002) and

Table 1 Plasma metabolite concentration in buffaloes exhibiting retained placenta

Parameters	Day 0(Normally calved)	RP(After 12 h of parturition)
NEFA (µmol/L)	406.69±20.60	418.49±34.00
β-HBA (µmol/L)	301.61±32.74^{A}	591.20±32.74^{B}
Glucose (mg/dL)	47.81±2.32	47.61±2.94
BUN (mg/dL)	21.24±2.17	13.72±0.82
Calcium (mg/mL)	6.24±0.24	5.40±0.58

Bearing superscripts AB in rows differ significantly (P < 0.05) from each other

Table 2 Plasma immune response mediators in buffaloes exhibiting retained placenta

Para-meters	Day 0(Normally calved)	RP(After 12h of parturition)
IgG (mg/mL)	17.41±1.68^{A}	8.84±0.85^{A}
IL-6 (pg/mL)	25.00±4.33	19.72±1.29
TAA (mmo/L)	1.88±0.16	1.15 ±0.09
Nitric oxide (µmol/L)	50.88 ±1.41^{A}	60.97 ±2.96^{B}

Bearing superscripts AB in rows differ significantly (P < 0.05) from each other

Pandey et al. (2009) reported significantly lower (P<0.05) glucose levels in buffaloes with RP than in normal parturient buffaloes.

The plasma calcium level was not significantly lower in buffaloes suffering from RP (5.40±0.58 mg/mL) compared to normal parturient buffaloes (6.24±0.24 mg/mL). This result was in agreement with the earlier reports (Abo El Maaty et al. 2021). In contrast to our results, various reports revealed that inadequate calcium concentrations are the predisposed risk factor for occurrences of RP in cattle and buffalo (Hashem and Amer 2008; Pandey et al. 2009). Mohanty et al. (1994) suggested that probably less availability of glucose and calcium to the uterine tissues results in atony of the uterus, with decreased contraction and hence the retention of fetal membranes. Calcium deficiency could act as a predisposing factor for uterine inertia leading to dystocia, RP, and metritis (Mohanty et al. 1994).

Plasma BUN level was not significantly lower in buffaloes suffering from RP (13.72±0.82 mg/ dL) compared to normal parturient ones (21.24±2.17mg/dL) with an odds ratio of 0.002. Our result was in agreement with previous studies on dairy cows (Lu et al. 2020). However, they predicted that serum BUN levels above 10.25 mg/ dL on day 7 relative to parturition are the predisposing risk factors for occurrences of RP in cows (Lu et al. 2020).

The changes in immune response mediator indicators IgG, IL-6, TAA, and NO levels in buffaloes suffering from RP compared to normal parturient buffaloes is presented in Table 2. The level of IgG was significantly (P<0.05) lower in buffaloes suffering from RP (8.84±0.85 mg/ mL) compared to normal parturient buffaloes (17.41±1.68 mg/mL). However, the TAA level was non significantly low in buffaloes suffering from RP (1.15 ±0.09 mmol/L) compared to normal parturient buffaloes (1.88±0.16 mmol/L). Mili et al. (2015a) reported a gradual drop in IgG and TAA levels in buffaloes from day 56 before parturition to the lowest levels on the day of calving. Hence, reduced availability of antioxidant defenses, gradual changes of humoral immune response (IgG and IgM) to a cell-mediated immune response with progressive oxidation of the cell membrane during the transition period may contribute to periparturient disorders including RP in dairy cows (Miller et al. 1993; Gitto et al. 2002).

Plasma IL-6 levels were also non significantly low in buffaloes suffering from RP (19.72±1.29 pg/mL) compared to normal parturient buffaloes (25.00±4.33 pg/mL). Our result was in agreement with the earlier reports (Dervishi *et al.* 2016). They revealed that the overall serum IL-6 concentration was not different between RP and normal parturient cows. But, serum IL-6 concentration in RP cows at 8 weeks before parturition was significantly higher than normal parturient cows. IL-6 plays a significant role during the transition from innate to adaptive immunity. Elevated levels of both IL-1 and IL-6 suggested the presence of an inflammatory insult. Hence, deviations from the normal reference ranges of both IL-1 and IL-6 during the transition period are the risk indicators of the development of RP in dairy cows (Dervishi *et al.* 2016).

The plasma nitric oxide levels were significantly (P<0.05) high in buffaloes suffering from RP (60.88±2.96 µmol/L) compared to normal parturient buffaloes (50.88 ±1.41 µmol/L). This result was in agreement with the earlier reports (Abo El Maaty *et al.* 2021). They reported significantly higher NO in cows suffering from RP (33.49±5.80µmol/L) compared to normal parturient cows (26.83±1.81µmol/ L).

Conclusion

It was concluded that alteration of plasma metabolites and humoral immune response coupled with a low antioxidant defense system as monitored by β-HBA, IgG, and NO levels during the transition period might be the risk indicators with the occurrence of retained placenta in buffaloes. However, large-scale studies are required to determine the crucial threshold levels of these attributes to trace the onset/ early diagnosis of RP in buffaloes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thankfully acknowledge the Director, ICAR – National Dairy Research Institute, Karnal, Haryana, India, for the research grant to conduct this study.

References

Abo El Maaty AM, Aly MA, Kotp MS, Ali AH, El Gabry MA (2021) The effect of Seasonal heat stress on oxidants–antioxidants biomarkers, trace minerals and acute phase response of peri parturient Holstein Friesian cows supplemented with adequate minerals and vitamins with and without retained fetal membranes. Bull Natl Res Cent 45:8

Dervishi E, Zhang G, Hailemariam D, Dunn SM, Ametaj BN (2016) Occurrence of retained placenta is preceded by an inflammatory state and alterations of energy metabolism in transition dairy cows. J Anim Sci Biotechnol 7:26

Esposito G, Irons PC, Webb EC, Chapwanya A (2014) Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim Reprod Sci 144:60–71

Gitto E, Reiter RJ, Karbownik M, Tan DX, Gitto P, Barberi S, Barberi I (2002) Causes of oxidative stress in the pre- and perinatal period. Biol Neonate 81 146- 157

Hashem MA, Amer HA (2008) Hormonal and biochemical anomalies in dairy cows afected by retained fetal membranes. Int J Vet Med 185:1517–1519

Lazlo K, Otto S, Viktor J, Laszlone T, Beckers JF, Endre B (2009) Examination of some reproductive indices of peripartal period in relation with energy metabolism in dairy cows. Magyar Allatorvosok Lapja 131:259–69

Leblance S J, Leslie K E, Duffieid T D (2005). Metabolic predictors of displaced abomasum in dairy cattle. J Dairy Sci 88:159-170

Lu W, Sun H, Xu M, Luo Y, Jin J, Hongze Shaod Zheng-Mei Xua, Shaoa L, Fua S, Jin C (2020) Blood urea nitrogen may serve as a predictive indicator of retained placenta in dairy cows. Anim Reprod Sci 218:106481

Li Y, Zhao Z, Yu Y, Liang X, Wang S, Wang L, Cui D, Huang M (2021) Plasma Metabolomics Reveals Pathogenesis of Retained Placenta in Dairy Cows. Front Vet Sci 8: 1-12

Mahnani A, Sadeghi-Sefidmazgi A, Ansari-Mahyari S, Ghorbani GR, Keshavarzi H (2021) Farm and cow factors and their interactions on the incidence of retained placenta in holstein dairy cows. Theriogenology 159:87–97

Mandali GC, Patel PR, Dhami AJ, Raval SK, Christi KS (2002) Biochemical profile in buffaloes with periparturient reproductive and metabolic disorders. Indian J Anim Reprod 23: 130-134

Mili B, Pandita S, Bharath Kumar BS, Parmar MS (2015b) Changes in Hormones of Somatotropic Axis during Transition Period in Murrah Buffaloes (*Bubalus bubalis*) Supplemented with Vitamin E. J Anim Res 5: 27-30

Mili B, Pandita S, (2021) Changes in hormones of the somatotropic axis associated with postpartum reproductive infections in Murrah buffaloes (Bubalus bubalis). Indian J Dairy Sci 74: 1-7

Mili B, Pandita S, Mohini M, Ashutosh M, Bharath Kumar BS (2014) Effect of vitamin E supplementation to dry Murrah buffaloes on dry matter intake, body condition score, Metabolic shifts pre and postpartum. Indian J Anim Res 48: 556-563

Mili B, Pandita S, Mohini M, Ashutosh M, Kumar BBS (2015a) Effect of vitamin E supplementation on antioxidant status and selective humoral and cellular immune responses in peripartuient buffaloes. Indian J Anim Sci 85: 853–855

Miller JK, Brzezinska-Slebodzinska E, Madsen FC (1993). Oxidative stress antioxidants and animal function. J Dairy Sci 76:2812

Mohanty KC, Mohanty BN, Ray S K H, Mohanty DN (1994) Levels of glucose, calcium and alkaline phosphatase in blood with relation to retention of placenta in bovines. Indian J Anim Reprod 15: 21-23

Moretti P, Probo M, Morandi N, Trevisi E, Ferrari A, Minuti A (2015) Early post-partum hematological changes in Holstein dairy cows with retained placenta. Anim Reprod Sci 152:17–25

Pandey AK, Shukla SP, Pandey SK, Sharma YK (2009) Haemato-biochemical profile in relation to normal parturient buffaloes and buffaloes with retained fetal membrane. Buffalo Bull 26: 46-49

Quiroz-Rocha GF, LeBlanc S, Duffield T, Wood D, Leslie KE, Jocobs RM (2009) Evaluation of prepartum serum cholesterol and fatty acids concentrations as predictors of postpartum retention of the placenta in dairy cows. J Am Vet Med Assoc 234:790–793

Seifi HA, Dalir B, Farzaneh N, Mohr M, Gorji- Dooz M (2007) Metabolic changes in cows with or withour retain fetal membranes in transition period. J Vet Med 54:92–7

Shipe WF, Senyk GF, Fountain KB (1980) Modified copper soap solvent extraction method for measuring free fatty acids in milk. J Dairy Sci 63: 193-198

Shoker AS, Humanly Yang Murabit M A, Hadeeah J, AL- Ghoul A, Kamal O (1997). Analysis of *in vitro* effect of exogenous nitric oxide on human lymphocytes. Mol Cell Biochem 171:75-83