



## Effect of different parameters on the serum levels of MDA, NBT, and NO in Andaman local goat breeds

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Received: 7 February 2022; Accepted: 14 September 2023

### ABSTRACT

The present study was carried out to assess the effect of rearing systems, breeds, number of pregnancies, and frequency of multiple births on physiological oxidative stress markers (OSMs) in indigenous goat breeds of Andaman and Nicobar Islands, India. Does (n=98) with more than one kidding record maintained in the organized goat breeding farm and at the farmer's field were selected for the study. Pregnancy records were grouped as more than or less than three pregnancies. Breeds were grouped such as Andaman local goat (ALG) and other indigenous goat breeds. Birth frequency was grouped as single and multiple births. Oxidative stress markers such as malondialdehyde (MDA), superoxide anions, and nitric oxide were analysed in blood serum with respect to the different experimental variables. The free radical superoxide and endogenous nitrite values concerning the breed, multiple birth frequency, number of kidding, and rearing systems were non-significant. Lipid peroxidation through TBARS and nitrate values for the breed, multiple birth frequency, and rearing system were non-significant. However, the pregnancy-wise TBARS and nitrate concentration were significant. Estimates of TBARS were significantly high in the animals with lesser pregnancies (1-3 kidding) while nitrate concentration was significant in higher pregnancies (> 3 kidding). This study revealed that these experimental variables had considerable effects on OSMs in goat breeds under the hot humid tropical island ecosystem of Andaman and Nicobar Islands.

**Keywords:** Andaman local goats, Breed admixture, Lipid peroxidation, Nitrate, Nitrite, Rearing system, Superoxide anion

Andaman and Nicobar Islands (ANI) is an archipelago with a hot humid climate (Inbaraj *et al.* 2017) having two indigenous goat breeds such as Andaman local and Teresa goats (Sunder *et al.* 2016). Reactive oxygen species (ROS) are generated during the normal oxygen metabolic process and are formed as a byproduct of aerobic metabolism (Wang *et al.* 2017). Decreased antioxidant levels and increased ROS production induces oxidative stress (Halliwell. 2006). Free radicals are two types: ROS [includes superoxide ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl ( $OH^{\bullet}$ )] and reactive nitrogen species [NOS - includes nitric oxide (NO) and nitrogen dioxide] (Agarwal *et al.* 2005). Nitroblue tetrazolium (NBT) compound measures indirectly the amount of ROS generated in cells like oocytes, cumulus cells, or embryos (Javvaji *et al.* 2020), leukocytes, and spermatozoa (Esfandiari *et al.* 2003). NO is a gaseous radical generated from L-arginine to yield L-citrulline and NO by Nitric oxide synthase (NOS) enzymatic catalysis (Dede *et al.* 2002). NO is an endogenous smooth muscle relaxant and has a role during the pregnancy of mammals

for the flow of blood to the uterine and fetoplacental unit (Abdel-Ghani *et al.* 2016). It is considered a gold-standard angiogenic marker (Abdel-Ghani *et al.* 2016). The unstable NO is immediately oxidized into stable  $NO_2$  (nitrite) and  $NO_3$  (nitrate). Thus monitoring the level of the two end products through a spectrophotometer gives the total nitric oxide values (Moshage. 1997).

ROS damages lipids present on the membrane as polyunsaturated phospholipids during the process of lipid peroxidation. Malondialdehyde (MDA) reacts with thiobarbituric acid and produces a red pigment as thiobarbituric acid reactive substances (TBARS) which is measured through UV-absorbance (Adenkola *et al.* 2018). It is considered the best predictor of ROS that causes lipid peroxidation (Georgieva 2005). During the pregnancy period, the energy metabolism is upregulated and necessary production of ATP for foetal growth is inevitable. As the process proceeds, huge production of ROS in foetal and placental mitochondria was observed. Increased MDA level in placentomes, hypothesized the occurrence of oxidative stress (Mutinati *et al.* 2013); further, the level increases proportionally to the number of foetuses during pregnancy (Gür *et al.* 2011). In ruminants, MDA has been widely used as a stress marker in many studies

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like pregnant and non-pregnant ewes (Erisir *et al.* 2009), pregnancy and foetal number effects (Gür *et al.* 2011), pregnancy and nutrition influence (Nawito *et al.* 2016) and in different reproductive stages of does (Jimoh *et al.* 2019). Specifically MDA level has been measured in ALG under walking stress period (Perumal *et al.* 2019). Therefore the present study was designed to assess the oxidative stress markers status in different breeds and their rearing systems (farmers flock versus farm), kidding number (1-3 versus 3-6), and multiple birth frequency (high versus low).

## MATERIALS AND METHODS

**Experimental animals and sample collection:** The present study was conducted at an organized goat breeding farm and unorganized goat farms under a farmer's field, Andaman and Nicobar, India. The goats were maintained in the semi-intensive system where they were allowed for grazing from 0700 to 1200 h, and fed with commercially available concentrate pellets @250-300 g/day/animal mixed with a mineral supplement, AGRIMIN® (Virbac, India). Data from a total of 98 non-gravid does belonging to Andaman local goats and other admixtures with more than one kidding record were grouped based on their multiple birth frequency which was calculated by dividing the number of kidding having multiples (twinning/triplets) to the total number of kidding. All the procedures were followed after the ethical approval of the Institute Animal Ethics Committee (IAEC) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Blood samples were collected aseptically from the jugular vein and the serum was separated by centrifugation at 6000 rpm for 30 min and stored at -80°C until further analysis.

**LPO, NBT and NO assays:** The level of lipid peroxidation was estimated using Thiobarbituric acid reactive substance assay (TBARS) (EZAssay™, CCKO23, HiMedia Pvt Ltd, Mumbai, India) through malondialdehyde (MDA) substance. Superoxide anion ( $\text{O}_2^-$ ) was estimated by spectrophotometric nitroblue tetrazolium (NBT) reduction assay as described (Verma *et al.* 2020) with minor modifications. NO assay was performed as per manufacturer instructions (EZAssay™ Nitric Oxide estimation kit, CCK061, HiMedia Pvt Ltd, Mumbai, India), for the determination of the nitrate and nitrite concentrations.

**Data collection and statistical analysis:** The stress markers data was analysed for outliers using the box-plot method and assessed for normality using Kolmogorov-Smirnov and Shapiro-Wilk normality tests. All test

data were normally distributed. Homoscedasticity of variance, tested using Leven's test, was equal. Data were expressed as mean±S.E.M. A p-value≤0.05 was considered significant. The effects of breed (Andaman Local goat vs other admixture), rearing condition (Farmers flock vs Farm flock), the effect of kidding number (1-3 vs 3-6), and multiple birth frequency (High and Low) were analyzed using the PROC GLM model of Statistical Analysis Software (SAS Institute Inc., Cary, NC, USA 2002). The following model was used for analysis:

$$Y_{ijkl} = \mu + A_i + P_j + M_k + J_l + e_{ijkl}$$

Where,  $Y_{ijkl}$ , Antioxidant parameter of the doe;  $\mu$ , overall mean;  $A_i$ , fixed effect of  $i^{\text{th}}$  admixture group;  $P_j$ , effect of  $j^{\text{th}}$  multiple birth group;  $M_k$ ,  $k^{\text{th}}$  effect of the management group;  $J_l$ , effect of  $l^{\text{th}}$  kidding group;  $e_{ijkl}$ , error fraction.

## RESULTS AND DISCUSSION

The compiled results are presented in Fig. 1 and basic data is presented in the Table 1. The free radical superoxide and endogenous nitrite values with respect to breed, multiple birth frequency, and number of kidding and rearing systems were non-significant. Lipid peroxidation through TBARS and nitrate values concerning breed, multiple birth frequency, and rearing system were non-significant. However, the pregnancy-wise TBARS ( $p<0.05$ ) and nitrate concentration ( $p<0.05$ ) were significant. Estimates of TBARS were significantly high in the animals with lesser pregnancies (1-3 kidding) while nitrate concentration was significant ( $p<0.05$ ) in higher pregnancies (> 3 kidding).

ROS predominantly has superoxide anion which acts as a precursor for the generation of other radical species and a mediator for oxidative reactions. Thus monitoring the level of superoxide provides overall information on ROS status (Javvaji *et al.* 2020). NBT has been widely applied for the assessment of superoxide anion by relatively easy colorimetric tests. The specificity of NBT is based on the level of superoxide present intracellularly. Though NBT has been applied to analyse bovine serum fructosamine concentration (Megahed *et al.* 2018), assessment of the superoxide anion in cattle is not reported. Similarly in goats, the NBT application for the determination of superoxide was lacking; thus present study provides baseline data in relation to breed admixture, rearing system, parity (number of pregnancies/kidding), and multiple birth rate. The present study superoxide radical values were non-significant between the experimental groups in the experimental variables analysed.

During normal pregnancy, lipid peroxidation level was

Table 1. Basic data on different stress markers on Andaman local goats

| Marker                  | Minimum | Maximum | Mean    | Std. Error | CV      |
|-------------------------|---------|---------|---------|------------|---------|
| NBT (OD620)             | 0.9865  | 2.4795  | 1.8151  | 0.02673    | 0.13735 |
| MDA (μM)                | 0.5819  | 2.1738  | 0.99442 | 0.02405    | 0.23699 |
| Endogenous Nitrite (μM) | 0       | 0.244   | 0.11846 | 0.00654    | 0.345   |
| Nitrate (μM)            | 0       | 0.287   | 0.147   | 0.01011    | 0.42933 |

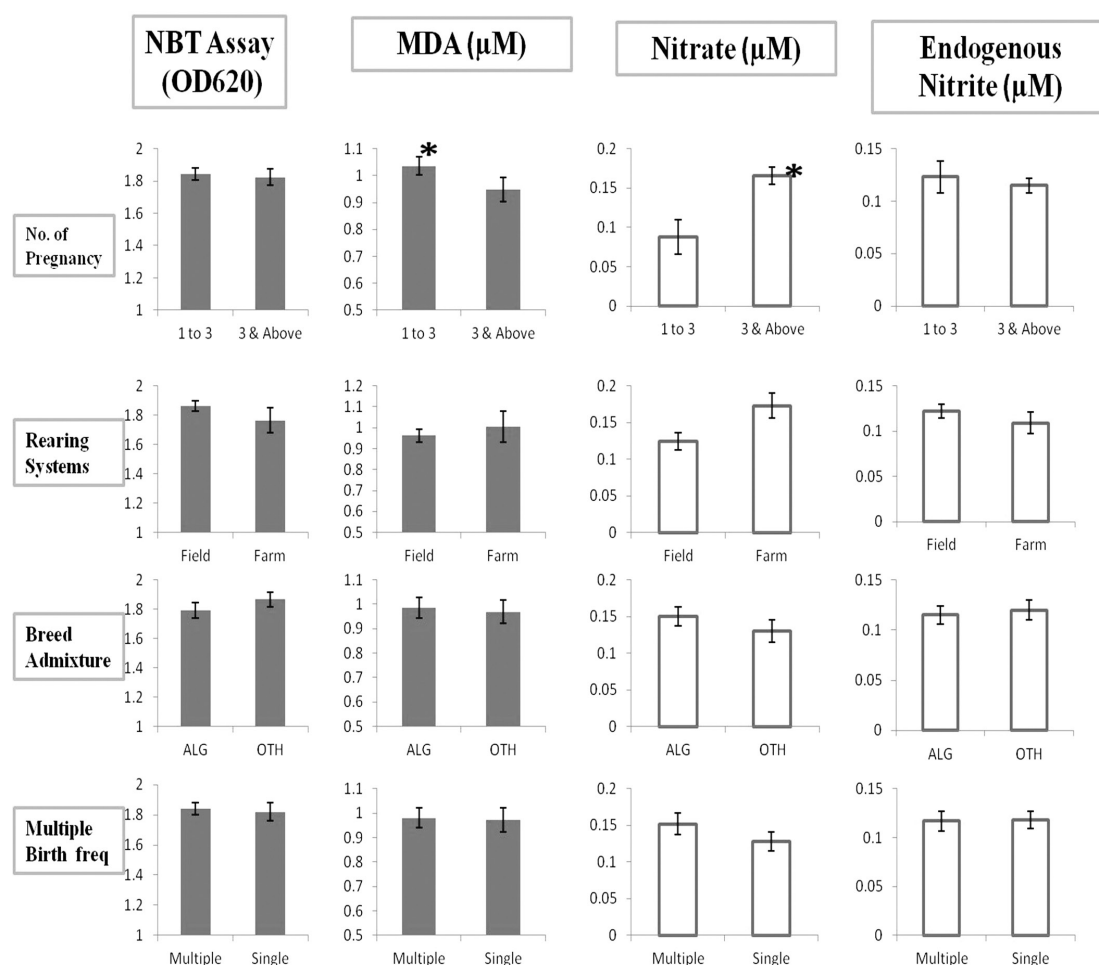


Fig. 1. Effect of different parameters on the serum levels of MDA, NBT, and NO in Andaman local goat breeds. Data were expressed as LSM±S.E.M. Significant at  $p < 0.05$  indicated by an asterisk.

higher in humans and rats (Erisir *et al.* 2009). MDA is known as an oxidative damage marker (Abd El Hameed *et al.* 2018). The results of present study were quite lower than the value of non-pregnant healthy Anatolian goats ( $1.43 \pm 0.48$  nmol/ml) (Kizil *et al.* 2007). The MDA value of adult clinically healthy Iranian native goats from both sexes ( $0.346$ – $0.801$  μmol/L) (Nazifi *et al.* 2009) was described as a reference value. The study value was found slightly higher than the reference range. The lipid peroxidation with respect to breed, multiple birth frequency and rearing system were non-significant. But with parity (number of pregnancies or number of kidding), TBARS were significant ( $p < 0.05$ ) with 1-3 parity numbers. Study throughout the pregnancy period of Saidi goats, MDA level was non-significant during the first month of all singletons, twin, and multiple bearing goats. Whereas the level was found higher during 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> months of pregnancy in twin and multiple bearing than in singleton goats (Abdel-Ghani *et al.* 2016). In another study, multiple bearing does reported higher MDA levels than in singleton and non-pregnant animals (Abd El Hameed *et al.* 2018). However, in this study, animals given singleton and multiple birth frequencies were similar and found non-significant. This may be because the present study estimated the base levels

parameters of different oxidative stress markers in non-gravid goats to use them as stress markers under different parameters studied while other studies used gravid and non-gravid animals that are at different stages of pregnancy. Hence, these findings indicate that the multiple-bearing goats and does at their initial pregnancies are under stress, especially at later stages of pregnancy, however estimating MDA levels in the non-gravid stage may not yield as useful marker for selecting them for multiple birth frequency.

Pregnant goats that were fed either concentrate or allowed for natural grazing had higher MDA levels than non-gravid goats (Nawito *et al.* 2016); however, the present values were lesser and non-significant between the rearing systems. The value of MDA in the present study for ALG and other breeds was lower than the earlier report in the indigenous goat breeds of ANI (Perumal *et al.* 2019). Similarly, the MDA value of the current study was lower than reported in the three different goat breeds Changthangi, Sirohi- and their Crossbred (Changthangi × Sirohi;  $6.90 \pm 1.13$  nmol/ml) (Kumar *et al.* 2019). In the present study, goats with 1-3 parity had significantly higher MDA does with >3 kidding history. The present study result can be compared with Dorset × Suffolk cross ewes that MDA level was found higher in 1<sup>st</sup> ( $4.02 \pm 0.13$  nmol MDA/ml) and 2<sup>nd</sup>

( $3.69 \pm 0.13$  nmol MDA/ml) parity than  $>4^{\text{th}}$  ( $3.49 \pm 0.13$  nmol MDA/ml) parity (Rios *et al.* 2017). The findings of these studies indicate a significant influence of the parity number of the does on the MDA estimates.

Nitric oxide (NO) plays a key role in vasculogenesis and angiogenesis which acts also as a uterine relaxant. Endothelial NO levels will be higher in the uterine artery as pregnancy proceeds (Xiao *et al.* 1999). During the pregnancy period, uteroplacental blood flow is increased up to 30-50 fold for maintenance of the growth and development of the foetus (Vonnahme *et al.* 2005). It is also important for the development of follicles, ovulation, steroidogenesis, and luteolysis. Therefore during the gestation period, NO level has been increased in the blood of goats (Kumar *et al.* 2012). Also, increased levels of arginine (a potential source of NO) are reported in the ovine uterine fluids (Kwon *et al.* 2003). However, the stability of NO is normally 5 sec (Lacasse *et al.* 1996) whereas nitrate and nitrite stability are longer which makes direct estimation difficult (Rosselli *et al.* 1994). Therefore measurement of these stable products provides the overall status of NO. In goat plasma, the reported level of NO ranged from 10.6 to 11.8  $\mu\text{M}$  (Kumar *et al.* 2012). In this study, nitrate and endogenous nitrite concentrations were determined (Table 1). Their levels show no significant differences between breeds, rearing systems (farmers flock versus farm flock), parities (kidding number 1-3 vs 3-6), and multiple birth frequencies (high and low). The only exception was levels of sodium nitrate estimated at different parity. It was significantly higher in goats with low parity (1-3) than more ( $>3$ ) kidding/parity. In another study, parasite infested ( $7.25 \pm 1.31$  and  $1.52 \pm 0.39$   $\mu\text{g/ml}$ ) goats had significantly higher nitrate and nitrite concentration than those in control groups ( $4.69 \pm 0.32$  and  $1.64 \pm 0.19$   $\mu\text{g/ml}$ ) (Dede *et al.* 2002). The findings of this study may only indicate that higher vascular and endothelial functions are expected in animals with higher parity.

The lack of variation in the oxidative marker profiles between breeds may indicate a similar adaptive mechanism. In a previous report, a lack of breed-wise variation in the antioxidant profiles of these animals was reported (Alyethodi *et al.* 2022). This may be due to the geographical isolation of these breeds in this island ecosystem that may be acquired due to their prolonged inhabitation to this geographically isolated island and animal import is largely restricted here due to biosecurity reasons. The effect of the rearing system (Farm vs Field) did not yield any difference in their oxidative profiles. On the Institutional farm, the goats were reared under a semi-intensive system. Animals were allowed grazing and fed with commercially available concentrate pellets along with mineral mixture. At the farmers' flock, the goats were maintained under the extensive system, neither concentrate feeding nor mineral mixture supplementation was practiced. Similar to the present findings, the levels of TBARS in goats reared under the semi-intensive and free-range system were reported non-significant (So-In, Charinya *et al.* 2023). The authors also report that rearing the goats in cages (intensive system)

significantly increases the TBARS levels indicating higher oxidative stress on the animals. These findings indicate the importance of the provision of grazing to farm animals.

Goats experience oxidative stress caused by superoxide anion and nitric oxides. The results of the present study revealed non-significant differences in the stress markers under studied variables except MDA and nitrate under the parity variable. Measurement of oxidative stress markers in goats of ANI was very scarce and their assessment is very crucial in the case of multiparous and multiple foetuses-producing animals. The stress levels of goats are varied with the availability of feed, pastures, and climatic conditions. Therefore, assessment of oxidative stress markers can be very advantageous for mitigating stress through antioxidant supplements and their data can be used as baseline value for further studies. The findings of this study also indicate that young animals are more susceptible to oxidative stresses and higher parity may improve the vascular and endothelial functions of the uterus as indicated by higher nitric oxide levels. It is concluded that values of antioxidants in the study can be considered as baseline data for the ALG and other goat breeds of Andaman and Nicobar Islands and other islands or topographies with similar ecosystems for further studies.

#### ACKNOWLEDGEMENT

We are thankful to the Director, ICAR-Central Island Agricultural Research Institute, Port Blair, for providing the necessary facilities to carry out the present research.

#### REFERENCES

- Abd El Hameed A R, Mahmoud K G M, Nawito M F, Sosa A S A and Ezzo O H. 2018. Progesterone concentration, oxidants and antioxidants indices in singleton and multiple bearings ewes and does. *Bioscience Research* **15**: 3869–75.
- Abdel-Ghani M A, El-Sherry T M, Hayder M and Abou-Khalil N S. 2016. Profile of peroxidative injury and antioxidant indicators in singleton, twins and multiple bearing goats throughout pregnancy. *Asian Pacific Journal of Reproduction* **5**: 400–05.
- Adenkola A Y, Adah A S and Azeez O M. 2018. Oxidative stress and haematological profiles of goats reared under different management systems. *Malaysian Journal of Veterinary Research* **9**: 19–29.
- Agarwal A, Gupta S and Sharma R K. 2005. Role of oxidative stress in female reproduction. *Reproductive Biology and Endocrinology* **3**: 1–21.
- Alyethodi R R, Sunder J, Karthik S, Perumal P, Bala P A, Muniswamy K, Arun K De and Bhattacharya D. 2022. Influence of breed, production system and fecundity on serum antioxidant profiles of goats reared in the tropical Island conditions. *Indian Journal of Animal Sciences* **92**: 1236–40.
- Dede S, Değer Y, Kahraman T, Değer S, Alkan M and Cemek M. 2002. Oxidation products of nitric oxide and the concentrations of antioxidant vitamins in parasitized goats. *Acta Veterinaria Brno* **71**: 341–45.
- Erisir M, Benzer F and Kandemir F M. 2009. Changes in the rate of lipid peroxidation in plasma and selected blood antioxidants before and during pregnancy in ewes. *Acta Veterinaria Brno* **78**: 237–42.

- Esfandiari N, Sharma R K, Saleh R A, Thomas A J and Agarwal A. 2003. Utility of the Nitroblue tetrazolium reduction test for assessment of reactive oxygen species production by seminal leukocytes and spermatozoa. *Journal of Andrology* **24**: 862–70.
- Georgieva N. 2005. Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems—A review. *Bulgarian Journal of Veterinary Medicine* **8**: 1–11.
- Gür S, Türk G, Demirci E, Yüce A, Sönmez M, Özer Ş and Aksu E H. 2011. Effect of pregnancy and foetal number on diameter of corpus luteum, maternal progesterone concentration and oxidant/antioxidant balance in Ewes. *Reproduction in Domestic Animals* **46**: 289–95.
- Halliwell B. 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology* **141**: 312–22.
- Inbaraj S, Kundu A, De A K, Sunder J and Sejian V. 2017. Seasonal changes in blood biochemical and endocrine responses of different indigenous goat breeds of tropical island agro-ecological environment. *Biological Rhythm Research* **1016**: 1–10.
- Javvaji P K, Dhali A, Francis J R, Kolte A P, Mech A, Roy S C, Mishra A and Bhatta R. 2020. An efficient nitroblue tetrazolium staining and bright-field microscopy based method for detecting and quantifying intracellular reactive oxygen species in oocytes, cumulus cells and embryos. *Frontiers in Cell and Developmental Biology* **8**: 1–12.
- Jimoh A O, Ojo O A, Daureen U and Ihejirika G. 2019. Metabolic and oxidative status of West African dwarf does at different reproductive stages in southwest Nigeria. *Bulletin of the National Research Centre* **43**: 190.
- Kumar A, Dangi S S, Mahla A S, Rajoriya J S and Purohit G N. 2012. Role of nitric oxide in animal reproduction: A review. *Theriogenology Insight* **2**(2): 73–72.
- Kumar P, Giri A, Bharti V K, Kumar K and Chaurasia O P. 2019. Evaluation of various biochemical stress markers and morphological traits in different goat breeds at high-altitude environment. *Biological Rhythm Research* **52**: 331–41.
- Kizil O, Ozdemir H, Karahan M and Kizil M. 2007. Oxidative stress and alterations of antioxidant status in goats naturally infected with *Mycoplasma agalactiae*. *Revue de Medecine Veterinaire* **158**(6): 326–30.
- Kwon H, Spencer T E, Bazer F W and Wu G. 2003. Developmental changes of amino acids in ovine fetal fluids. *Biology of Reproduction* **68**: 1813–20.
- Lacasse P, Farr V C, Davis S R and Prosser C G. 1996. Local secretion of nitric oxide and the control of mammary blood flow. *Journal of Dairy Science* **79**: 1369–74.
- Megahed A A, Hiew M W H and Constable P D. 2018. Clinical utility of plasma fructosamine concentration as a hypoglycemic biomarker during early lactation in dairy cattle. *Journal of Veterinary Internal Medicine* **32**: 846–52.
- Moshage H. 1997. Nitric oxide determinations: Much ado about NO•-Thing? *Clinical Chemistry* **43**: 553–6.
- Mutinati M., Piccinno M, Roncetti M, Campanile D, Rizzo A and Sciorsci R. 2013. Oxidative stress during pregnancy in the sheep. *Reproduction in Domestic Animals* **48**: 353–57.
- Nawito M F, Abd A R, Hameed E, Sosa A S A and Mahmoud K G M. 2016. Impact of pregnancy and nutrition on oxidant / antioxidant balance in sheep and goats reared in South Sinai, Egypt. *Veterinary World* **9**: 801–05.
- Nazifi S, Saeb M, Ghafari N, Razeghian I, Razavi S M, Vosoughi F, Dehghani F and Orangi H. 2009. Reference values of oxidative stress parameters in adult native Iranian goats. *Bulgarian Journal of Veterinary Medicine* **12**: 119–24.
- Perumal P, De A K, Bhattacharya D, Sunder J, Bhowmick S, Kundu A and Muniswamy K. 2019. Walking and dry season stresses modulates the physiological, hematological and biochemical profiles of indigenous local goats in Andaman and Nicobar Islands. *International Journal of Bio-resource and Stress Management* **10**: 597–605.
- Rios T S, Esqueda M T S T, Cruz A D, Mora J L C, Perrusquia R G, Morales J L R, Velasco J L F and Bautista J H. 2017. Oxidative state of ewes with different number of parity during gestation and lactation. *Pesquisa Veterinaria Brasileira* **37**: 1405–10.
- Rosselli M, Imthurn B, Macas E, Keller P J and Dubey R K. 1994. Circulating nitrite/nitrate levels increase with follicular development: Indirect evidence for estradiol mediated NO release. *Biochemical and Biophysical Research Communications* **202**(3):1543–52.
- So-In, Charinya and Nuchsupha Sunthamala. 2023. Influence of goat management systems on hematological, oxidative stress profiles, and parasitic gastrointestinal infection. *Veterinary World* **16**: 483–90.
- Sunder J and Kundu A. 2016. Haemato-biochemical profile of the Teresa goat: An Indigenous goat of A & N. *Journal of Immunology and Immunopathology* **18**: 47.
- Verma N, Alyethodi R R, Kathuria A, Alex R, Hussain S, Singh U, Tyagi S, Sirohi A S, Kumar S, Deb R, Sengar G S, Raja T V and Prakash B. 2020. Effect of heat stress on superoxide anion production in native and crossbred cattle under *in vitro* whole blood culture model. *Journal of Thermal Biology* **87**: 1–6.
- Vonnahme K A, Wilson M E, Li Y, Rupnow H L, Phernetton T M, Ford S P and Magness R R. 2005. Circulating levels of nitric oxide and vascular endothelial growth factor throughout ovine pregnancy. *Journal of Physiology* **565**: 101–09.
- Wang S, He G, Chen M, Zuo T, Xu W and Liu X. 2017. The role of antioxidant enzymes in the ovaries. *Oxidative Medicine and Cellular Longevity* **2017**: 4371714.
- Xiao D, Liu Y, Pearce W J and Zhang L. 1999. Endothelial nitric oxide release in isolated perfused ovine uterine arteries: Effect of pregnancy. *European Journal of Pharmacology* **367**: 223–30.