



Nitric oxide and prostaglandin metabolites profiling in buffaloes with impending postpartum reproductive disorders

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ABSTRACT

The study was conducted to correlate the periparturient immune status in terms of plasma nitric oxide (NO) and prostaglandin $F_{2\alpha}$ metabolites (PGFM) profile with impending postpartum reproductive disorders in buffaloes. Pregnant buffaloes (40) were observed for occurrence of postpartum reproductive disorders (PRD), i.e. metritis, endometritis and delayed uterine involution during 1 week prepartum to 4 weeks postpartum period. A representative number (6) of buffaloes that did not develop any PRD were included in Gr. 1 (healthy); and the animals with PRD were assigned into Gr. 2 (8). The blood samples were collected at weekly interval from 1 week prepartum to 4 weeks postpartum period. Plasma NO and PGFM were estimated. A significantly higher NO concentration was found at calving (49.00 ± 7.06 vs. 34.44 ± 3.70 μ M) and second week (43.60 ± 5.57 vs. 24.25 ± 2.34 μ M) postpartum in buffaloes affected with PRD as compared to healthy buffaloes. The PGFM level was also higher in buffaloes that developed PRD throughout the periparturient period. However, the concentration of PGFM was significantly higher, particularly at calving (2.51 ± 0.43 vs. 1.267 ± 0.30 ng/ml) and second week (1.058 ± 0.22 vs. 0.431 ± 0.08 ng/ml) postpartum. Hence, monitoring of plasma nitric oxide and PGFM level would be effective to predict certain reproductive disorders at calving or immediately after parturition in buffaloes.

Key words: Buffalo, Nitric oxide, Prostaglandin metabolites, Reproductive disorders

Uterine defence mechanism plays crucial role to prevent uterine infections during peripartum period when animals undergo pronounced physiological changes that cause suppression of host defence including both of cellular and humoral immune responses. Besides, cellular and humoral immune responses certain inflammatory mediators like nitric oxide (NO) and prostaglandin $F_{2\alpha}$ metabolites (PGFM) are also produced that may be linked to the inflammatory status of animal. Higher nitric oxide production was observed in cows with mastitis, sub-clinical or clinical endometritis (De 2004, De Jun *et al.* 2010, Krishnan 2011). Several reports have described higher concentration of circulating PGFM in cows with uterine infections in dairy cows with heavy endometritis than mild endometritis (Mateus *et al.* 2003). However, there is no report available on plasma nitric oxide and PGFM profile and in buffaloes either during the peripartum period or with postpartum reproductive disorders. Therefore, the present study was conducted to elucidate the periparturient NO and PGFM profile in buffaloes developing certain reproductive disorders at postpartum period.

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MATERIALS AND METHODS

Experimental animals: This experiment was conducted on pregnant Murrah buffaloes (40) of second to fourth parity, maintained under uniform feeding and management practices at the Institute. The events of calving, expulsion of fetal membranes and occurrence of postpartum reproductive disorder (PRD) including metritis, endometritis and delayed uterine involution were observed during the course of 1 week prepartum to 4 weeks postpartum period. The occurrence of metritis was adjudged based on enlarged uterus with foul smelling, watery or purulent reddish brown exudates along with rise of body temperature at 3 to 8 days postpartum. The clinical endometritis was inferred when animals were observed with purulent or mucopurulent discharge on vaginal inspection at 3 weeks postpartum. Uterine involution was also monitored at weekly interval by per rectal examination of genitalia from 1 week postpartum to till 5 weeks postpartum period.

Sample collection: Out of 40 pregnant buffaloes observed during periparturient period, 5 animals were diagnosed with metritis, 3 with clinical endometritis concurrent with delayed uterine involution. None of the animals suffered from dystocia or retention of fetal membrane. Two experimental groups were retrospectively formed. Those buffaloes did not develop any PRD

throughout the peripartum period, of which a representative number (6) was included in Gr. 1 (healthy), while the animals which experienced PRD were assigned into Gr. 2 (8). The blood samples (20 ml) were collected in heparinized tubes from all the animals at 1 week before expected calving to 4 weeks post-calving period at weekly interval. Plasma was separated and stored in -20°C till the analysis of NO and PGFM being completed.

Nitric oxide assay: The concentration of nitric oxide (NO) in plasma was estimated in buffaloes that did or did not develop postpartum reproductive disorders (PRD). Plasma nitrate and nitrite were estimated as per Sastry *et al.* (2002). The nitrate content was reduced to nitrite by activated copper-cadmium (Cu-Cd) alloy followed by colour development with Griess reagents. The standard curve (Fig. 1) was made using 5 to 100 μM concentration of potassium nitrate (KNO_3) solution following the same procedure. The final concentration of nitric oxide (μM) in

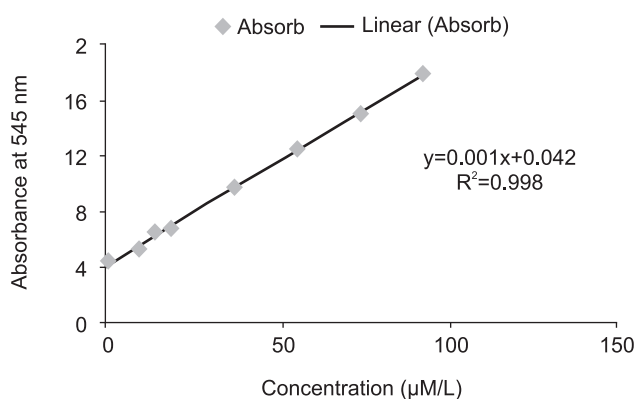


Fig. 1. Typical curve for standardizing the plasma nitric oxide (NO) assay.

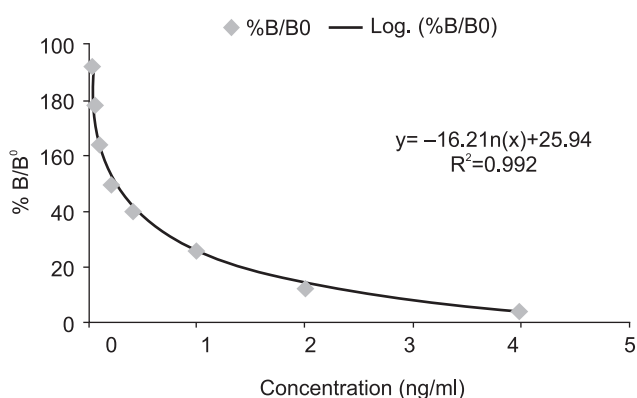


Fig. 2. Typical curve for standardizing the prostaglandin metabolites (PGFM) assay based on competition ELISA.

Table 1. Plasma nitric oxide concentration ($\mu\text{M/L}$) in buffaloes with or without postpartum reproductive disorders (PRD)

Group	-1 wk	0 wk	1 wk	2 wk	3 wk	4 wk
Healthy (Gr. 1, n=6)	53.20 $\pm$ 4.56 ^{aA}	34.44 $\pm$ 3.70 ^{cB}	31.07 $\pm$ 6.66 ^B	24.25 $\pm$ 2.34 ^{aB}	28.75 $\pm$ 2.33 ^B	30.57 $\pm$ 3.90 ^B
PRD (Gr. 2, n=8)	36.40 $\pm$ 2.08 ^{bAB}	49.00 $\pm$ 7.06 ^{dA}	38.00 $\pm$ 4.49 ^{AB}	43.60 $\pm$ 5.57 ^{bAB}	30.83 $\pm$ 4.60 ^B	28.80 $\pm$ 2.68 ^B
p value	0.007	0.083	0.423	0.016	0.681	0.725

Values (mean $\pm$ SEM) with different superscripts (^{a, b}) and (^{c, d}) in a column differ significantly at $P < 0.05$ and $P < 0.10$, respectively. Values with different superscripts (^{A, B, C}) in row differ significantly at $P < 0.05$.

test samples was calculated using the regression equation [$y = 0.001x + 0.042$, where x , concentration of NO and y , absorbance, R^2 , 0.998] derived from the standard curve.

Plasma PGFM assay: The plasma PGFM was estimated using the commercial kit based on competitive ELISA. This test was operated on the basis of competition between the enzyme conjugate and the PGFM in sample for limited binding sites on the antibody coated plate. The standard curve was graphed by plotting the binding percentage ($\%B/B_0$) on Y axis and concentration in X-axis. A logarithmic regression curve was drawn by using trend line analysis (Fig. 2).

The concentration of the unknown sample was calculated from the regression equation [$y = -16.2 \ln(x) + 25.94$, y , binding percentage; x , antilog value of concentration; R^2 , 0.992] generated out of the standard curve. Final concentration was calculated by multiplying with the dilution factor and expressed in ng/ml.

Statistical analysis: All the data were analyzed using the statistical package SPSS 16.0. Independent t-test was used for comparison of mean values between 2 groups at each point of time and the effect of sampling time on plasma nitric oxide and PGFM was analyzed by one-way ANOVA with Duncun multiple range test (DMRT) as post hoc analysis. The result presented as mean $\pm$ SEM. The difference of mean values for all data analyzed with $P < 0.05$ was considered as significant, whereas $0.05 < P < 0.10$ was considered as tendency.

RESULTS AND DISCUSSION

The results depicted the differential NO and PGFM concentration in plasma of the 2 groups of animals over the sampling days. A significantly higher NO concentration was found at the prepartum period in healthy buffaloes as compared to those with PRD ($P < 0.05$, Table 1). Piccinini *et al.* (2004) also found similar trend in plasma NO level during pre- to post- calving period in healthy cows.

However, the NO level was significantly higher ($P < 0.10$) on day of calving in buffaloes with PRD, which could exert inhibitory effect on superoxide production by PMN cells as observed by Patra (2012) and is also supported by Blum *et al.* (2000) and Mehrzad *et al.* (2002).

The plasma NO level was similar throughout the postpartum period in Gr. 1, whereas in PRD buffaloes, the level remained significantly higher up to second week and gradually declined to a lower level at third and fourth week postpartum ($P < 0.05$). The higher level of NO in PRD buffaloes during second week post calving indicating the prevailing inflammatory situation. This finding is well

Table 2. Plasma 13,14-dihydro-15-keto-prostaglandin F₂α (PGFM) concentration (ng/ml) in buffaloes with or without postpartum reproductive disorders (PRD)

Group	-1 wk	0 wk	1 wk	2 wk	3 wk	4 wk
Healthy (Gr. 1, n=6)	1.594±0.75 ^A	1.267±0.30 ^{aAB}	0.86±0.19 ^{ABC}	0.431±0.08 ^{aBC}	0.34±0.06 ^C	0.408±0.18 ^{BC}
PRD (Gr. 2, n=8)	1.71±0.32 ^{AB}	2.51±0.43 ^{bA}	1.881±0.52 ^{AB}	1.058±0.22 ^{bBC}	0.536±0.10 ^C	0.432±0.11 ^C
p value	0.201	0.033	0.113	0.017	0.102	0.391

Values (mean±SEM) with different superscripts (^{a, b}) in a column and (^{A, B, C}) in a row differ significantly (P<0.05).

suited with the reports of other researches who have observed a significantly higher NO level in plasma of endometritis and sub-clinical endometritis cows as compared to healthy (De Jun *et al.* 2010, Krishnan 2011) and in cows with LPS induced mastitis (Bouchard *et al.* 1999) or naturally infected mastitis cows (De 2004). The possible source of NO are the macrophages, neutrophils, epithelial and endometrial cells of uterus and facilitates the recruitment and activation of more immune cells, stimulates secretion of acute phase proteins and it participates in microbial killing by reactive nitrogen intermediates (Hirvonen *et al.* 1999).

Similarly, significant effect of time on concentration of PGFM both in healthy and PRD buffaloes was observed in present study. The plasma PGFM concentration was higher in buffaloes that developed PRD as compared to those of healthy, during all sampling days, although the mean values were statistically significant (P<0.05) at calving and second week postpartum (Table 2) that indicated a kind of inflammation prevailing during the periparturient period. Our contention is well supported by Delvecchio *et al.* (1994).

The plasma PGFM level at day of calving and at second week postpartum in buffaloes may be used as an indicator of uterine inflammation/infection, and associated with subsequent development of PRD, which is well supported by the evidence of significant higher plasma PGFM concentration in heavy endometritis than in mild endometritis group at the time of diagnosis and during spontaneous recovery as observed by Mateus *et al.* (2003).

In healthy buffaloes the PGFM levels gradually declined from a significant higher concentration at prepartum to the basal level at subsequent postpartum period which could be correlated with the normal physiological process of uterine and cervical involution as indicated by Kaidi *et al.* (1991).

From this experiment it could be concluded that sustained elevation in plasma levels of nitric oxide and prostaglandin metabolites in the buffaloes with PRD might be due to uterine infections in postpartum period. This could be used for monitoring the status of uterine infection at calving or early postpartum period and for determining suitable management strategies to cut-off the incidence of uterine infection at later postpartum period.

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