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Season and Sex Dependent Variations in Free Radicals In Gonads and Their Correlation with Melatonin and Cortisol in Indian Goat *Capra Hircus*

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Abstract: *In the body physiology, gonads (i.e. testes and ovaries) are most important tissues in terms of metabolism and dynamicity. As functional activities of gonads 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 gonads. To explore the level of free radical status in gonads we studied free radical scavenging enzymes (Super Oxide Dismutase; SOD, Catalase; Cat, Glutathione Peroxidases; GPx) and Malonaldehyde (MDA; marker of lipid peroxidation) in gonads of goats by standard biochemical methods. Total Antioxidant Status (TAS) was measured by ABTS estimation. We noted significantly high levels of SOD, Catalase, GPx activities and ABTS levels in gonads of goats during monsoon and significantly low during winter. However, MDA was significantly low in gonads during winter. Thus, we may conclude that, winter is a season of maximal stress in gonads of goats due to high reproductive performance and gestational stress particularly in females.*

Keywords: *ABTS, CAT, Free radicals, goats, SOD, gonad, GPx, MDA.*

1. Introduction

In the body physiology, gonads (i.e. testes and ovaries) are most important tissues because of their spontaneous involvement in gametogenesis. As spermatogenesis and oogenesis both are multistep and energy consuming processes [1], 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 [2, 3]. 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 [4], metabolism [5] etc. In case of short day breeders the reports are inadequate to depict the role of free radicals in regulation of reproduction.

Neurohormone melatonin is regarded as most important anti – stress hormone [6]. Melatonin itself or its metabolite 5-Sulfatoxy melatonin can directly scavenge free radicals [7] or it can up regulate the expressions of a number of free radical scavenging enzymes [8]. In case of goats, the circulatory level of melatonin is highest during winter and winter is the period of gestation for female goats [9]. 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 glucocorticoids [10] which is also anti-gonadotrophic [11] in nature. Till date only partial reports are available [12] 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 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 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 Fandos et al. (1993), [13]. 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 (*Linumusit atissimum*) 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), [14]. 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 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, Uttar-Pradesh.

2.3. Blood Sampling

For the assessment of peripheral hormone, one night before the slaughtering, blood of male and female goats was collected from left jugular vein by venipuncture applying minimum stress [9]. Blood samples were obtained during the night time (3 hours after sunset) in a 10 mL dispovan syringe coated with 10% EDTA (anticoagulant). All the goats were sampled within 40 minutes under dim red light (less than 1 lux at a distance of 20 cm) to avoid a direct illumination to the eyes of the goats. Blood was centrifuged (3000 × g) for collection of plasma and was immediately stored at -20^0C until the analysis of hormones (melatonin and cortisol).

2.4. Sampling of testes and ovaries

The animals were electrically stunned and bled immediately till death after terminal cervical incision [9]. The desired tissues (testes and ovaries) were collected aseptically, weighed (Kern Instruments, Germany), and a small portion was cut, washed in PBS for three times then weighed and was kept in a sterile vial containing chilled PBS for assessment of enzymatic parameters.

2.5. Circulatory Level of Cortisol

The ELISA kit of cortisol was generously gifted by Prof. T. G. Srivastava, National Institute of Health and Family Welfare (NIHFW), New Delhi, India. The estimation was carried out following manufacturer's protocol. According to the manufacturer's instruction, 25 µL of standard, control and samples were added in each well of ELISA plate followed by 100 µL of enzyme conjugate solution. The wells were then incubated with mild shaking at room temperature for two hours. The wells were then aspirated and washed three times with wash solution. Then, 100 µL of the TMB chromogenic solution (substrate) was added to each well and plate was incubated at room temperature for 30 minutes in dark. Finally, 100 µL of stop solution was added in each well and absorbance was recorded at 450 nm. The co-efficient of variation between intra and inter – assay variations ranged from 3.38% to 5.56% and 5.69% to 7.84% respectively. The recovery was 92% with an accuracy of 98.7%. The sensitivity or lower level of detection was 0.27µg/dL. The assay was carried out in triplicate.

2.6. Circulatory Level of Melatonin

Peripheral melatonin level was measured in the blood collected at night with the help of a commercial kit (Biosource, Nivelles, Belgium; Cat. No. KIPL3300) according to the manufacturer's protocol.According to the manufacturer's instruction, 25 µL of standard, control and samples were added in each well of ELISA plate followed by 100 µL of enzyme conjugate solution. The wells were then incubated with mild shaking at room temperature for two hours. The wells were then aspirated and washed three times with wash solution. Then, 100 µL of the TMB chromogenic solution (substrate) was added to each well and plate was incubated at room temperature for 30 minutes in dark. Finally, 100 µL of stop solution was added in each well and absorbance was recorded at 450 nm. Analytic sensitivity (limit of detection) for melatonin serum was 2 pg/mL. Inter and intra-assay variations were between 9.0% and 15%, respectively. The assay was carried out in triplicate.

2.7. 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 [15]. 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, Winooski VT, USA). One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under assay conditions.

2.8. Estimation of catalase activity

Catalase (CAT; EC 1.11.1.6) activity was measured following the procedure of Sinha, 1972 [16]. 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 4^0C. 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, Winooski VT, USA). The activity of CAT was expressed as amount of H_2O_2 degraded per minute.

2.9. Estimation of glutathione peroxidase (GPx) activity

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was assayed as described by Mantha et al., 1993 [17]. 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, 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.

2.10. 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 [18]. 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 [19]. The absorbance of the upper phase was measured at 534 nm (ELx-800, Biotek Instruments, 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.

2.11. 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 [20]. 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, Winooski VT, USA).

2.12. Statistical analysis

The data were presented as the means ± Standard Error of Mean (SEM). Variation in lymphoid tissue level activities of SOD, CAT, GPx, TBARS, ABTS levels of male and female goats were analyzed by one-way ANOVA. To evaluate the interactive effect (male *vs* female), the Duncan multiple range t test was used. 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 [21].

3. RESULTS

3.1. Circulatory Level of Cortisol

Circulatory level of cortisol was significantly high in both the sexes during monsoon ($p < 0.01$) and winter ($p < 0.05$; Fig. 1) There was no sex dependent variation or sex * season interactive effect ($p > 0.05$)

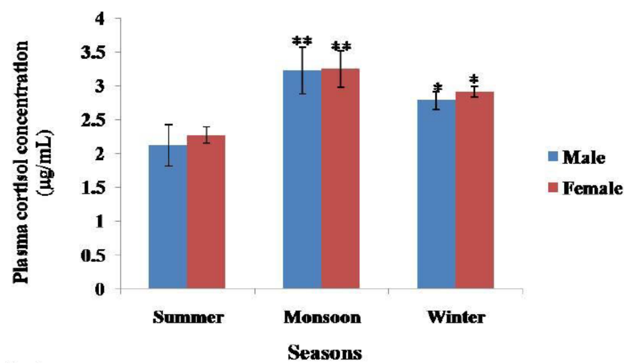

Fig1. *Season and sex dependent variations in plasma cortisol level in male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter*

3.2. Circulatory Level of Melatonin

Circulatory level of melatonin was significantly high in both in cases of males and females during monsoon ($p < 0.05$) and winter ($p < 0.01$) in comparison to summer. Females always presented a significantly higher level of melatonin in comparison to males during summer ($p < 0.05$) and monsoon ($p < 0.01$) and summer and winter ($p < 0.01$; Fig. 2).

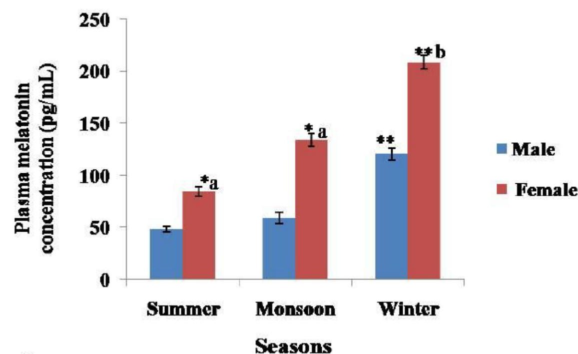

Fig2. *Season and sex dependent variations in plasma melatonin level in male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p < 0.01; summer vs monsoon and winter. a p< 0.05, b p< 0.01; male vs female*

3.3. SOD activity in gonads

SOD activity in gonads of both the sexes was significantly high during monsoon ($p < 0.01$) and winter ($p < 0.05$; Fig. 3A). Sex dependent variations were not observed.

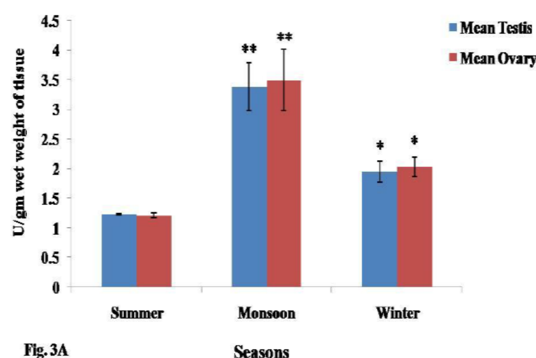

Fig3A. *Season and sex dependent variations in Super Oxide Dismutase (SOD) activity in gonads of male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter*

3.4. Catalase activity in gonads

Catalase activity in testes and ovaries of both the sexes was significantly high ($p < 0.01$) during monsoon and winter ($p < 0.05$) in comparison to summer (Fig. 3B). Sex dependent variations were not observed.

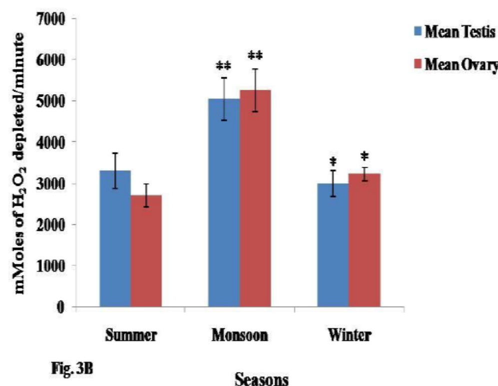

Fig3B. *Season and sex dependent variations in Catalsaseactivity in gonads of male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter.*

3.5. GPx activity in gonads

Glutathione peroxidase (GPx) activity in testes and ovaries of both the sexes was significantly high during monsoon ($p < 0.05$) and winter ($p < 0.01$; Fig. 3C). However there was no sex dependent variation.

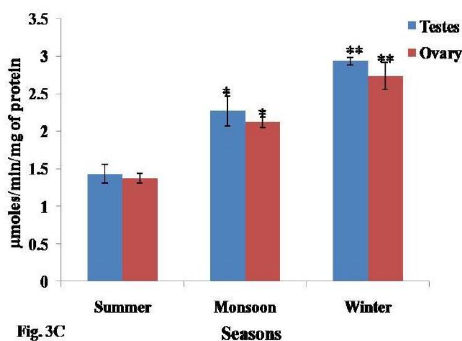

Fig3C. *Season and sex dependent variations in Glutathione Peroxidase (GPx) activity in gonads of male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter.*

3.6. TAS level in gonads

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; Fig. 3D). However, sex dependent variations were not observed.

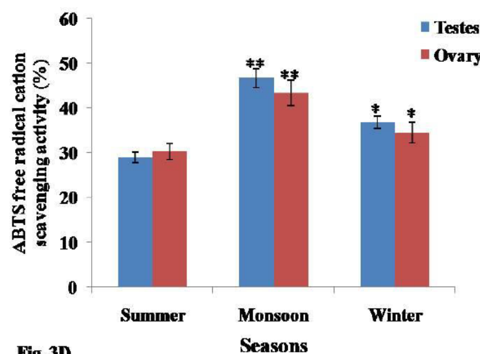

Fig3D. *Season and sex dependent variations in 2,2'- azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS)activity in gonads of male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter.*

3.7. MDA level in gonads

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 ($p < 0.01$; Fig. 3E).

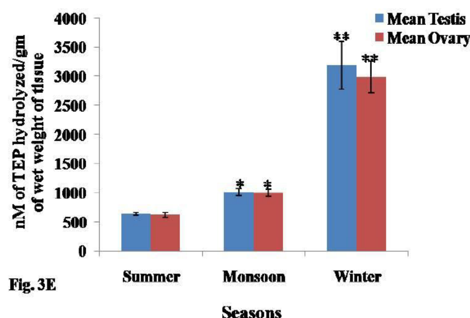

Fig3E. *Season and sex dependent variations in Malonaldihyde (MDA) level in gonads of male and female goats, C. hircus. Data represents mean ± SEM, N=18/sex/season. Vertical bar on each point represents standard error of mean (SEM). *p< 0.05, ** p< 0.01; summer vs monsoon and winter.*

4. Discussion

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 (unpublished data). 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 [22, 23]. 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 [24]. 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 [25]. 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 CAT).

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

In conclusion we may suggest that monsoon and winter are the most important seasons in terms of stress to both the sexes of goats as suggested by elevated level of free radical scavenging enzymes, stress hormone glucocorticoid and its receptor. Monsoon is stressful due to reproductive preparatory phase but winter is stressful for maintenance of gestation particularly in females. In this context, the role of neurohormone melatonin is noteworthy. Melatonin acted as a "coupler" which not only increased the free radical scavenging enzymes but also scavenged free radicals as an amphipatic free molecule particularly during winter.

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REFERENCES

[1] Jouaux A., Blin J.L., Adeline B., Heude-Berthelin C., Sourdaine P., Mathieu M. and Kellner K., Impact of energy storage strategies on gametogenesis and reproductive effort in diploid and triploid Pacific oysters *Crassostrea gigas* — Involvement of insulin signaling, Aquaculture. 388, 173 (2013).

[2] Chemineau P., Guillaume D., Migaud M., Thiéry J.C., Pellicer-Rubio M.T. and Malpaux B., Seasonality of Reproduction in Mammals: Intimate Regulatory Mechanisms and Practical Implications, Reprod. Domest. Anim. 43, 40 (2008).

[3] Ungerfeld R. and Bielli A., Seasonal and Social Factors Affecting Reproduction Animal Reproduction in Livestock, Chapter in the book: Encyclopedia of Life Support System, U.S.A.: UNESCO, 2012.

[4] Knight J. A., Free radicals, antioxidants, and the immune system, Ann. Clin. Lab. Sci. 30, 145 (2000).

[5] Droge W., Free Radicals in the Physiological Control of Cell Function, Physiol. Rev. 82, 47 (2002).

[6] Vishwas D.K., Mukherjee A, Haldar C., Dash D. and Nayak M.K., Improvement of oxidative stress and immunity by melato.nin: An age dependent study in golden hamster, Exp. Gerontol. 48, 168 (2013).

[7] Tan D.X., Manchester L.C., Reiter R.J., Qi W.B., Karbownik M. and Calvo J.R., Significance of melatonin in antioxidative defense system: reactions and products, Biol. Signals. Recept. 9, 137 (2000).

[8] Toma´s-Zapico C. and Coto-Montes A., A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes, J. Pineal. Res. 39, 99 (2005).

[9] Kaushalendra and Haldar C., Correlation between peripheral melatonin and general immune status of domestic goat, *Capra hircus*: A seasonal and sex dependent variation, Small. Rumin. Res. 107, 147 (2012).

[10] Gupta S. and Haldar C., Physiological crosstalk between melatonin and glucocorticoid receptor modulates T-cell mediated immune responses in a wild tropical rodent, *Funambulus pennanti*, J. Steroid. Biochem. Mol. Biol. 134, 23 (2013).

[11] van Diepen H.A., Preclinical pharmacological profile of nomegestrol acetate, a synthetic 19-nor-progesterone derivative, Reprod. Biol. Endocrinol. 10, 1 (2012).

[12] Veeraswamy S., Srivastava R.S. and Varshney V.P., Pineal-adrenal relationship: modulating effects of glucocorticoids on pineal function to ameliorate thermal-stress in goats, Asian-Aust. J. Anim. Sci. 21, 988 (2008).

[13] Fandos P., Orueta J.F. and Olanda A., Tooth wear and its relation to kind of food: the repercussion on age criteria in *Capra pyrenaica,* Acta. Theriol., 38, 93 (1993).

[14] Chowdhury S.A., Bhuiyan M.S.A. and Faruk S., Rearing Black Bengal goat under semi-intensive management 1. Physiological and reproductive performances, Asian-Aust. J. Anim. Sci. 15, 477 (2002).

[15] Das K., Samanta L. and Chainy G.B.N., A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals, Ind. J. Biochem. Biophys. 37, 201 (2000).

[16] Sinha A.K., Colorimetric assay of catalase, Anal. Biochem. 47, 389 (1972).

[17] Mantha S.V., Prasad M., Kalra J. and Prasad K., Anti oxidant enzymes in hypercholesteromia and effect of vitamin E in rabbits, Atherosclerosis, 101, 135 (1993).

[18] Sharma S., Haldar C. and Chaube S.K., Effect of exogenous melatonin on X-ray induced cellular toxicity in lymphatic tissue of Indian tropical male squirrel, *Funambulus pennant*, Int. J. Radiat. Biol. 84, 363 (2008).

[19] Ohkawa H., Ohishi N. and Yagi K., Reaction of linoleic acid hydroperoxide with thiobarbituric acid, J. Lipid. Res. 19, 1053 (1978).

[20] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free. Rad. Biol. Med. 26, 1231 (1999).

[21] J.L. Bruning and B.L. Knitz, Computational handbook of statistics, Scott, F and Company Eds, 2nd Ed. Illinois, USA (1977).

[22] Jana K., Samanta P.K. and De D.K., Nicotine Diminishes Testicular Gametogenesis, Steroidogenesis, and Steroidogenic Acute Regulatory Protein Expression in Adult Albino Rats: Possible Influence on Pituitary Gonadotropins and Alteration of Testicular Antioxidant Status, Toxicol. Sci. 116, 647 (2010).

[23] Das U.B., Mallick M., Debnath J.M. and Ghosh D., Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic and androgenic disorders in male rats, Asian. J. Androl. 4, 201 (2002).

[24] Ghosh S., Singh A.K., and Haldar C., Seasonal modulation of immunity by melatonin and gonadal steroids in a short day breeder goat *Capra hircus,* Theriogenol. 82, 1121 (2014).

[25] Rosen R., Hu D.N., Perez V., Tai K., Yu G.P., Chen M., Tone P., McCormick S.A. and Walsh J., Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients, Mol. Vis. 15, 1673 (2009).

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