Oxidative stress molecules as indicators of uterine health in Murrah buffaloes during peripartum period

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Abstract: The present study was carried out to know the changes in concentration of oxidative stress molecules in peripheral blood during peripartum period in relation to uterine health in Murrah buffaloes (n=24). Blood samples were collected from day -7 to day +35 of calving and serum was used for analysis. Based on assessment of uterine fluid scoring, trans-rectal USG, clinical signs uterine health of buffaloes were determined and they were classified into healthy and uterine infected groups. Results indicated that nitric oxide (NO) concentration was significantly (P<0.05) higher in serum during peripartum period i.e. on day -7 to +35, whereas malondialdehyde (MDA) concentration in serum was elevated throughout peripartum period in uterine infected Murrah buffaloes. Total antioxidant capacity (TAC) was significantly higher (P<0.05) on day -7, 0, +7, +14, +21, +35 postpartum in healthy buffaloes as compared to uterine infected buffaloes. From this study it can be concluded that in Murrah buffaloes NO and TAC in serum may be used as promising indicators for predicting uterine health before onset of clinical infections.

Keywords: Nitric oxide (NO), Malondialdehyde (MDA), Total antioxidant capacity (TAC)

Introduction
India is considered as the dairy hub of the world as it holds the largest stocks of cattle and buffaloes. But the poor production performance is associated with compromised reproductive efficiency such as peripartum uterine infection, infertility, subfertility, anestrus, delayed onset of puberty, mastitis, metritis, endometritis, 1; whereas the incidence of metritis, endometritis and subclinical endometritis has been reported to be about 20, 20 and 30%, respectively (Markusfeld, 1987; LeBlanc et al. 2002; Kasimanickam et al. 2014; Goshen and Shpigel, 2006; Hammon et al. 2006). Presence of high progesterone (Tizard, 1991) during gestation period and high cortisol around parturition (Magnusson and Fossum, 1992) results in immune suppression and animals become susceptible to various infectious diseases during this stage (Castillo et al. 2005). Further it has been reported that during peripartum period there is enhanced production of reactive oxygen and nitrogen species (ROS and RNS) (Rizzo et al. 2012) which cause damage of macromolecules such as proteins, lipids and DNA (Trevisan et al. 2001) and it is controlled by cellular antioxidant defence systems. Oxidative stress results when ROS are produced faster than their neutralization by antioxidant mechanisms (Trevisan et al. 2001). There are reports that during the peripartum period oxidative stress is a major threat and the incidence of health problems is clearly a huge complicating factor for subsequent reproductive performance. As a part of immune response phagocytes produce Nitric oxide (NO) during inflammatory diseases. Productions of NO occurs as a result of inflammation and is produced by a wide range of cells including macrophages, neutrophils, epithelial and endothelial cells and have an important role in immunity and inflammation (Frean et al. 1997; Korhonen et al. 2005). A well-established mechanism of oxidative damage caused by reactive oxygen species is Lipid per-oxidation and estimation of the melondialdehyde (MDA) provides a convenient index of lipid peroxidation (Nielsen et al. 1997). When MDA reacts with thiobarbituric acid formation of a red pigment occurs in the form of thiobarbituric acid reactive substance (TBARS) which is measured by spectrophotometry (Janero, 1990). By measuring the total antioxidant capacity (TAC) of body the plasma antioxidant status can be measured. It is the outcome of the interaction of many different compounds and systemic metabolic...
interactions (Ghiselli et al. 2001). Early prediction of diseases before occurrence of clinical signs will be helpful in improving management strategies and productivity of Murrah buffaloes.

Keeping this in view the present study was undertaken to examine the changes in concentration of oxidative stress molecules NO, MDA and TAC in peripheral blood of Murrah buffaloes during peripartum period.

Materials and Methods

Location and climatic conditions

The present research was conducted at Livestock Research Centre (LRC), National Dairy Research Institute (NDRI), Karnal, Haryana, India. The experimental duration was mid September to end of May, 2018. Climatic condition of the place touches both the extremes i.e. cold (approximately 3°C) and hot (approximately 48°C). Relative humidity ranges between 15 and 80%. Average annual rainfall is about 90 to 120cm.

Experimental animals and collection of blood samples

Twenty four Murrah buffaloes of 2nd parity having body weight around 400kg were selected at peripartum period (10 days before expected date of calving to 35 days after calving). Optimum conditions were maintained for all experimental animals and were kept in general herd in open housing system. Blood samples were collected on day -7, 0, 7, 21 and 35 of calving from jugular vein of all the experimental animals both in anticoagulant containing vacuum tubes for serum separation and non vacuum coagulation tubes for differential count of blood. After that coagulation tubes are kept in slanting manner for 1hr and serum was separated by centrifugation at 3000rpm for 10 minutes at 4°C. Serum samples were stored at -20°C for further analysis of different parameters. Uterine fluid was collected using blue sheath (IMV technology, France) fitted in Universal AI gun by inserting it into vagina till it reaches the uterine horn guided by per rectal palpation on day +7, +14, +21, +35 of parturition and it was scored as the method described by Sheldon et al. 2009. Buffaloes which had undergone normal puerperium without any postpartum complications were classified as healthy animals.

Estimation of NO concentration by modified Greiss method

Using modified Griess reaction by Shoker et al. (1997) the NO concentration in serum was estimated. Precipitation of serum protein was done using acetonitrile (1:1 volume) and vortexed thoroughly, followed by holding at room temp for 1hr. Then centrifugation was done at 7000rpm for 5min and the supernatant was transferred to a 2ml eppendorf tube followed by evaporation at 37°C. Adding equal amount of milli Q water (same as serum sample taken initially for deproteinization) the contents were kept in room temperature for 1hr to ensure complete solubilisation. Standard was prepared using 1.56 to 100µM concentration of Sodium nitrate (NaNO₂). For estimation of NO 100µl of samples and standards were taken in the wells of 96 well flat bottomed plate and 100µl vanadium chloride was added in each well following addition of 100µl Greiss reagents (Greiss 1 reagent; 2% sulphanilamide in 5% HCl and Greiss 2 reagent; 0.1% N-Naphthylethylenediamine dihydrochloride). Then the plate was kept in incubator for 30 min. at 37°C and absorbance was taken at 540nm wavelength in spectrophotometer (TECAN, Seestrasse 103, Switzerland). Using linear regression equation the final concentration of nitric oxide (µM) in samples was estimated.

Estimation of serum malondialdehyde (MDA) using TBARS method

Malondialdehyde (MDA) in serum was estimated by the method of Kaushal and Kansal (2012). MDA concentration was measured by this method due to its reactivity with thiobarbituric acid (TBA) in acidic conditions to generate a pink colour chromophore which was read at 535nm. 0.2ml of serum was added to 2.8ml of TCA (Trichloro acetic acid):TBA:HCl reagent solution and the mixed solution was heated for 15 min. in boiling water. Then the contents were mixed vigorously after cooling in room temperature. After centrifuging at 1000rpm for 10 min at room temperature supernatant was taken in cuvet and absorbance was taken at 535nm against the blank. A series of standard solution (8-40nmol) were also treated in similar manner. The MDA content was calculated from the calculation curve and expressed as nmoles/ mg protein (1,1,3,3- tetraethoxypropane was used as a standard).

Estimation of Total antioxidant capacity (TAC) using ELISA kit:

Standards were prepared, optical densities of samples were taken and TAC was calculated as per the protocol given in the ELISA kit (CAYMAN total antioxidant assay kit).

Statistical analysis

Descriptive statistics were calculated for different biochemical and blood parameters for both healthy and uterine infected group and the results were expressed as mean± SE. Within group comparisons were performed using independent sample T test. One way ANOVA was used to compare between groups. Group wise multiple comparisons were performed using Tukey’s post hoc test. The difference of means was considered significant when the probability (P value) was <0.05. All the analysis was performed using IBM SPSS Statistics 22, Prism.

Results and Discussion

Based on the uterine discharge scoring and per rectal examination the Murrah buffaloes were classified into healthy and uterine infected groups. The healthy group consisted of animals that had undergone normal puerperium without development of uterine infection. Buffaloes with mucopurulent or purulent or fetid uterine discharge during postpartum period i.e. up to day 35...
postpartum with or without systemic signs were classified as uterine infected animals. In Murrah buffaloes (n=24), a total number of 11 were healthy and 13 animals developed uterine infection.

Nitric oxide (NO)

The mean serum NO concentration was significantly (p<0.05) higher on day -7 and 0 as compared to other days of sampling period i.e. on day +7, +14, +21 and +35 in healthy group of buffaloes. When comparison was made between two groups, serum NO concentration was significantly higher (P<0.05) on day 0, +7, +14, +21 and +35 in uterine infected buffaloes as compared to healthy buffaloes. The pattern of changes in NO concentration as observed in the present study was similar to the observations of previous studies in Sahiwal cows (Baithalu et al. 2016). Enhanced NO level has been observed in uterine infected buffaloes than in healthy buffaloes (Mili and Pandita, 2014). In the present study, the elevated levels of NO from the day of parturition to day 35 in serum might have shown the damaging effect on uterine health resulting into uterine infection.

Malondialdehyde (MDA)

The mean serum MDA concentration was significantly (p<0.05) higher on days 0, +7, +14, +21 and +35 in healthy group of buffaloes when compared with uterine infected buffaloes. When comparison was made across the days in healthy group of buffaloes, serum MDA concentration although decreased (P>0.05) on day 0 from that of day -7 but remain elevated throughout postpartum period. However, in uterine infected buffaloes TAC concentration was significantly decreased (P<0.05) on day 0 from that of day -7 and concentration elevated afterwards and maximum concentration was obtained on day +35. Ghiselli et al. (2001) reported the decreased levels of TAC to provide information regarding the dynamic equilibrium between pro-oxidants and antioxidants in the plasma. Reduction in the level of antioxidants renders system incapable to protect the cellular components resulting in reduced immunity and onset of various infections (Miller et al. 1993; Sordillo and Atiken, 2009). The pattern of changes in TAC concentration observed in the present study was similar to the observations made by earlier workers (Castillo et al. 2005; Baithalu et al. 2016).

Conclusions

From the above study it can be concluded that higher concentration of TAC and lower production of MDA and NO in healthy buffaloes indicate better anti-oxidant status to combat primary immunosuppression problems resulting into uterine infection.

| Group          | Day -7 | Day 0 | Day +7 | Day +14 | Day +21 | Day +35 |
|----------------|--------|-------|--------|---------|---------|---------|
| Healthy buffaloes | 22.24±5.90 | 13.82±1.75 | 8.77±0.87 | 6.6±0.61 | 6.29±0.94 | 4.32±0.72 |
| Infected buffaloes | 28.88±3.28 | 25±2.29 | 25.92±2.62 | 28.47±2.29 | 26.47±2.53 | 34.45±6.98 |
| Healthy buffaloes | 2.09±0.29 | 2.42±0.56 | 2.00±0.56 | 1.94±0.34 | 1.26±0.15 | 1.16±0.25 |
| Infected buffaloes | 7.45±0.84 | 9.10±1.32 | 9.81±1.54 | 10.80±2.08 | 11.96±1.66 | 11.02±1.36 |
| Healthy buffaloes | 127.86±12.36 | 113.01±4.92 | 148.20±14.51 | 121.72±9.01 | 138.09±13.88 | 172.78±31.02 |
| Infected buffaloes | 102.28±5.56 | 68±8.04 | 93.68±7.35 | 96.23±5.24 | 102.94±5.79 | 113.20±7.15 |

Means bearing different superscripts (A,B,C,D) in column and superscripts (a,b,c,d) in row differs significantly (P<0.05)
against oxidative stress. On the other hand, the higher concentration of serum NO and MDA in buffaloes that developed uterine infection indicate damaging effects on uterine health. Further it may be concluded that serum NO and TAC can be used as indicators for monitoring of uterine health in Murrah buffaloes.

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References
Baithalu RK, Singh SK, Kumaresan A, Mohanty AK, Mohanty TK, Kumar S, Kerketta S, Maharana BR, Patbandha TK, Attupuram N, Agarwal SK (2016) Transcriptional abundance of antioxidant enzymes in endometrium and their circulating levels in Zebu cows with and without uterine infection. Anim Reprod Sci 177: 79-87
Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL (2005) Oxidative status during late pregnancy and early lactation in dairy cows. Vet J 169:286–292
Frean SP, Bryant CE, Fröling IL, Elliott J, Lees P (1997) Nitric oxide production by equine articular cells in vitro. Equine Vet J 29: 98-102
Ghiselli A, Serafini M, Natella F, Scaccini C (2001) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. In Bio-Assays for Oxidative Stress Status 219-227
Goshen T, Shpigel NY (2006) Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. Theriogenology 66: 2210-2218
Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL (2006) Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopath 113: 21-29
Heidarpour M, Mohri M, Borji H, Moghdass E (2012) Oxidative stress and trace elements in camel(Camels dromedarius) with liver cystic echinococcosis. Vet Parasitol187: 459-463
Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury . Free Radic Biol Med 9: 515-540
Kasimanickam R, Asay M, Schroeder S, Kasimanickam V, Gay JM, Kastelic JP, Hall JB, Whittier WD (2014). Calm temperament improves reproductive performance of beef cows. Reprod Domest Anim 49: 1063-1067
Kaushal D, Kansal VK (2012) Probiotic Dahi containing Lactobacillus acidophilus and Bifidobacterium bifidum alleviates age-inflicted oxidative stress and improves expression of biomarkers of ageing in mice. Mol Biol Rep 39: 1791-1799
Kaya S, Üdün M, Özen H, Kuru M, Şahin L, Küürt A, Kacak C (2017) The Impact of Endometritis on Specific Oxidative Stress Parameters in Cows. J Vet Med Soc 68: 231-236
Korhonen R, Lahti A, Kankaanranta H, Moilanen E (2005) Nitric oxide production and signaling in inflammation. Curr Drug Targets-Inflamm Allergy 4: 471-479
LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH (2002) Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci 85: 2223-2236
Magnusson U, Fossum C (1992) Effect of estradiol-17 beta treatment of gilts on blood mononuclear cell functions in vitro. Am J Vet Res 53: 1427-1430
Markusfeld O (1987) Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. J Dairy Sci 70: 158-166
Mili B, Pandita S, Singh AK, Mohini M, Ashutosh M (2013) Xanthine oxidase activity during transition period and its association with occurrence of postpartum infection in Murrah buffalo (Bubalus bubalis). Afr J Biotechnol 12 :5101-5104
Miller JK, Brzezinska-Slebdzinska E, Madsen FC (1993) Oxidative stress, antioxidants, and animal function. J Dairy Sci 76: 2812-2823
Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P (1997) Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem 43: 1209-1214
Rizzo A, Roscino MT, Binetti F, Scirosi RL (2012) Roles of reactive oxygen species in female reproduction. Reprod Domest Anim 47: 344-352
Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ (2009) Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 81: 1025-1032
Shoker AS, Yang H, Murabit MA, Jamil H, Al-Ghoul A, Okasha K (1997) Analysis of the in vitro effect of exogenous nitric oxide on human lymphocytes. Mol Cell Biochem 171: 75-83
Sordillo LM, Aitken SL (2009) Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopath 128:104-109
Tizard I (1991) Use of immunomodulators as an aid to clinical management of feline leukemia virus-infected cats. J Am Vet Med Assoc 199: 1482-1485
Trevisan M, Browne R, Ram M, Muti P, Freudenheim J, Carosella AN, Armstrong D (2001) Correlates of markers of oxidative status in the general population. Am J Epidemiol 154: 348-356