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Research Article

PROFILES OF SERUM NITRIC OXIDE AND ASCORBIC ACID IN EARLY PREGNANT AND NON-PREGNANT EWES DURING BREEDING SEASON

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ABSTRACT

The present study was designed to determine the levels of serum nitric oxide (NO) and ascorbic acid (AA) in pregnant and non-pregnant cross bred sheep during early stages of pregnancy. Healthy cross bred ewes (n=29) were selected and randomly allotted to three groups with 10 animals in natural tupping (NT) and 10 in laparoscopic artificial insemination (LAI) group and remaining 9 ewes in cervical artificial insemination (CAI) group. All the ewes were subjected to the same estrus induction protocol that involved insertion of progesterone intravaginal sponges for 10 days followed by an injection of 500 IU eCG at the time of sponge withdrawal. Blood samples were collected on different days for the analysis of serum NO and AA concentration. The serum NO concentrations were significantly higher ($P<0.05$) in pregnant than non-pregnant ewes on day 17 and day 35 post tupping/insemination. The serum AA concentrations were significantly higher ($P<0.05$) in pregnant than non-pregnant ewes on day 35 post tupping/insemination. In conclusion, from the third week of pregnancy, the serum NO and during fifth week, the AA concentrations are significantly higher in pregnant than non-pregnant ewes.

Keywords: Ascorbic Acid, Breeding season, Ewe, Insemination techniques, Nitric oxide

Pregnancy is a physiological process characterized by a drastic increase in energetic and oxygen demands to ensure an adequate fetal development and growth (Nawito *et al.*, 2016). It is associated with significant alterations in various biochemical constituents including nitric oxide (NO) and ascorbic acid (AA). As an endogenous vasodilator, NO is involved in vascular relaxation and angiogenesis (Xuan and Cheng, 2002). Only few studies have reported high NO levels from mid to late gestation in ewes (Vonnahme *et al.*, 2005). In animals, three main biological actions of AA include collagen synthesis, hormone secretion and anti-oxidant action (Luck *et al.*, 1995). In females, the AA has an important role in collagen biosynthesis, steroidogenesis and apoptosis during ovarian folliculogenesis (Luck and Zhao, 1993). Considering the importance of NO and AA in the regulation of uterine blood vascularity and luteal tissue functions respectively, the current study was designed to investigate the levels of NO and AA in ewes during early stages of pregnancy following hormonal treatment during breeding season.

MATERIALS AND METHODS

Study period and Area: The present study was conducted on cross bred ewes maintained at Mountain Research Centre for Sheep and Goats, FVSc & AH, SKUAST-Kashmir, Shuhama, Srinagar, Kashmir, India (34° 08¹ N 74° 28¹ E).The treatment was initiated with the onset of breeding season which corresponded to Autumn (August-November) during temperate climatic conditions of Jammu and Kashmir.

Experimental design: Twenty nine multiparous cross bred healthy ewes (NARI-Swarna ram x non-descript ewes) weighing 36.41 ± 4.25 kg (Mean ± S.E.M) with body condition score between 2.5-3.5 (Scale of 0-5) were selected. The ewes were randomly allotted to three groups, with 10 each in natural tupping (NT) and laparoscopic artificial insemination (LAI) group and remaining 9 in cervical artificial insemination (CAI) group. Ewes in all the treatment groups were subjected to the same estrus induction protocols which involved insertion of intravaginal progesterone sponges (AVIKESIL-S, CSWRI, Avikanagar) for 10 days only. Later, 500 IU eCG (Folligon®, MSD Animal Health, Pune, India) was given intramuscularly to all the animals at the time of sponge withdrawal (Fig. 1). The ewes to be used for LAI group were synchronized in such a way that only two animals

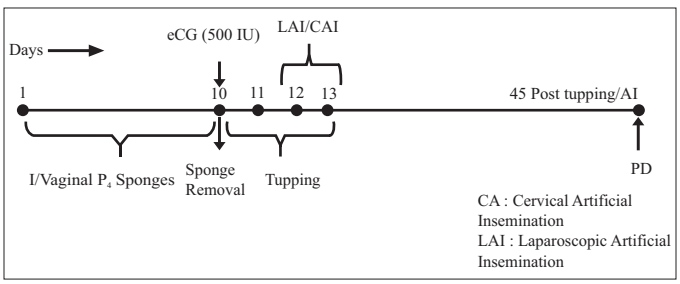

Fig. 1. Experimental design and timeline for treatment distribution

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were subjected for insemination per day. After removal of sponges and eCG injection, animals of NT group (n=10) were kept with proven breeding rams in the ratio of 10:1 for tupping upto 72 hours.

In ewes belonging to the CAI group (n=9), timed os-cervical insemination was performed using chilled semen at 48 h and 60 h after sponge removal and eCG injection. Ewes of group-3 (LAI; n=10) were subjected to timed LAI using chilled semen 48 h after sponge withdrawal. From all 29 ewes, the blood samples were collected on the day of start of hormone treatment, day of insemination/tupping (day 0), day 10, 17 and 35. The blood samples were analyzed for serum NO and AA concentrations.

Serum Nitric Oxide concentration: The concentration of nitric oxide in the serum samples was determined as per the method described by Sastry *et al.* (2002).

Serum Ascorbic Acid concentration: The concentration of ascorbic acid was determined in the serum as per the method described by Zannoni *et al.* (1974).

Statistical Analysis: The data obtained were analysed statistically by paired t-test and One- way ANOVA using statistical software version SPSS-20 (Snedecor and Cochran, 1994). Post-hoc analysis was done by DMRT. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The mean nitric oxide concentration (μM) in the serum of pregnant and non-pregnant cross bred ewes following different insemination techniques is presented in Table 1. The mean NO concentration between pregnant and non-pregnant ewes varied non-significantly (P>0.05) on pretreatment day, day 0 and day 10 but difference was significantly more (P<0.05) on day 17 (142.89 ± 5.68 μM vs 121.85 ± 3.43 μM) and Day 35 (154.48 ± 4.13 μM vs 113.06 ± 12.25 μM). In the non-pregnant ewes, the mean serum NO concentration varied non-significantly on all the days. However, in pregnant ewes, the mean serum NO concentration increased significantly (P<0.05) from pretreatment day (123.95 ± 3.01 μM) to day 35 (154.48 ± 4.13 μM).

Table 1

Serum nitric oxide (nitrite plus nitrate) concentration (Mean ± SEM) in cross bred ewes during breeding season

Stage/Day	Nitric oxide concentration (μM)	
	Pregnant (n=16)	Non-Pregnant (n=13)
Pre treatment	123.95 ± 3.01^{aA}	126.26 ± 3.19^{aA}
Day 0	133.48 ± 4.70^{abA}	133.27 ± 5.90^{aA}
Day 10	139.39 ± 4.86^{bA}	130.84 ± 5.04^{aA}
Day 17	142.89 ± 5.68^{bcA}	121.85 ± 3.43^{aB}
Day 35	154.48 ± 4.13^{cA}	113.06 ± 12.25^{aB}

Means bearing different superscript (a, b, c) within columns and (A, B) within rows differ significantly (P < 0.05)

Table 2

Serum Ascorbic acid concentration (Mean ± SEM) in cross bred ewes during breeding season

Stage/Day	Ascorbic acid concentration (μg/ml)	
	Pregnant (n=16)	Non-Pregnant (n=13)
Pre treatment	33.84 ± 6.33^{aA}	33.84 ± 7.62^{aA}
Day	043.34 ± 7.17^{abA}	35.01 ± 6.47^{aA}
Day10	55.59 ± 6.76^{bA}	26.17 ± 5.84^{aB}
Day17	46.73 ± 5.67^{abA}	29.04 ± 8.06^{aA}
Day35	56.63 ± 5.48^{bA}	30.18 ± 6.19^{aB}

Means bearing different superscript (a, b, c) within columns and (A, B) within rows differ significantly (P < 0.05)

The mean serum AA concentration (μg/ml) in pregnant and non-pregnant ewes following different insemination methods is presented in Table 2. The mean serum AA in pregnant ewes was non-significantly higher on day 0 (43.34±7.17 μg/ml vs 35.01±6.47 μg/ml), but significantly (P<0.05) higher on day 10 (55.59 ± 6.76 μg/ml vs 26.17 ± 5.84 μg/ml) and day 35 (56.63±5.48 μg/ml vs 30.18±6.19 μg/ml). In non-pregnant ewes, the mean serum AA concentration varied non-significantly (P>0.05) between different days. However in pregnant ewes, AA level increased significantly (P<0.05) from day 0 to day 10 and varied non-significantly thereafter.

The results of serum nitric oxide in current study are concomitant with the findings of Rasool *et al.* (2018), Malik *et al.* (2018) and Vonnahme *et al.* (2005). Malik *et al.* (2018) reported significantly higher levels of NO in pregnant than non-pregnant ewes at day 17 and day 35 post-tupping/insemination. Higher serum NO levels are documented at all stages of gestation in pregnant sheep. Vonnahme *et al.* (2005) found substantial increase in NO metabolites in ewes during early (20-39 days) pregnancy. The arterial plasma nitrate concentrations in pregnant sheep are significantly (P<0.05) elevated compared to the levels in non-pregnant ones (Yang *et al.*, 1996). These workers concluded that in ewes increased NO levels during pregnancy mediates the cardiovascular adaptations acquired in normal pregnancy period. In order to maintain the high oxygen and nutrient delivery required for normal fetal growth and development, utero-placental blood flow increases 30 to 50 fold during pregnancy (Sladek *et al.*, 1997, Magness *et al.*, 2001). To ensure dramatic increase in the blood flow, the uterine vascular bed must vasodilate and proliferate. The NO being vasodilator is the major mediator for augmented placental-fetal blood flow during pregnancy

(Rosenfield *et al.*, 1996). Other studies claimed that NO is involved in maintaining myometrial quiescence during pregnancy (Ali *et al.*, 1997). The progesterone secretion from corpus luteum is accompanied by the development of an extensive vascular system due to vasodilation of corpus luteum mediated by NO (Augustin, 2001).

The results of present study indicate that the mean serum AA concentrations were higher in pregnant compared to the non-pregnant ewes. Our findings are in agreement with the results of previous workers (Malik *et al.*, 2018, Rasool *et al.*, 2018, Miszkiel *et al.*, 1999). The fully mature corpus luteum contains maximum levels of ascorbic acid. The concentrations remain high remain high during pregnancy and decrease with the regression of the corpus luteum (Petroff *et al.*, 1997). Excessive demands for AA are put forth by the fetus during pregnancy right from first trimester (Wilson and Loh, 1973). The underproduction of ascorbate results in sub-fertility in domestic animals (Luck *et al.*, 1995). AA is cofactor for collagen biosynthesis in luteal extracellular matrix (Luck *et al.*, 1995). The steroidogenic activity of the corpus luteum increases with the increase in ascorbic acid concentration (Miszkiel *et al.*, 1999). The protection of corpus luteum from luteolysis is essential for maintenance of pregnancy (Mohebbi-Fani *et al.*, 2011).

CONCLUSIONS

In pregnant ewes, significantly ($P>0.05$) higher concentrations of serum NO and AA were recorded during third to fifth week of pregnancy. However upto day 10, the levels remain similar between pregnant and non-pregnant animals.

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