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Serum nitric oxide and ascorbic acid levels in pregnant and non-pregnant ewes during non-breeding season

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Abstract
The objective of this study was to determine the levels of nitric oxide (NO) and ascorbic acid (AA) in pregnant and non-pregnant cross bred sheep during non-breeding season. The esperiment was conducted at Mountain Research Centre for Sheep and Goat, FVSc & AH, SKUAST-Kashmir, Shuhama from March to May 2017. Cross bred ewes (n=24) were subjected to estrus induction protocol involving insertion of intravaginal sponges for 10 days followed by an injection of 500 IU eCG at the time of sponge withdrawal. Subsequently the animals were subjected to natural tupping, cervical insemination or laparoscopic insemination (LAI). Blood samples were collected on different days for 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 (157.19±7.13 µM vs 138.58±3.36 µM) and day 35 (161.63±5.07 µM vs 142.20±3.14 µM) post tupping/insemination. The serum AA levels were also significantly higher ($P<0.05$) in pregnant than non-pregnant ewes on day 17 (77.21±7.71 µg/ml vs 54.49±4.97 µg/ml) and day 35 (81.95±4.85 µg/ml vs 60.37±5.10 µg/ml) post tupping/insemination.

Keywords: ascorbic acid, ewes, insemination techniques, nitric oxide

1. Introduction
Pregnancy is a unique ephemeral state showing alterations in animal physiology particularly endocrine and cardiovascular systems leading to dramatic changes in the concentration of various blood biochemical constituents [1]. These physiological changes are important for normal embryonic, placental and fetal development [2]. Nitric Oxide (NO) is an important regulatory agent needed in various female reproductive events [3]. NO is also implicated in peripheral vasodilation during pregnancy and in the control of blood flow in the fetoplacental circulation [4]. Other studies demonstrate that NO is involved in maintaining myometrial quiescence during pregnancy [5]. Ascorbic acid has three biological actions of particular relevance to reproduction; each one dependent on its role as a reducing agent. In domestic animals low levels of ascorbate result in underproduction of hormones and subsequently sub-fertility [6]. It was found that vitamin C injected to the sheep during the breeding season increased the levels of plasma oestrogen and progesterone in addition to the fertility [7]. The present study was attempted to determine the levels of nitric oxide (NO) and ascorbic acid (AA) in serum harvested from ewes following hormonal treatments during the off-season (spring) in a temperate zone.

2. Materials and Methods
2.1 Study period and Study Area
The present study was conducted in ewes maintained at Mountain Research Centre for Sheep and Goat, FVSc & AH, SKUAST-Kashmir, Shuhama. The treatment was initiated during off-season which corresponded to spring (March-May, 2017) in the temperate climatic conditions of Jammu and Kashmir.

2.2 Methodology
Ewes in all the treatment groups were subjected to the same estrus induction protocols which included insertion of intravaginal progesterone sponges (AVIKESIL-S, CSWRI, Avikanagar) for 10 days only. 500 IU eCG (Folligon®, MSD Animal Health, Pune, India) was given to all the animals at the time of sponge withdrawal. After removal of sponges, animals of control

group (n=6) were kept with proven breeding rams for tupping upto 72 hours. In ewes belonging to the second group (n=8) timed cervical insemination performed using chilled semen 48 hours (day 12 evening) after sponge removal. The AI was repeated after 12 hours in all these animals. Ewes belonging to the third group (n=10) were subjected to timed laparoscopic insemination using chilled semen 48 hours after sponge withdrawal. 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 nitric oxide and ascorbic acid concentration.

2.3 Serum nitric oxide concentration
The concentration of nitric oxide in the serum samples was determined as per the method described by Sastry *et al,* [8].

2.4 Serum ascorbic acid concentration
The concentration of ascorbic acid was determined in the serum as per the method described by Zannoni *et al,* [9].

2.5 Statistical analysis
The data obtained was analysed statistically by students t-test and DMRT [10] using statistical software version SPSS-20. Differences were considered significant at P < 0.05.

3. 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. There was a significant difference (P<0.05) in mean nitric oxide concentration between pregnant and non-pregnant ewes on day 17 (157.19±7.13 µM vs 138.58±3.36 µM) and day 35 (161.63±5.07 µM vs 142.20±3.14 µM) post tupping/ insemination. In the pregnant ewes, the mean serum nitric oxide concentration showed a gradual and significant increase (P<0.05) from pre-treatment to day 35 post-insemination. However, in non-pregnant ewes the changes in mean serum nitric oxide concentration was non-significant at all the stages. The mean ascorbic acid (µg/ml) level in the serum of pregnant and non-pregnant cross bred ewes following different insemination techniques is presented in Table 2. There was a significant difference (P<0.05) in mean ascorbic acid concentration between pregnant and non-pregnant ewes at day 17 (77.21±7.71 µg/ml vs 54.49±4.97 µg/ml) and day 35 (81.95±4.85 µg/ml vs 60.37±5.10 µg/ml) post tupping/insemination. In non-pregnant animals, the serum ascorbic acid concentration increased non-significantly from day 0 to day 35 post tupping/ insemination. However, in pregnant ewes, the mean serum ascorbic acid concentration showed a significant increase (P<0.05) persistently from day 0 to day 35.

The significantly higher levels of serum NO in pregnant than non-pregnant ewes are consistent with the findings of Rasool [11], Mushtaq [12] and Vonhamme *et al,* [2]. However, Rasool [11] reported significantly higher levels of serum NO in pregnant ewes on day 35 only. Higher concentrations of serum NO were determined in pregnant ewes compared to cyclic and non-cyclic non-pregnant ewes [12]. The studies of Vonnahme *et al* [2] demonstrated biphasic temporal pattern of nitric oxide metabolite in blood of pregnant ewes with increased levels starting from day 20 to 39 and continuing upto day 40 to 69 of gestation. The values return to non-pregnant concentration from day 70 to 100. Thereafter higher concentration is again noticed till full term. Kwon *et al* [13] demonstrated that ovine placentome produces NO in a similar biphasic manner. They reported that increase in NO synthesis specific activity is observed from day 30 to 60 of gestation. This activity declines on day 80 and increases again on day 100. In order to maintain oxygen and nutrient delivery for proper fetal growth and development, uteroplacental blood flow increases 30-50 folds during pregnancy [14, 15]. The NO, a key angiogenic factor has a critical role in proliferation and dilatation of vascular bed to accommodate the increased blood flow [16]. Ku *et al.* [17] observed that vascular endothelial growth factor (VEGF) leading to relaxation of coronary arteries is due to the release of endothelium derived NO. Uterine and /or placental NO synthase (NOS) specific activity increases in early pregnancy both in humans [18] and sheep [13]. The higher levels of serum ascorbic acid (AA) determined in pregnant than non-pregnant ewes is in close agreement with the findings of Rasool [11], Mushtaq [12], Miszkiel *et al* [19] and Petroff *et al,* [20]. Following ovulation AA stimulate development of ascorbic acid rich corpus luteum [21]. The synthesis of higher quantity of collagen during luteal development and maturation is supported by higher levels of ascorbic acid [22]. The AA content is maximum in fully mature corpus luteum, remains high throughout pregnancy and decreases with the regression of corpus luteum [23]. The bovine and porcine corpus luteum formed during estrous cycle as well as pregnancy has high concentration of AA [20, 21]. Excessive demands for AA are made by the fetus during pregnancy [24]. The AA has important role in formation and function of corpus luteum. Protection of corpus luteum from luteolysis is critical for establishment and maintenance of pregnancy [25].

4. Conclusion
In sheep the NO and AA concentrations increase significantly during pregnancy. Increased NO in the maternal circulation may help cardiovascular adaptations during pregnancy. Higher levels of AA are crucial for functioning of corpus luteum and therefore maintenance of pregnancy. For maintenance of uneventful/smooth gestation in livestock the management should give due attention to the levels of such critical blood biochemical indices.

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Table 1: Serum nitric oxide (nitrite plus nitrate) concentration (Mean±S.E.M) in cross bred ewes irrespective of the insemination technique used during non-breeding season

Stage/Day	Nitric oxide concentration (µM)	
	Pregnant (n=6)	Non-Pregnant (n=18)
Pretreatment	142.46±5.07^{aA}	144.96±2.56^{aA}
Day 0	148.01±4.46^{abA}	149.46±3.55^{aA}
Day 10	151.32±6.51^{abA}	144.92±4.54^{aA}
Day 17	157.19±7.13^{abA}	138.58±3.36^{aB}
Day 35	161.63±5.07^{bA}	142.20±3.14^{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±S.E.M) in cross bred ewes irrespective of the insemination technique used during non-breeding season

Stage/Day	Ascorbic acid concentration (µg/ml)	
	Pregnant (n=6)	Non-Pregnant (n=18)
Pretreatment	47.29 ± 5.59^{aA}	46.69 ± 5.35^{aA}
Day 0	46.14 ± 5.98^{aA}	43.82 ± 6.01^{aA}
Day 10	63.08 ± 12.15^{abA}	47.93 ± 10.13^{aA}
Day 17	77.21 ± 7.71^{bA}	54.49 ± 4.97^{aB}
Day 35	81.95 ± 4.85^{bA}	60.37 ± 5.10^{aB}

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

6. References

1. Magness RR. Maternal cardiovascular and other physiologic responses to the endocrinology of pregnancy. In The Endocrinology of Pregnancy, ed. Bazer FW, pp. 507-539. Human Press Inc., Totowa, NJ.
2. Vonnahme KA, Wilson ME, Li Y, Rupnow HL, Phernetton TM, Ford SP, Magness RR. Circulating levels of nitric oxide and vascular endothelial growth factor throughout ovine pregnancy. J. Physiology. 2005; 565(1): 101-109.
3. Atakisil O, Atakisil E, Pancarci SM, Ozcanl A. Plasma and Amniotic Fluid Nitric Oxide Level and Adenosine Deaminase Activity in Sheep. J. Appl. Anim. Res. 2009; 35:197-199.
4. Xuan Z, Cheng Z. Nitric oxide in female reproductive system. Sci. Bull. 2002; 47(16):1321-1324.
5. Ali M, Buhimschi I, Chwalisz K. Changes in expression of the nitric oxide synthase isoforms in rat uterus and cervix during pregnancy and parturition, Mol. Hum. Reprod. 1997; 3:995.
6. Luck MR, Jeyaseelan I, Scholes RA. Ascorbic Acid and Fertility. Biol. Reprod. 1995; 52:262-266.
7. Halıloglu S, Serpek B. The effects of plasma vitamin C and ceruloplasmin levels and exogen vitamin C supplementation on reproduction in sheep. Turk. J. Vet. Anim. Sci. 2000; 24:403-411.
8. Sastry KVH, Moudgal RP, Mohan J, Tyagi JS, Rao GS. Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal. Biochem. 2002; 36:79-82.
9. Zannoni V, Lynch M, Goldstein S, Satao P. A rapid micro method for determination of ascorbic acid in plasma and tissues. Biochem. Med. 1974; 11:41-48.
10. Snedecor GW, Cochran WG. Statistical Methods, 8th ed. Iowa State University Press, Ames, Iowa, 1994.
11. Rasool F. Fertility of ewes following hormone based estrous synchronization and chemical induced cervical ripening. MVSc Thesis submitted to SKUAST-Kashmir. 2016.
12. Mushtaq F. Studies on biochemical and endocrine profile at gonadal level in ewes with special reference to conception. MVSc Thesis submitted to SKUAST-Kashmir, 2014.
13. Kwon H, Wu G, Meininger CJ, Bazer FW, Spencer TE. Developmental changes in nitric oxide synthesis in the ovine placenta. Biol. Reprod. 2004; 70:679-686.
14. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. Am. J. Physiology. Regul. Integr. Comp. Physiology. 1997; 272:R441-R463.
15. Magness RR, Sullivan JA, Li Y, Phernetton TM, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. VI. Ovarian and pregnancy effects of eNOS and NOx. Am. J Physiology. 2001; 280:H1692-H1698.
16. Rosenfield CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen induced vasodilation of the ovine uterine circulation. J. Clin. Invest. 1996; 98:2158-2166.
17. Ku DD, Zaleski JK, Liu S, Brock TA. Vascular endothelial growth factor induces EDRF dependent relaxation in coronary arteries. Am. J. Physiol. Heart. Circ. Physiol. 1993; 265: H586-H592.
18. Williams DJ, Vallace PJ, Neild GH, Spencer JA, Imms FJ. Nitric oxide mediated vasodilation in human pregnancy. Am. J. Physio. Heart. Circ. Physiol. 1997; 272:H748-H752.
19. Miszkiel G, Skarzynski D, Bogacki M, Kotwica J. Concentrations of catecholamines, ascorbic acid, progesterone and oxytocin in the corpora lutea of cyclic and pregnant cattle. Reprod. Nutr. Dev. 1999; 39:509-516.
20. Petroff BK, Ciereszko RE, Dabrowski K, Ottobre AC, Pope WF, Ottobre JS. Depletion of vitamin C from pig corpora lutea by prostaglandin $F_{2\alpha}$ induced secretion of the vitamin. J. Reprod. Fertil. 1998; 112:243-247.
21. Hurley WL, Doane RM. Recent developments in the roles of vitamins and minerals in reproduction. J Dairy Sci. 1987; 72:784-804.
22. Luck MR, Zhao Y. Identification and measurement of collagen in bovine corpus luteum and its relationship with ascorbic acid and tissue development. J.Reprod. Fertil. 1993; 99:647-652.
23. Petroff BK, Dabrowski K, Ciereszko RE, Ottobre JS. Ascorbate and dehydroascorbate concentrations in porcine corpora lutea, follicles and ovarian stroma throughout the estrous cycle and pregnancy. Theriogenology. 1997; 47:1265-1273.
24. Wilson CWM, Loh HS. Vitamin C and fertility. Lancet. 1973; 859.
25. Mohebbi-Fani M, Mirzaei A, Nazifi S, Shabbooie Z. Changes of Vitamins A, E and C and lipid peroxidation status of breeding and pregnant sheep during dry seasons on medium to low quality forages. Trop. Anim. Health. Prod. 2011; 44:259-265.