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Roles of free radicals in reproduction and immune modulations in goats: A review

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Abstract

In the body physiology, gonads (i.e. testes and ovaries) and lymphoid organs are most important tissues in terms of metabolism and dynamicity as functional activities of lymphoid organ and gonad are multistep and energy consuming processes they are always performing a number of biochemical reactions. As a causative effect of the same generation of free radicals are quite obvious. Thus, enormous production of free radicals can limit the proper functional management of lymphoid organ and gonad. We noted significantly high levels of SOD, Catalase, GPx activities and ABTS levels in lymphoid organs and gonads of goats during monsoon and significantly low during winter particularly in lymphoid organs. However, all the parameters were significantly high in gonads during winter only. Malonaldihyde; MDA a marker for lipid peroxidation presented a reverse pattern of activity level than SOD being significantly high during summer but the level was significantly low during monsoon and winter. The glucocorticoid receptor expression was significantly high in spleen and thymus of males during monsoon but the level was significantly high only during monsoon in lymphoid organs of males. All the free radical parameters cumulatively suggest that, monsoon and winter are the seasons of stress for both the sexes of goats as suggested by elevated level of glucocorticoid receptor and thus to counteract the elevated stress level melatonin acted as a "coupler" which not only increased the free radical scavenging enzymes but also scavenged free radicals as an amphipatic free molecules particularly during winter. Winter is the most challenging season for female goats due to "cold stress" as well as "gestational stress" along with energy demanding mega two major events i.e. Maintenance of immunity and gestation is occurring simultaneously.

Keywords: Catalase; Free radicals; Glucocorticoids; Goats; Gonad; GPx; Lymphoid organs; MDA; Melatonin

1 Introduction

The caprine species (particularly the goats) have to survive in different adverse climatic conditions in different parts of the world. In tropical environments (like India) they are under the threat of huge changes in the environmental temperature and humidity levels during different seasons [1]. During summer (April – June) they are under "heat stress" due to high temperature and scorching heat. Monsoon (July – September) is favourable season for growth of different pathogens due to high humidity levels [2]. Being the free grazing animals the goats are easily infected by all of these pathogens (like bacteria, coccidian, nematodes etc.) during monsoon. Further, monsoon is the reproductive preparatory phase for goats and thus, during monsoon the circulatory levels of different gonadal steroids are also high in both the sexes [3]. Being the ruminant short day breeder, winter season (November – January) is stressful particularly for female goats due to "gestational" as well as "cold" stress. Simultaneously, in the internal body milieu a number of physiological processes are going on in a regular manner to maintain the body homeostasis. Further, also to cope up with the environmental stress some important physiological metabolic functions were also elevated. All the physiological responses of elevated environmental stress and routine metabolic processes of the body can give rise to a number of free radicals which responsible for immune compromised condition for the animal which may be due to elevated level of apoptosis, [4] thus can limit their mortality and productivity as well [5]. In the body physiology, gonads (i.e. testes

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and ovaries) are most important tissues because of their spontaneous involvement in gametogenesis. As spermatogenesis and oogenesis both are multistep and energy consuming processes [6] they are always performing a number of biochemical reactions. As a causative effect of the same generation of free radicals are quite obvious. In the seasonal breeders, the gametogenesis process is dependent upon the status of reproductive physiology like active and inactive phases of reproduction [7, 8]. In the long day breeders (like squirrels and hamsters) and even in humans there are reports depicting the roles of free radicals in modulation of different physiological processes like immunity [9], metabolism [10] etc. In case of short day breeders the reports inadequate to depict the role of free radicals in regulation of reproduction.

Neurohormone melatonin is regarded as most important anti – stress hormone [11]. Melatonin itself or its metabolite 5-Sulfatoxy melatonin can directly scavenge free radicals [12] or it can up regulate the expressions of a number of free radical scavenging enzymes [13] In case of goats, the circulatory level of melatonin is highest during winter and winter is the period of gestation for female goats [1]. Thus, the role of melatonin as a pro-gonadotrophic and anti-stress hormone is mostly prevalent in goats. Further, it is also well reported that physiological manifestation of elevated stress is high circulatory level of glucocorticods [14] which is also anti-gonadotrophic [15] in nature. Till date only partial reports are available [16] demonstrating the levels of glucocorticoids and melatonin in circulation under thermal stress. But, detailed study considering the oxidative load in gonads and lymphoid organs of goats is totally lacking.

Therefore, objective of the present study was to note the seasonal and sex dependent variations on oxidative load/status in gonads and lymphoid organs of Indian goat *Capra hircus*. To establish the above objective we noted Total Anti-oxidant Status (TAS), levels of lipid peroxidation (by estimation of TBARS), different free radical scavenging enzyme (SOD, CAT, GPx) activities in lymphoid organs and gonads of goats.

2 Material and methods

2.1 Animals and maintenance

Goats of approximately same age (~1 year) and weight (~20 ± 2 kg) were procured from commercial goat raiser and then were housed in goat shelter under natural conditions of Varanasi ($25^0$18' N, 83^0 01' E, India) in order to maintain a consistency in food and hygiene throughout the year. At the time of procurement, the goats were weighed (Calf Weighing Sling, Munk's Livestock, Kansas, USA) and the age was determined by dentition as described by [17]. The male and female goats were kept separately to avoid mating or pheromonal effects. The detection of heat period was purely based on the visual observations i.e. more vocalization, reddening of vulva and mucorrhea. Goats were fed with usual ration of roughages (dry and green) and concentrate as suggested by Central Institute for Research on Goats, (CIRG), Mathura, Uttar-Pradesh, India. Single goat generally requires 4-5 kg of fodder/day and was fed with usual ration made up of roughages (dry and green) and concentrate. Dry roughages contained crushed barley (*Hordeum vulgare*, 1 part), crushed maize (*Zea mays*, 2 parts), linseed (*Linumusita tissimum*) or mustard seed cake (*Brassica juncea*, 2.25 parts), rice bran (*Oryza sativa*, 2 parts) along with small amount of molasses or a pinch of salt when required. Green roughages contained maize (*Zea mays*), elephant grass (*Pennisetum purpureum), pearl millet (Pennisetum glaucum*), sorghum (*Sorghum* sp.) and oat (*Avena sativa*). The concentrate contained oilseed cakes and soaked gram (*Cicer arietinum*) and water *ad libitum*. They were exposed to 8 hours outdoor for free grazing and 16 hours indoor (during night) conditions. Health of the goats was monitored by noting down the body temperature (normal rectal temperature, 102.5^0F–103^0F) and rumen movement by authorized veterinary doctors. Goats were treated with helminthicide twice per year and 0.5% solution of Malathion (acaricidal baths) as described by Chowdhury et al. (2002) [18]. The slaughtering of the goats was performed according in the city abattoir to the Slaughter of Animal Act under "Central Provinces Gazette" 1915 and modified in 2002. All the experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional practice within the framework of revised Animal (Specific Procedure) Act of 2007 of Government of India on animal welfare. The study was carried out during three major seasons of a year i. e. summer, monsoon and winter. Thus, the climatic condition during summer months was (April–June, temperature $43.87^0 \pm 1.02^0$ C, percent relative humidity [%RH] 36.74 ± 4.28%, day length, light–dark cycle-13.42 hours:10.18 hours), monsoon months (July–September, temperature $28.68^0 \pm 2.76^0$ C, %RH 87.04 ± 3.50%, day length, light–dark cycle-12 hours:12 hours), and winter months (November–January, temperature $10.76^0 \pm 3.63^0$ C, %RH 64.12 ± 3.05%, day length, light–dark cycle 10.35 hours: 13.25 hours). All of the results were validated with the samples collected from CIRG in a seasonal manner.

2.2 Experimental design

In order to study the free radical parameters in gonads and lymphoid organs of goats throughout the year, a total number of 108 male and female goats were included for the study. The study was conducted during three seasons, i.e.,

summer (April–June), monsoon (July–September) and winter (November–January). A total number of 12 goats (six males and six females) were selected from the flock for every month of a season (i.e. n = 6/sex/every month of season) and were numbered on ears. Thus, for summer, the total numbers of male goats were 18 and the total numbers of female goats were also 18. Hence, for summer the total number of males and females were 36 (18 males + 18 females). The same numbers of goats were used for monsoon and winter months. The results were validated with the samples collected from CIRG, Mathura, and Uttar-Pradesh.

2.3 Gonads, spleen and thymus sampling

Samples of desired tissues were collected following the method of Kaushalendra and Haldar (2012) [1]. Briefly, the animals were electrically stunned and bled immediately till death after terminal cervical incision in the city abattoir. The desired tissues (spleen, thymus and gonads**)** were collected aseptically, weighed (Kern Instruments, Germany), and a small portion was cut, washed in PBS for three times then weighed and kept in a sterile vial containing chilled PBS for assessment of enzymatic parameters. Further, left tissues were kept in -20^{o}C for different biochemical parameters.

2.4 Estimation of Superoxide Dismutase (SOD) activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the method of Das et al. (2000) [19]. 10% homogenates of tissues were prepared in 150 mM phosphate buffered saline (PBS, pH 7.4) and centrifuged for 30 min at 12,000 g at 4 °C. The supernatant was again centrifuged for 60 min at 12,000 × g at 4 °C and then processed for enzymatic activity based on a modified spectrophotometric method using nitrite formation by superoxide radicals. A 0.5 mL of homogenate was added to 1.4 mL of reaction mixture comprised of 50 mM phosphate buffer (pH 7.4), 20 mM L-methionine, 1% (v/v) Triton X- 100, 10 mM hydroxylamine hydrochloride, 50 mM ethylene diaminetetraacetic acid (EDTA) followed by a brief pre-incubation at 37 °C for 5 min. Next, 0.8 mL of riboflavin was added to all samples along with a control containing buffer instead of sample and then exposed to two 20W fluorescent lamps fitted parallel to each other in an aluminium foil coated wooden box. After 10 min of exposure, 1 mL of Greiss reagent was added and absorbance of the colour formed was measured at 543 nm on a spectrophotometer (ELx-800, Biotek Instruments, and Winooski VT, USA). One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under assay conditions. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

2.5 Estimation of Catalase activity

Catalase (CAT; EC 1.11.1.6) activity was measured following the procedure of Sinha (1972) [20]. This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture. There is production of green color at the end of the process. 10% homogenate of tissues were prepared in PBS (10 mM; pH 7.0) and then centrifuged at 12,000 × g for 20 min at 4^{0} C. Supernatant was taken for enzyme estimation. 5 mL of PBS was added to 4 mL of H_2O_2 (200 mM) and then 1 mL of enzyme extract was added. After 1 min 1 mL of this solution was taken in a tube and 2 mL of $K_2Cr_2O_7$ (5%) solution was added. Then it was boiled for 10 min and absorbance was measured at 570 nm (ELx-800, Biotek Instruments, and Winooski VT, USA). The activity of CAT was expressed as amount of H_2O_2 degraded per minute. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

2.6 Estimation of Glutathione Peroxidase (GPx) activity

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was assayed as described by Mantha et al. (1993) [21]. The reaction mixture (1 mL) contained 50 µL sample (10% tissue homogenates prepared in chilled PBS and centrifuged at 12, 000 × g), 398 µL of 50 mM phosphate buffer (pH 7.0), 2 µL of 1 mM EDTA, 10 µL of 1 mM sodium azide, 500 µL of 0.5 mM NADPH, 40 µL of 0.2 mM GSH and 1 U glutathione reductase. The reaction mixture was allowed to equilibrate for 1 min at room temperature. After this, the reaction was initiated by addition of 100 mM H_2O_2. The absorbance measured kinetically at 340 nm (ELx-800, Biotek Instruments, and Winooski VT, USA) for 3 min. The GPx activity was expressed as nmol of NADPH oxidized to $NADP^+$ per min per mg of protein using an extinction coefficient (6.22 mM/cm) for NADPH. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

2.7 Estimation of lipid peroxidation (LPO) assay by thiobarbituric acid reactive substances (TBARS) level

Tissues of goats were weighed and homogenized in a tenfold excess of 20 mMTris–HCl buffer (pH 7.4) and the 10% homogenates were centrifuged for 15 min at 3000 × g at 4 °C. The supernatant was subjected to thiobarbituric acid (TBA) assay by mixing with 8.1% sodium dodecyl sulphate (SDS), 20% acetic acid, 0.8% TBA and then digested it for 1

h at 95 °C [22]. The reaction mixture was immediately cooled in running water, vigorously shaken with 2.5 mL of n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 × g [23]. The absorbance of the upper phase was measured at 534 nm (ELx-800, Biotek Instruments, and Winooski VT, USA). Total thiobarbituric acid reactive substances (TBARS) were expressed as malondialdehyde (MDA; nmol/g tissue weight) taking 1, 1, 1, 1-tetraethoxy propane (TEP) as standard. The standard curve was calibrated using different dilutions of 10 nM TEP. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

2.8 Estimation of Total Antioxidant Status (TAS)

The free radical scavenging activity of antioxidants for 2, 2′- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cations was measured according to the method of Re et al. (1999) [24]. This method measures the antioxidant activity determined by decolorization assay of the ABTS radical cation, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. A stock solution of ABTS radical cations was prepared one day before the assay by mixing 5 mL of 7 mM ABTS with 1 mL of 14.7 mM potassium persulfate, followed by storage in the dark at room temperature. The stock solution of ABTS radical cations was diluted with water or ethanol. ABTS radical cation was generated by oxidation of ABTS with potassium persulfate. 2.95 mL of ABTS cation solution was mixed with 50 μL of 10% tissue homogenates and the decrease in absorbance was monitored for 10 min at particular interval of time at 734 nm (ELx-800, Biotek Instruments, and Winooski VT, USA).

2.9 Western blot analysis of glucocorticoid receptor expression pattern in gonads and lymphoid organs of goats

The Western blot analysis was performed according to the method published elsewhere [25]. Briefly, the thymus and spleen tissues were dissected in chilled PBS, homogenized, and lysed in lysate buffer. The protein content of the lysates was quantified using the Bradford method. The aliquots containing 100 μg protein of thymus were resolved on 10% (GR) of SDS-PAGE. Electrophoresis was followed by electrotransfer (Biometra, Goettingen, Germany) to nitrocellulose membranes (Bioscience, Keene, NH, USA) for 1 hour. The membranes were then blocked in Tris-buffered saline (TBS; Tris 50 mM, pH 7.5, NaCl 150 mM) solution containing 5% fat-free dry milk and 0.1% Tween-20 and were incubated with primary antibody against GR (anti-GR, N-20, sc-2045, at a dilution of 1:250. The membranes were washed thrice in TBS–Tween-20 and were incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) (donkey anti-rabbit HRP-IgG for GR at a dilution of 1:500). Finally, the blots were washed thrice with TBS and developed with Super Signal West Pico Chemiluminescent substrate (#34080; Thermo Scientific, Rockford, USA). Further, the membranes were stripped with stripping buffer (10% sodium azide) and were immunostained with β-actin antibodies in 1:1000 dilutions (A-2228; Sigma–Aldrich) as internal loading control. Immune detection of β-actin was performed with donkey anti-mouse IgG-HRP (1:1000). Bands were quantified by the measurement of O.D. using Scion Image Analysis Software (Scion Corporation, MD, and USA). Values were expressed as the ratio of the density of the specific signal to the β-actin signal. The ratio of density was calculated with respect to β-actin (house-keeping gene) and expressed as percent relative integrated density value of GR. The value presented was as percent band intensity ± standard error of the mean.

2.10 Statistical Analysis

The data were presented as the means ± Standard Error of Mean (SEM). Variation in tissue (gonads and lymphoid organs) level activities of SOD, CAT, GPx, TBARS, and ABTS levels of male and female goats were analyzed by two-way ANOVA. The expressions of GR was analyzed by one-way ANOVA followed by post hoc test i.e. Dunnett test (2-sided). In Dunnett t-test, male and female goats of summer season were treated as control and compared with all other groups. The mean difference was considered to be statistically significant at the 0.05 level ($p < 0.05$). Statistical analyses were done with Statistical Package of Social Sciences (SPSS) software version 17.0 and in accordance with Bruning and Knitz (1977) [26].

3 Results

It is to be noted that the studies are under way (unpublished data) the glimpses of the results will be presented.

3.1 SOD activity in lymphoid organs and gonads

SOD activity in spleen and thymus were significantly high in both the sexes during monsoon ($p < 0.01$) and significantly low during winter ($p < 0.05$ in male spleen and female thymus and $p < 0.01$ in male thymus). Further, SOD activity in gonads of both the sexes was significantly high during monsoon ($p < 0.01$) and winter ($p < 0.05$; Fig. 1C). However there was no sex dependent variation.

3.2 Catalase activity in lymphoid organs and gonads

Catalase activity in spleen and thymus of both the sexes were significantly high during monsoon ($p < 0.05$) and significantly low during winter ($p < 0.01$). Further, Catalase activity in testes and ovaries of both the sexes was significantly high ($p < 0.01$) during monsoon and significantly low ($p < 0.05$) winter in comparison to summer. Sex dependent variations were not observed.

3.3 Glutathione peroxidase (GPx) activity in lymphoid organs and gonads

Glutathione peroxidase (GPx) activity in spleen and thymus of both the sexes were significantly high during monsoon and winter ($p < 0.01$). Glutathione peroxidase (GPx) activity in testes and ovaries of both the sexes was significantly high during monsoon ($p < 0.05$) and winter ($p < 0.01$). However there was no sex dependent variation.

3.4 ABTS level in lymphoid organs and gonads

During monsoon the Total Antioxidant Status (TAS) of lymphoid organs of both the sexes were significantly high ($p < 0.01$). During wither also the similar trend was observed. The TAS level was significantly high in spleen ($p< 0.05$) and thymus ($p < 0. 01$) of both the sexes. During monsoon and winter the Total Antioxidant Status (TAS) of gonads of both the sexes were significantly high ($p < 0.01$ during monsoon and $p < 0.05$ during winter). However, sex dependent variations were not observed.

3.5 Estimation of lipid peroxidation (LPO) assay by thiobarbituric acid reactive substances (TBARS) level

In case of both thymus and spleen during summer the level was highest. But, the level was significantly low monsoon ($p < 0.01$ in female spleen and male thymus). In case of female thymus, the level was significantly low ($p < 0.01$) during summer in comparison to males and the level was significantly high ($p < 0.01$) during monsoon in comparison to males as well as in comparison to summer. In case of both testes and ovaries during summer the level was lowest in both the sexes. But, the level was significantly high in gonads of both the sexes during monsoon ($p < 0.05$) and winter ($p < 0.01$).

3.6 Western Blot analysis of expression of Glucocorticoid Receptor (GR)

The glucocorticoid receptor (GR) expression pattern in males was significantly high in thymus, spleen and testes during monsoon and winter ($p < 0.05$). But in case of female spleen, thymus and ovaries the level was significantly high only during monsoon ($p < 0.05$).

4 Discussion

Pesticides are well known for increasing the free radical loads of different metabolically active organs such as kidney [27], liver [28], brain [29] and immune system [30] of animals including human being [31]. In the present agro-ecosystem, the use of pesticides and chemical fertilizers is increasing continuously to fulfil the requirement of food for ever expanding population. But, pesticides pose a large threat to primary consumers. Hence, like other herbivores, goats being free grazing animals are directly exposed to pesticides and other environmental stresses during different seasons of the year. All these factors cumulatively have weakened their immune system. Reports are available regarding oxidative and nitrosative stress in lymphoid organs and gonads in different seasonal [32] and spontaneous breeders [31]. But, reports on the free radical load in goats are totally lacking. In this context our results are significant and first of its kind depicting the free radical status in gonads and lymphoid organs of goats.

The generation of reactive oxygen species by aerobic organisms comes with a high physiological price, which can be lowered by antioxidants such as melatonin [33] Endogenously produced melatonin may have a significant role in deferring a number of free radical-related disease and some patho-physiological changes [34]. Being amphipathic molecule this indoleamine is acting as free radical scavenger because it has the capability of penetrating all physiological barriers and can enter all sub-cellular compartments. Thus, high level of circulatory melatonin during winter season might be responsible for lowered lipid peroxidation and increased antioxidant enzyme level in lymphoid organs of goats. The antioxidant enzymes (SOD, CAT, GPx) showed a clear-cut variation in a season dependent manner. Maximum levels of antioxidant enzymes (SOD, CAT and GPx) were observed in all the groups of goats during monsoon and winter season when the peripheral melatonin level was high [2]. Our data gets support from the studies of small mammals suggesting that changes in oxidative load are dependent on the circadian melatonin rhythm [35]. Therefore, a physiological level of melatonin appears to be adequate to alter the antioxidant defence system as reflected by the level of activities of antioxidant enzymes in goats. During monsoon the circulatory level of melatonin is moderately high but cortisol level is highest [2]. This season is also the reproductive preparatory phase of goats with higher levels of gonadal steroids [3]. Due to high level of temperature and humidity, monsoon is the most important season for parasitic growth

and infections. Being free grazing animals goats are under inflammatory stress and can generate high level of free radicals. To scavenge them the free radical scavenging enzyme level and TAS levels were also high. During winter, both the male and female goats are under cold stress and particularly females are under gestational stress. Melatonin might be stimulating the protective activity of antioxidant enzymes as designated by ABTS radical cation reduction. The more free radicals, the less ABTS percentage inhibition occurs and *vice-versa*. The present results also suggested that physiological level of melatonin in circulation of a seasonal breeder is highly relevant in terms of total antioxidant capacity of lymphoid organs and gonads. Effects of lipid per-oxidation in particular are under intense investigation because of their involvement in several pathological conditions. The unsaturated lipids are more prone to free radicals damage and hence, lipid per-oxidation is considered as the biomarker of free radical load [36]. As oxidative stress is indicative imbalance between oxidants and antioxidants, methods for quantifying oxidative stress mostly include direct or indirect estimation of oxidants and antioxidants. Malonaldehyde (MDA) is a low molecular weight end product which is generated as the free radical damages the lipids [37]. Further, melatonin reduced lipid peroxidation during monsoon and winter. This supports our observation of seasonal variation in enzyme activity, TAS levels as recorded in lymphoid organs and gonads of goats.

In general, the level of melatonin has inverse correlation with the cortisol level. Whenever the goats were under ecological stress during monsoon (seasonal infection etc.) and winter (cold stress and gestational stress) the level of melatonin was moderate and high but the cortisol level was high. This suppressed the general immunity and increased the free radical load. Our data gets further support from the result of up regulated GR expression during monsoon and winter in male and female goats. During monsoon, the levels of cortisol and its receptor (GR) in lymphoid organs of both the sexes were significantly high. This may be due to elevated inflammatory stress and moderately high level of melatonin. During winter, moderately high level of cortisol and GR expression suggest that due to high level of melatonin the level of free radical was decreased. This is an adaptive modification particularly suggested for female goats for maintenance of pregnancy and perfect gestation.

We noted higher level of free radical scavenging enzyme parameters (SOD, CAT and GPx) and TAS level in gonads during monsoon. This may be due to higher metabolic activity of gonads of both the sexes in terms of steroidogenesis and gametogenesis. The sophisticated machinery of steroidogenesis and gametogenesis might have involved a number of local biochemical activities which in turn have generated huge amount of free radicals which is equivocal with the reports of other workers [38, 39]. Thus, to cope up with the elevated level of free radicals, the free radical scavenging enzyme activities and TAS levels were high. Another reason may be that, during monsoon melatonin level was moderately high and the free radical scavenging activity of melatonin as a free molecule was low. So, the gonadal level of stress management was solemnly performed by gonads it selves. This point was further supported by moderately high level of free radical scavenging enzymes and TAS level during winter when plasma level of melatonin attended its yearly peak. Being an amphi-pathic molecule, melatonin might have crossed the Blood Testes Barrier (BTB) in males and scavenged free radicals. Further, it is also well reported that both testes and ovaries are well equipped with melatonin synthesizing machinery and locally can synthesize melatonin at tissue level [3]. Thus, this local melatonin and one of its metabolite (5-sulfatoxymelatonin) can scavenge free radicals as 5-sulfatoxymelatonin is more potent free radical scavenger than melatonin itself [40]. Another possibility is that, tissue melatonin might have elevated some of the key enzyme for free radical scavenging (like GPx) during winter which either alone was sufficient enough to scavenge free radicals or have elevated the levels of other free radical scavenging enzymes (like SOD and CAT0 [41].

We noted higher level of MDA (a marker of lipid peroxidation) in gonads during monsoon and winter in both the sexes. The reason behind is that, high cholesterol was taken up by both testes and ovaries during monsoon and winter. But the paths of utilization in both the sexes were different. During monsoon it was utilized by both the sexes for steroidogenesis and during winter it was utilized by females for maintenance of gestation and by males for spontaneous gametogenesis.

5 Conclusion

Being ruminant short day breeder maintenance of immunity and reproduction is a simultaneous process in goats. But, both the processes are highly energy demanding. Thus, our next aim was to note the path of energy allocation in goats, *Capra hircus* to modulate reproduction and immunity particularly during monsoon (which is the reproductive preparatory phase and season of inflammatory stress) and winter which is the season for cold stress for both the sexes and gestational stress particularly for females.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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