

Increased nitric oxide level around parturition in cows with or without postpartum uterine diseases

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ABSTRACT

Blood samples were collected from 41 advanced pregnant cows on 15 days prepartum (–15d), calving day (0d), 15 days (15d) and 30 days (30d) postpartum to study the nitric oxide profile in cows with Retained placenta (ROP), Clinical metritis (CM), Clinical endometritis (CE), Cervicitis (CT) and Delayed involution of uterus (DIU) during the periparturient period. The blood serum was used for estimation of nitric oxide (NO) using microassay. The nitric oxide level (μM) in the serum of cows was nonsignificantly higher for ROP, CM and CE than normal at –15d and the corresponding values were 31.88 ± 5.01 , 30.30 ± 4.41 , 32.08 ± 7.56 and 22.31 ± 2.69 at calving and 13.89 ± 2.72 , 14.70 ± 4.84 , 7.08 ± 2.13 and 11.94 ± 1.46 at 30d pp. The NO level increased from –15d to the day of calving (0d), thereafter a significant decrease was observed from 0d to postpartum days (15d and 30d) in ROP, CM, CE and normal cows. The significantly higher NO level in cows around the day of calving in all cows indicates its role on the physiology of calving. It is evident from the study that NO level declined significantly from calving to 15d and 30d postpartum in all postpartum cows irrespective of inflammatory status of the uterus. However, the level of NO in periparturient cows is not associated with the development of postpartum uterine diseases.

Key words: Nitric oxide, Periparturient cow, Postpartum reproductive diseases, Serum

The bacterial contamination of the uterus during parturition and postpartum period occurs in most of the cows due to the breached anatomical barrier during parturition and resulting into establishment of postpartum uterine diseases. Cows suffering from retention of placenta are the most likely to suffer from other postpartum reproductive diseases (Islam *et al.* 2014). These diseases prolong the calving interval and decrease the calving rate due to delay in complete regeneration of endometrium and failure to conceive on repeated inseminations (Hussain and Daniel 1991, Sheldon *et al.* 2009). Nitric oxide level in cows is reported to be associated with the occurrence of subclinical (Li *et al.* 2010, Krishnan *et al.* 2014, Song *et al.* 2015) and clinical endometritis (Li *et al.* 2010) in cattle. Subsequent to the pathogen recognition, immune cells (macrophages, neutrophils, epithelial and endothelial cells) release pro-inflammatory molecules and nitric oxide (Baumann and Gauldie 1994). Recently higher concentration of Interleukin –1, a pro-inflammatory cytokine in the circulation of the postpartum cows with retained placenta, Clinical metritis and clinical endometritis has been reported (Islam *et al.* 2013a).

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Nitric oxide (NO), a reactive radical gas, plays an important in the host defense by destroying microbes with the help of reactive nitrogen intermediates (Nathan 1992, Hirvonen *et al.* 1999). The concentration of NO in plasma and uterine fluid is related with the degree of endometritis in cattle (Li *et al.* 2010). However, the information on level of NO during different stages of periparturient period in relation to the occurrence of postpartum uterine diseases in cattle is not available. Therefore, the present study was undertaken with the objective to explore the possible association of NO profile during the periparturient period with the development of postpartum uterine diseases.

MATERIALS AND METHODS

Animals: The experimental animals used in this study were selected from Vrindavani Cattle herd maintained at Cattle and Buffalo Farm, Livestock production and Management section, Indian Veterinary Research Institute, Izatnagar, India. A total of 41 healthy advanced pregnant cows between second to fourth parity those had no reproductive disorder during the previous pregnancy/calving were selected randomly at 240 days of pregnancy.

Management of animals: The climate of Indian Veterinary Research Institute including the Cattle and Buffalo Farm is tropical. The environmental temperature during summer varied from 30°C to 45°C and during winter 10°C to 25 °C. The winter exists in the farm area from November to February. The animals were managed under

intensive system in the cattle sheds. During the day time they were allowed to remain in the paddock connected to the cattle shed. The feeding and watering of the cows were done in the paddock. All the animals were maintained under uniform feeding and managemental conditions throughout the year.

Gynaecological examination of the animals

Prepartum: The details of pregnancy diagnosis, selection, regular observation for external signs for the approaching parturition and fixing of prepartum sampling days (15 d prior to calving; -15d) of experimental cows has already been mentioned elsewhere (Islam *et al.* 2013a, Islam *et al.* 2013b). The ranges of the prepartum collection day fell between 10 (- 10d) to 22 days (- 22d) prior to calving.

Calving: The experimental cows were also monitored / observed on the day of calving (0d) for nature of parturition (normal/ abnormal), expulsion of placenta (retained fetal membranes) and lochia (Islam *et al.* 2013a, Islam *et al.* 2013b).

Postpartum: During the postpartum period, the individual cow was critically monitored for the diagnosis of post partum disorders like retained placenta (ROP), clinical metritis (CM), clinical endometritis (CE), Cervicitis (CT), delayed uterine involution (DIU) and also for ovarian activity at 15, 30 and 45 days postpartum by transrectal palpation as has already been mentioned elsewhere (Islam *et al.* 2013a, Islam *et al.* 2013b).

Nitric oxide assay: Nitric oxide level of blood serum was measured by nitrate reduction by copper-cadmium alloy (Cu-Cd alloy) followed by color development with Griess reagent as per the method described by Sastry *et al.* (2002).

Statistical analysis: Data on nitric oxide were analysed by one way analysis of variation (ANOVA) using statistical software SPSS version 16 for which 40 animals including 24 normal cows were included. The effects of postpartum reproductive diseases (Control, Retained placenta, Clinical metritis, Clinical endometritis and delayed involution), and periods (days of sample collection -15d, 0d, 15d and 30d) were observed. The cow was included in the analysis as a random effect. When an effect between groups and time was observed, post-hoc multiple comparisons were performed using Duncan's multiple range test and cross checked with LSD. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The concentration of nitric oxide (NO) in the serum of cows of different groups during periparturient period is presented in Table 1. The nitric oxide level was non-significantly higher for ROP, CM and CE than normal cows at -15d and 0d. At 30d, NO level was higher for ROP and CM. The cows with PRD had higher NO level compared to normal cows before calving and on the day of calving. Irrespective of the periparturient days, the overall NO

Table 1. Serum nitric oxide level (μM) during periparturient period in cows with or without postpartum reproductive diseases

Groups	Periparturient period (days)				Overall
	-15d	0d	15d	30d	
ROP (9)‡	27.64 $\pm 6.28^B$	31.88 $\pm 5.01^B$	12.44 $\pm 1.90^A$	13.89 $\pm 2.720^A$	21.10 ± 2.45
CM (5)**	22.70 $\pm 6.36^{AB}$	30.30 $\pm 4.41^B$	10.50 $\pm 2.23^A$	14.70 $\pm 4.84^A$	19.55 ± 2.77
CE (6)‡	30.70 $\pm 7.00^B$	32.08 $\pm 7.56^B$	10.17 $\pm 3.09^A$	7.08 $\pm 2.13^A$	19.54 ± 3.48
DIU (3)	17.67 ± 6.23	21.17 ± 7.00	6.33 ± 0.93	11.50 ± 4.64	14.17 ± 2.82
NM (24)‡	21.46 $\pm 1.26^B$	22.31 $\pm 2.69^B$	11.42 $\pm 1.14^A$	11.94 $\pm 1.46^A$	16.78 ± 1.01
Overall	23.38 ± 1.65	26.17 ± 2.06	11.03 ± 0.82	11.96 ± 1.11	

ROP, Retained placenta; CM, clinical metritis; CE, clinical endometritis; DIU, delayed involution of uterus; NM, normal; values are shown as mean $\pm$ SEM; means with different superscripts in a row (A, B) differ significantly; ** $P < 0.05$; ‡ $P < 0.01$; figures within parentheses indicate number of observations. All cows under CM and 2 under CE group of cows suffered from ROP.

concentration was higher for the cows with ROP, CM and CE than normal cows. The result of the present study is in partial agreement with the previous reports in cows with Clinical (Li *et al.* 2010) and subclinical endometritis (Li *et al.* 2010, Krishnan *et al.* 2014). However, the values in our study were slightly lower than Krishnan *et al.* (2014) and in close proximity with Li *et al.* (2010). The difference might be attributed to the variation in reproductive status of the cows. Higher level of NO in the blood (Li *et al.* 2010, Krishnan *et al.* 2014) and uterine fluids (Li *et al.* 2010, Song *et al.* 2015) and higher expression of nitric oxide synthase-2 (NOS2) in uterine biopsy has been reported in cows with endometritis (Li *et al.* 2010). Similarly, Janowski *et al.* (2013) also reported significantly higher expression of inducible nitric oxide synthase (iNOS) in the uterus of cows with subclinical endometritis.

In all group of cows, the NO level increased from -15d to the day of calving (0d), thereafter a significant decrease was observed from 0d to postpartum days (15d and 30d). Interestingly higher prepartum level of NO and subsequent increase on the day of calving might play some role for the physiological preparation of the cows for the calving as parturition is an inflammatory condition (Gabler *et al.* 2009, Chapwanya *et al.* 2009, Galvao *et al.* 2011). Immune cells release nitric oxide and pro-inflammatory molecules subsequent to the pathogen recognition (Baumann and Gauldie 1994). This study may take support in respect of higher NO production around calving from Herath *et al.* (2009) who reported increased expression of nitric oxide synthase 2 (NOS2) at 1wk pp. Nitric oxide plays a key role in the processes leading to cervical softening prior to labor and inducible nitric oxide synthase (iNOS) contributes most to the increased production of NO during labor (Aalberts

et al. 2007).

The declining trend in NO from calving to 15d pp in the present study is in agreement with the finding of Piccini *et al.* (2004). A progressive and significant decline in nitric oxide level from the pre-calving and calving to the post calving period has been reported (Piccinini *et al.* 2004). Though, NO level in blood serum was higher in cows suffering from reproductive disorders, but the difference is not significant as reported by the earlier workers for subclinical (Li *et al.* 2010, Krishnan *et al.* 2014) and clinical endometritis in cattle (Li *et al.* 2010). Significantly higher nitric oxide production has also been reported in cows with LPS induced mastitis (Bouchard *et al.* 1999). The difference in NO concentration in cows with PRD in this study than the previous reports might be due to higher concentration of IL-10 (Khalifeh *et al.* 2009). NO plays an important role in the host defense by destroying microbes (Nathan 1992) and its induction is highly affected by the types of cytokines and the infectious agents present (Khalifeh *et al.* 2009). Nonsignificantly higher NO concentration observed in our study might be attributed to the higher level of anti-inflammatory cytokine, IL-10 in the postpartum cows with uterine diseases (Islam *et al.* 2013b). It was reported that *in vitro* exposure to inhibitory cytokines such as IL-10 and TGF- β prior to MAP infection or LPS stimulation resulted in the down regulation of NO (Khalifeh *et al.* 2009). It has also been demonstrated that endogenous or exogenous IL-10 results in the down regulation of iNOS expression in cultured bovine monocyte-derived macrophages (Goff *et al.* 1998). NO content in the serum of IL-6 pre-treated and post-treated mice showed much reduction than that of only LPS-challenged mice (Nandi *et al.* 2010) may be due to the antiinflammatory nature of the IL-6 cytokine. The higher level of NO recorded around calving in this study may be physiological and essential for the preparation of cows for calving. Higher iNOS expression in the advanced pregnant cows indicated the important role of NO in prelabor cervical ripening of bovine (Albert *et al.* 2007).

In conclusion, the significantly higher NO concentration in cows around the day of calving in all cows indicates its role on the physiology of calving. It is evident from the study that NO level declined significantly from calving to 15d and 30d postpartum in all postpartum cows irrespective of inflammatory status of the uterus. However, the level of NO in periparturient cows is not associated with the development of postpartum uterine diseases.

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