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Season and Sex Dependent Variations in Energy Allocation for Reproduction and Immunity of Indian goat *C. hircus*

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KEYWORDS

Cholesterol, Energy allocation, Glucose, Glycogen, Gonads, Immunity, Lymphoid organs, Reproduction

A B S T R A C T

In most of the mammals including human the energy balance between reproduction and immune modulations are well managed due to their opportunistic behavior in reproduction. But, it is a matter of concern in seasonal breeders. The main problem of energy management is with the short day breeders (sheep and goats) where reproduction and immune modulations are occurring simultaneously. We noted significantly high level of cholesterol and glucose in thymus spleen and gonads of both the sexes during monsoon and winter but tissue level (liver, spleen and thymus) glycogen was significantly low during monsoon and winter. % uptake of glucose and cholesterol was significantly high in lymphoid organs. Marker of glycogenolysis, glycogen phosphorylase was significantly high in lymphoid organs and gonads during monsoon and winter in both the sexes. Thus, in conclusion it can be suggested that, the role of thymus is of highest importance (in terms of energetic), to maintain two energetically most important events i.e. immunity and reproduction simultaneously by acting as "local power house".

Introduction

Reproduction and immune modulation both are extremely dynamic biological mega events. Modulation in immunity is in need of a balance of mitosis and apoptosis. Reproduction in both males and females involves gametogenesis and steroidogenesis process. Hence, both reproduction and immune modulations are high energy demanding processes.

In most of the mammals including human the energy balance between reproduction and immune modulations are well managed due to their opportunistic behavior in reproduction (Garcia-Garcia, 2012). But, it is a matter of concern in seasonal breeders (Nelson and Demas, 1997). However, in the long day breeders (like squirrel, hamsters) the energy allocation pattern is partially documented.

Their reproductive phase is divided into two different phases Reproductive Active Phase (RAP) and Reproductive Inactive Phase (RIP). During, RAP the circulatory level of melatonin is low with basal level of immune parameters and high reproductive behavior and performances. But during RIP; reproductive performances are low while immunological parameters are high (Haldar and Ahmad, 2010; Demas and Nelson, 2003). The main problem is with the short day breeders (sheep and goats) where reproduction and immune modulations are occurring simultaneously. Thus, it is a matter of investigation that how both the high energetically important mega events are occurring simultaneously in short day breeders (Demas and Nelson, 1996; Ruckstuhl *et al.*, 2003). In sheep, the reproductive energy demand is partially documented (Sayed, 2009), but till date no report is available regarding the energy balance between reproduction and immunity in goats.

Further, in some animals (like mice and rats) it is evident that there may be a local hormonal circuit in peripheral endocrine organs (like ovary for GnIH and GnRH; Singh *et al.*, 2010), salivary gland (Harris and Kaufman, 1985), GI tract (Ahlman and Nilsson, 2001; Clarke *et al.*, 2014) etc. But, no report is available regarding the primary and secondary lymphoid organ can be regarded as endocrine organ and also a site for local micro circuit of hormones. This kind of results or even speculations are totally lacking in any seasonal (including long day or short day) breeders.

We identified the lacunae of previous studies and therefore, aim of the present study was to note the seasonal and sex dependent variations in energy allocation pattern in reproduction and immune modulation in goats with a special focus on hormonal microcircuit in lymphoid organs.

Materials and Methods

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). 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 (*Pennisetumpurpureum), 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). 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.

Experimental design

In order to study the energy allocation pattern in regulation of reproduction and immunity in 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.

Blood sampling

For the assessment of plasma parameters, one night before the slaughtering, blood of male and female goats was collected from left jugular vein by venipuncture applying minimum stress (Kaushalendra and Haldar, 2012). 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 biochemical (glucose, cholesterol etc.) parameters.

Sampling of spleen, thymus, liver and gonad

The animals were electrically stunned and bled immediately till death after terminal cervical incision (Kaushalendra and Haldar, 2012) in the city abattoir. The desired tissues (spleen, thymus, liver and gonads**)** were collected aseptically, weighed (Kern Instruments, Germany), and a small portion was cut, washed in PBS for three times then weighed. A small portion was weighed and kept in a sterile vial containing chilled PBS

were kept in -20^{o}C for different biochemical parameters.

Glucose estimation

The extraction of tissue glucose was done by the method of Moses *et al.* (2006). In brief the tissue was homogenized in 6 N perchloric acid solutions (10% V/V) and then was spun at 10,000 rpm for 10 minutes. Finally the supernatant was taken and glucose was estimated by commercially available glucose estimation kit (Beacon India Pvt. Ltd., Mumbai) following manufacturer's protocol. The plasma was directly used for the assay.

Cholesterol assay

The cholesterol estimation was done by method of Sackett (1969). The stock solution of cholesterol was made of 1 mg/mL. Then serial dilutions were made from 0-200µg/mL in chloroform for standard curve. A mixture of acetic anhydride and sulphuric acid (20:1) was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm. For experimental samples the cholesterol was extracted in a mixture of ether: ethanol (3:1) following homogenization 10% (V/V).

Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and evaporated to dryness in boiling water bath.

Finally it was reconstituted in 5 mL of chloroform and 1 mL of acetic anhydride and sulphuric acid (20:1) mixture was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm (ELx-800, Biotek Instruments, Winooski VT, USA). For the cholesterol assay in plasma instead of tissue homogenate, equal volume of (0.5 mL) plasma was used.

Glycogen estimation

The estimation process was done by anthrone-sulphuric acid method as suggested by Shavali and Haldar (1998). In brief a stock solution of glycogen was prepared (5mg/mL). It was then diluted in varying concentrations of glycogen (from 20µg/mL to 100µg/mL) for standard curve. A solution of anthrone (0.5%) in sulphuric acid was prepared. It was mixed with standard glycogen solution, placed in boiling water bath till the characteristic colour (pinkish to red) was obtained and absorbance was measured in a spectrophotometer at 620 nm. For experimental estimation, the tissue glycogen was extracted in 50% KOH solution and 4 mL of anthrone-sulphuric acid solution was added to each of samples. After mixing well, the samples were placed in boiling water bath till the characteristic colour of the solution were obtained and absorbance was measured in a spectrophotometer (ELx-800, Biotek Instruments, Winooski VT, USA) at 620 nm.

Glycogen phosphorylase estimation

The glycogen phosphorylase (GP; EC 2.4.1.1) activity was measured following the protocol of Mason and Fasella (1971) with a few modifications as suggested by Zhang *et al.* (2012). The reaction mixture consisted of 50mM sodium glycerolphosphate (pH 7.1), 10mM potassium phosphate, 5mM $MgCl_2$, 0.5mM NAD^+, 1mM DTT, 1.6 unit phosphorglucomutase, 1.6 unit glucose-6-phosphate dehydrogenase, and 0.2% glycogen in a total volume of 0.3mL. Reaction was started by adding 200 µL of glycogen phosphorylase (for standard) and 200 µL of 10% tissue homogenate to make the volume of 500 µL and reaction was started at 25°C. The reaction was monitored by measuring the increase of absorbance (Ex. 350nm, Em.470nm, HITACHI F-4600

UV- Visual- Fluorescence spectrophoto meter) for NADH generation and results were expressed as µmoles of NADH generated/min.

Estimation of tissue level of melatonin

For estimation of melatonin content in the lymphoid tissues commercial melatonin ELISA kit (RE54021, IBL, Hamburg, Germany) was used as per manufacturer's instruction. The tissues were homogenised in PBS with 0.1% ethanol and centrifuged. The supernatants were used for determinations of melatonin. Standards, controls and tissue homogenates were extracted using C18 reverses phase extraction columns according to the protocol of the manufacturer. The reaction was developed using p-nitrophenyl phosphate and optical densities were determined at 405 nm in an automatic microplate reader (Biotek, USA). The concentration of melatonin was expressed as pg/mg of protein measured in the tissue sample. The sensitivity of the melatonin assay was 1.6 pg/mL. Both the intra- and inter-assay coefficients of variation (CV) were 11.4% and 19.3% respectively.

Statistical analyses

The data were presented as the mean ± standard error of the mean (SEM). The results of plasma and tissue level of glycogen, glucose and cholesterol concentrations, and tissue level enzyme activity were analyzed by one way ANOVA followed by post hoc 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).

Results and Discussion

Glucose concentration in blood, lymphoid organs and gonads

We noted significantly high level of plasma glucose in females than males and other seasons ($p < 0.01$; Fig. 1A). In spleen the tissue level glucose was significantly high in both the sexes during monsoon ($p < 0.01$ in males and $p < 0.05$ in females) and winter ($p < 0.01$ in males and $p < 0.05$ in females; Fig. 1B). In thymus, the glucose level was significantly high during monsoon ($p < 0.01$ in males and $p < 0.05$ in females) and winter ($p < 0.01$) only in females than males and other seasons (Fig. 1C). In the gonads the glucose level is significantly high in females during monsoon ($p < 0.01$) than other seasons and ($p < 0.05$) than males. During monsoon the level is significantly low in males ($p < 0.01$) than other seasons and significantly high in females ($p < 0.01$) when compared with other seasons and males (Fig. 1D).

Cholesterol concentration in blood, lymphoid organs and gonads

We noted significantly high level of plasma cholesterol during monsoon and winter in females ($p < 0.01$) in comparison to males ($p < 0.05$ during monsoon and $p < 0.01$ during winter; Fig. 2A). Cholesterol concentration in female spleen was significantly high during monsoon and winter ($p < 0.01$) than summer and also in comparison to males ($p < 0.05$) particularly during monsoon. In male spleen the level was significantly high only during winter ($p < 0.05$; Fig. 2B). Cholesterol concentration in male thymus was significantly low during monsoon ($p < 0.01$) and was significantly high during winter ($p < 0.01$). However, in females'

cholesterol level was significantly high during monsoon ($p < 0.01$) and winter ($p < 0.05$). Females showed higher cholesterol level than males in monsoon ($p < 0.05$) and males showed higher cholesterol level than females ($p < 0.01$) during winter (Fig. 2C). Gonad level of cholesterol was significantly high in both the sexes during monsoon ($p < 0.05$ in males and $p < 0.01$ in females) and winter ($p < 0.05$ in males and $p < 0.01$ in females; Fig. 2D).

Glycogen concentration in liver, lymphoid organs and gonads

We noted a decreasing pattern in glycogen level in liver. The level was significantly low during monsoon ($p < 0.01$ in males and $p < 0.05$ in females) and winter ($p < 0.01$ in both the sexes). However, gender dependent variation in liver glycogen level is also prominent being significantly low in females than males during monsoon ($p < 0.05$) and winter ($p < 0.01$; Fig. 3A). Stored glycogen level in spleen was significantly low during monsoon and winter in females ($p < 0.01$) and in males it is significantly low ($p < 0.01$) only during winter (Fig. 3B). Stored glycogen level was significantly low in thymus of both the sexes ($p < 0.01$ in males and $p < 0.05$ in females) during monsoon and winter ($p < 0.01$ in both the sexes). However, during monsoon females showed higher level of glycogen ($p < 0.05$; Fig. 3C). In gonads, the stored glycogen was significantly low ($p < 0.05$) in testes only during monsoon. Gender dependent variation in gonadal glycogen storage was not significant (Fig. 3D).

% uptake of glucose by lymphoid organs from circulation

% uptake of glucose was significantly low in female spleen during monsoon ($p < 0.01$) but the level is significantly low in male spleen ($p < 0.01$) during winter (Fig. 4A). In case of thymus, the % uptake of glucose is significantly high in both the sexes during monsoon ($p < 0.01$) and winter ($p < 0.01$; Fig.4B).

% uptake of cholesterol by lymphoid organs from circulation

We noted significantly low level of % uptake of cholesterol by male spleen during monsoon and winter ($p < 0.05$) but the level was significantly high in female spleen during monsoon ($p < 0.05$) and winter ($p < 0.01$).

The female goats always showed significantly higher level of % uptake of cholesterol than males during monsoon ($p < 0.05$) and winter ($p < 0.01$; Fig. 5A). In case of male thymus, the % uptake of cholesterol was significantly low during monsoon ($p < 0.05$) and significantly high ($p < 0.01$) during winter. But, the female thymus showed significantly increased % uptake of cholesterol during monsoon ($p < 0.05$) and winter ($p < 0.01$; Fig. 5B).

Glycogen phosphorylase activity in lymphoid organs and gonads

The glycogen phosphorylase activity in spleen of both the sexes was significantly high during monsoon ($p < 0.05$) and winter ($p < 0.01$; Fig. 6A). In thymus of both the sexes the level was significantly high during monsoon ($p < 0.01$) and winter ($p < 0.05$; Fig. 6B). In gonads the level was significantly high in testes during monsoon ($p < 0.01$) only and in case of ovaries the level was significantly high during monsoon ($p < 0.01$) and winter ($p < 0.05$; Fig. 6C).

Estimation of tissue level melatonin in thymus

Tissue level melatonin was significantly high in thymus of both the sexes during

winter ($p < 0.01$; Fig. 7). The main energy distribution of the body is being channelized to modulate the two mega physiological events they are the modulation of immunity and maintenance of reproduction. However, some literature suggesting the energy allocation pattern and seasonal modulation of reproductive energetic has been reported by some authors (Nelson and Demas, 1997; Viney *et al.*, 2005; Martin and Festa-Bianchet, 2010) in sheep, seal and other animals residing in polar regions (e.g. polar bear). But, those reports are partial and never included patterns of immunity. Goats being the short day breeder provide an opportunity to study the path of energy allocation in both immunity and reproduction simultaneously. But, there is no literature available in this regard and our study provides first report to describe the energy allocation pattern primarily in immune organs as well as in gonads during reproductively active (i.e. winter) and inactive (i.e. summer and monsoon) phases of goats. To delineate the energy allocation pattern, we studied the glycogen, glucose and cholesterol content of both the tissue (lymphoid organs and gonads) as well as at circulatory level.

Fig.1A Season and sex dependent variations in glucose concentration in blood 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). ** $p < 0.01$; summer vs. monsoon and winter, bp < 0.01; male vs. female; **B** Season and sex dependent variations in glucose concentration in spleen 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

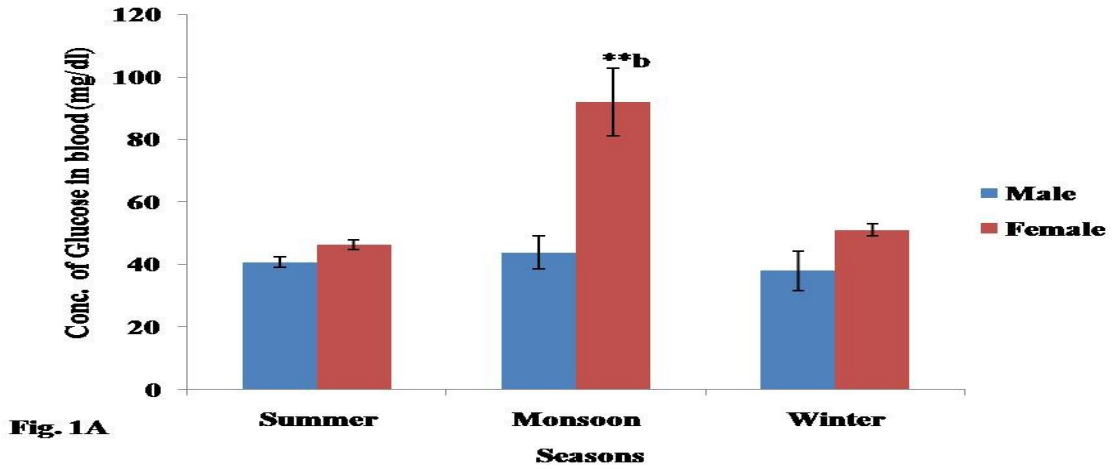

Fig. 1A

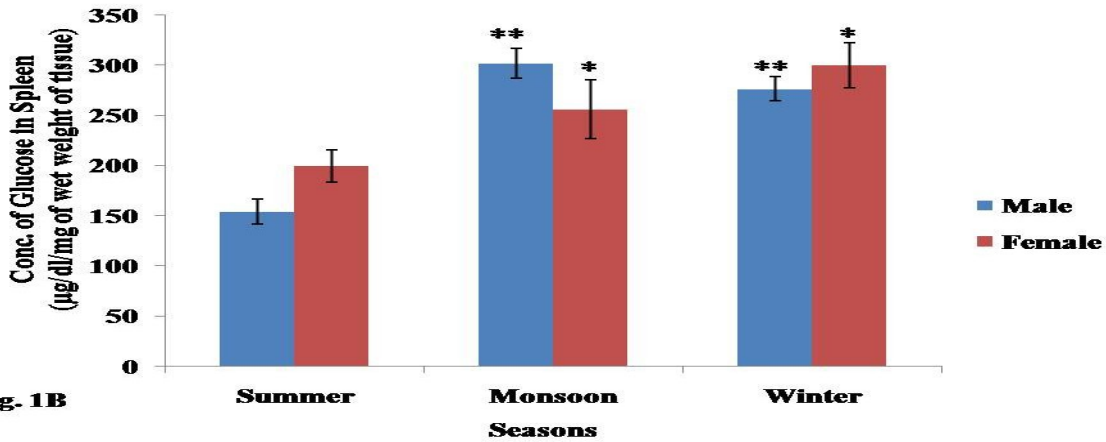

Fig. 1B

Fig.1C Season and sex dependent variations in glucose concentration in thymus 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, bp < 0.01; male vs female; **D** Season and sex dependent variations in glucose concentration 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< 0.01; summer vs. monsoon and winter, ap < 0.05, bp < 0.01; male vs. female.

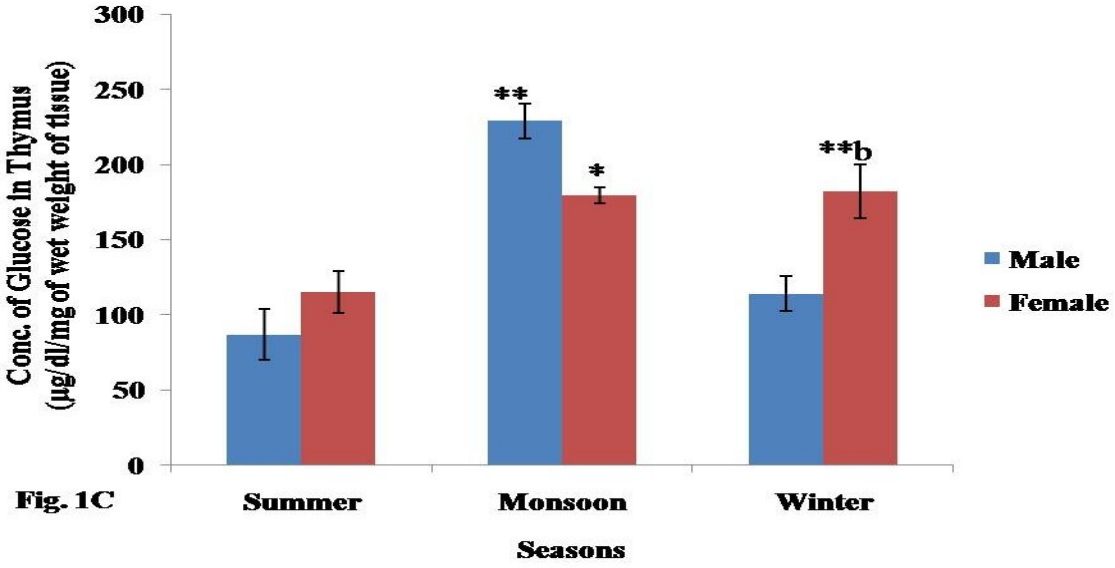

Fig. 1C

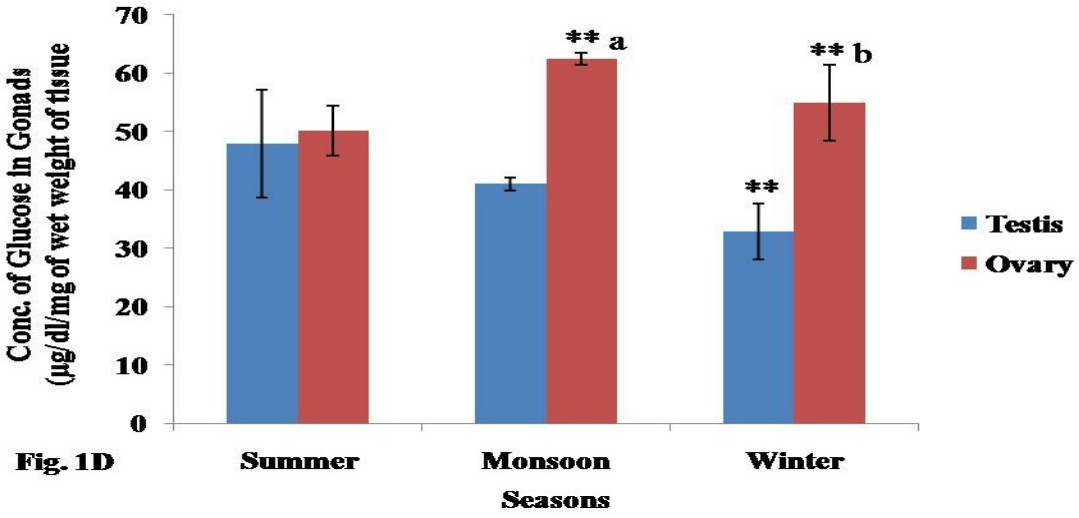

Fig. 1D

Fig.2A Season and sex dependent variations in cholesterol concentration in blood 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< 0.01; summer vs. monsoon and winter, ap < 0.05, bp < 0.01; male vs. female. **B** Season and sex dependent variations in cholesterol concentration in spleen 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, ap < 0.05; male vs. female.

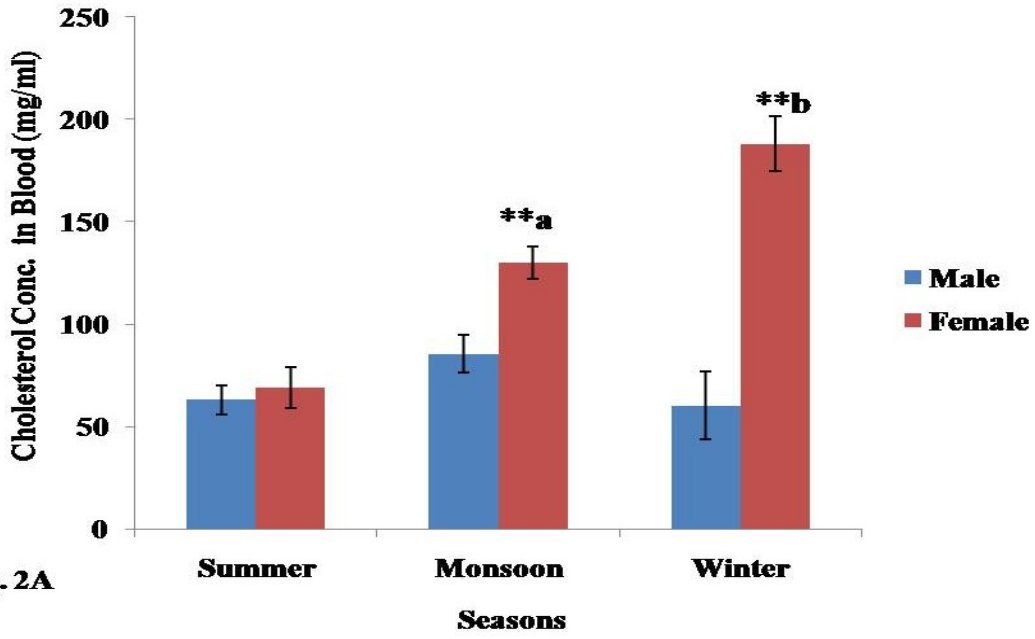

Fig. 2A

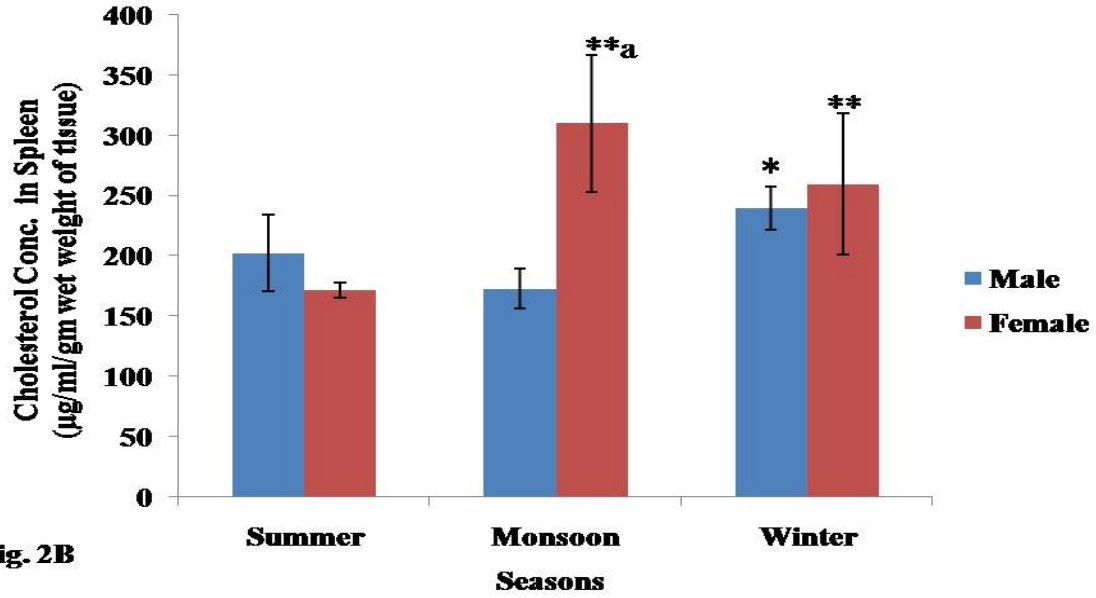

Fig. 2B

Fig.2C Season and sex dependent variations in cholesterol concentration in thymus 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, ap < 0.05, bp < 0.01; male vs. female; **D** Season and sex dependent variations in cholesterol concentration 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.

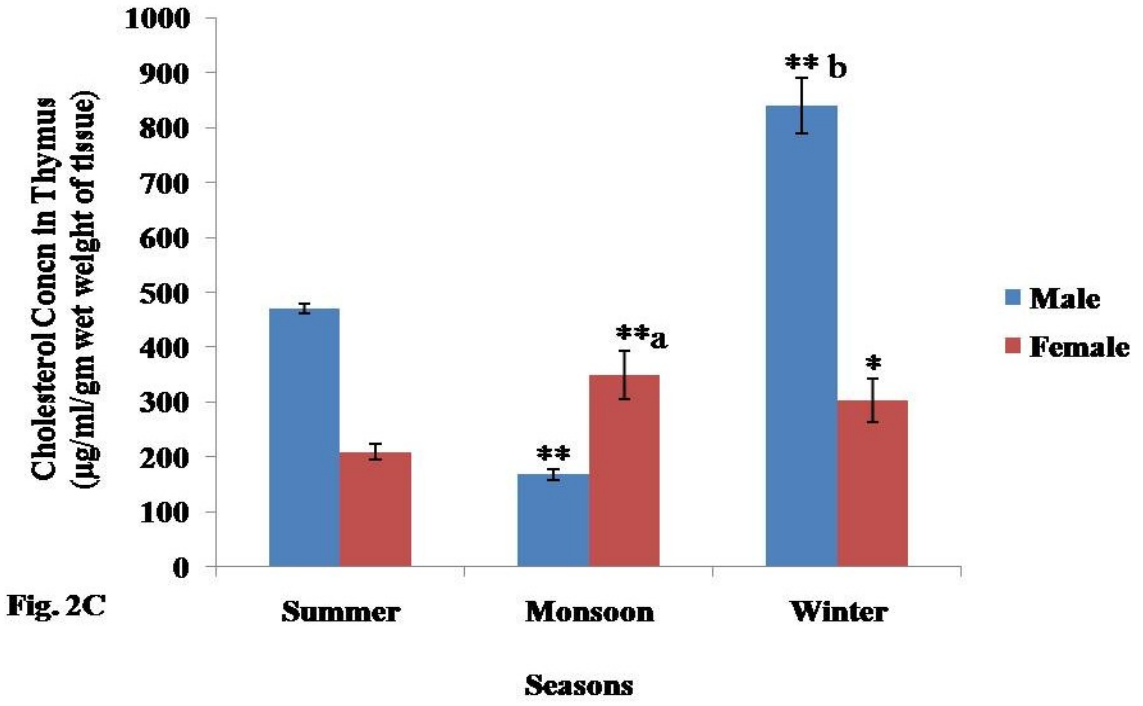

Fig. 2C

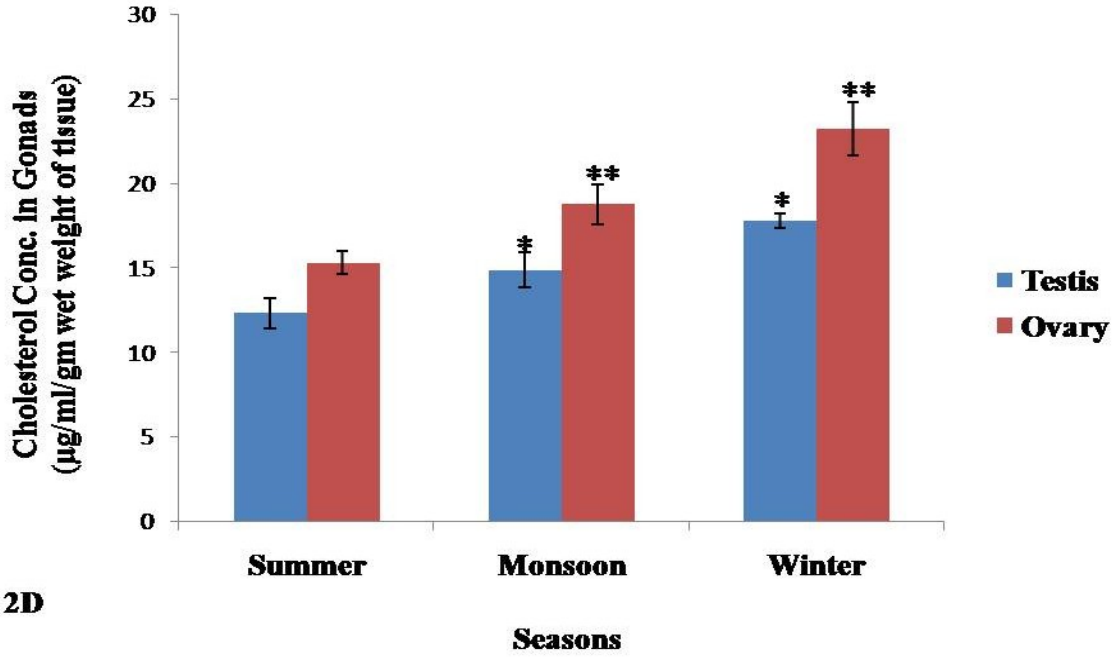

Fig. 2D

Fig.3A Season and sex dependent variations in glycogen concentration in liver 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, ap < 0.05, bp < 0.01; male vs. female; **B** Season and sex dependent variations in glycogen concentration in spleen 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< 0.01; summer vs. monsoon and winter.

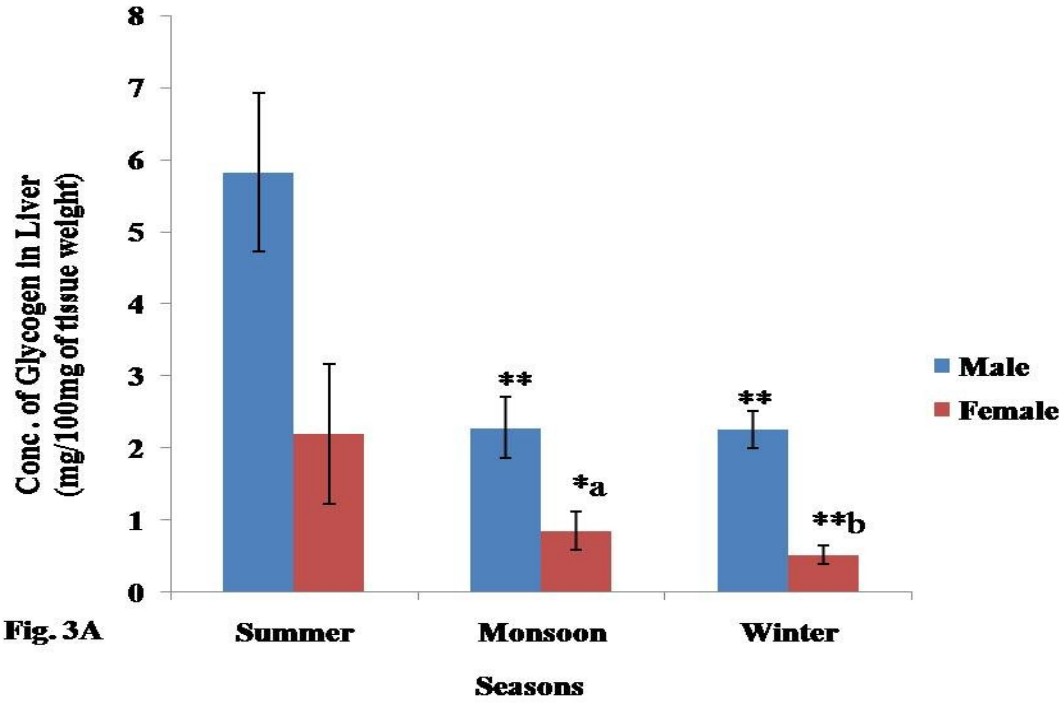

Fig. 3A

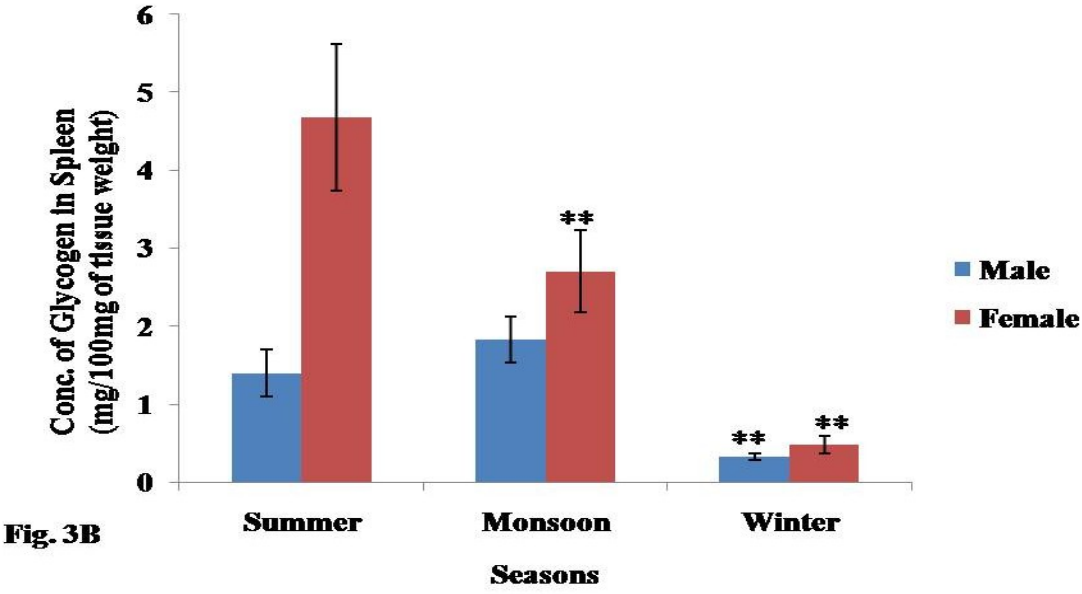

Fig. 3B

Fig.3C Season and sex dependent variations in glycogen concentration in thymus 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, ap < 0.05; male vs. female; **D** Season and sex dependent variations in glycogen concentration 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< 0.01; summer vs. monsoon and winter.

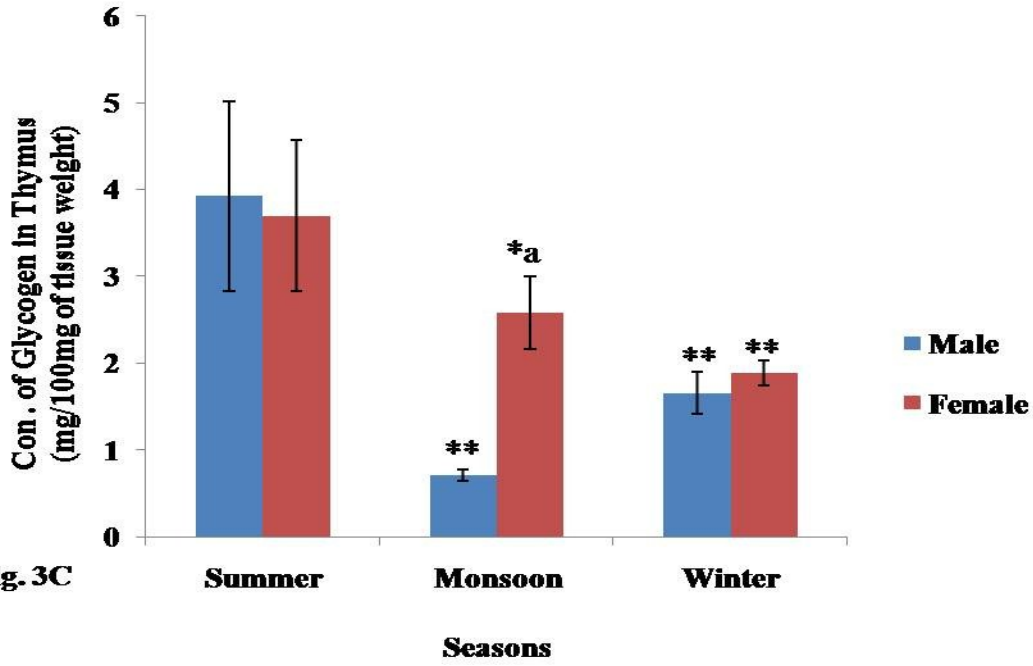

Fig. 3C

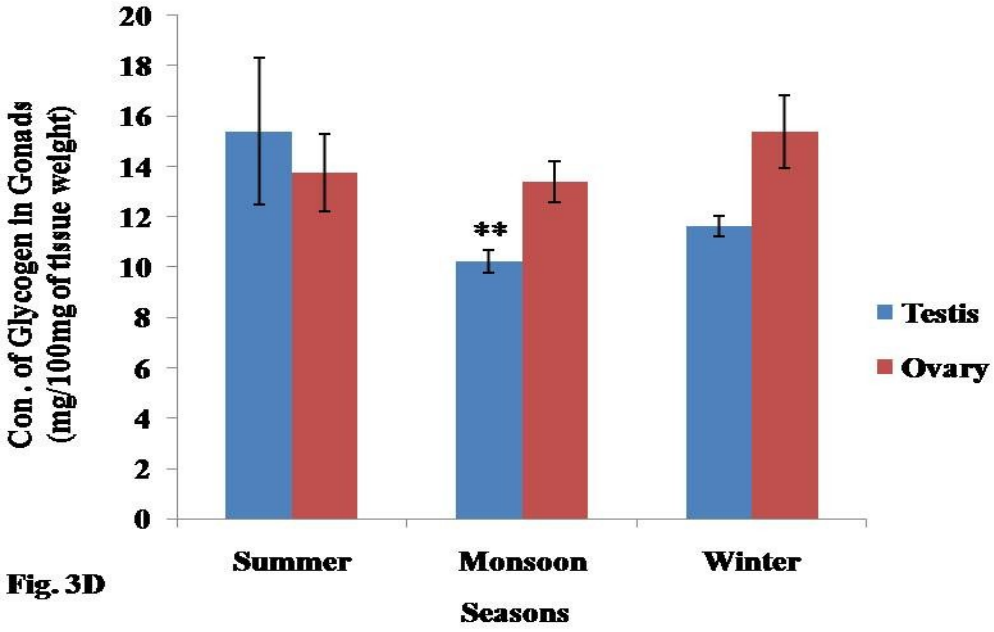

Fig. 3D

Fig. 4A Season and sex dependent variations in % up take of glucose from blood by spleen 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< 0.01; summer vs. monsoon and winter; **B**. Season and sex dependent variations in % up take of glucose from blood by thymus 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< 0.01; summer vs. monsoon and winter.

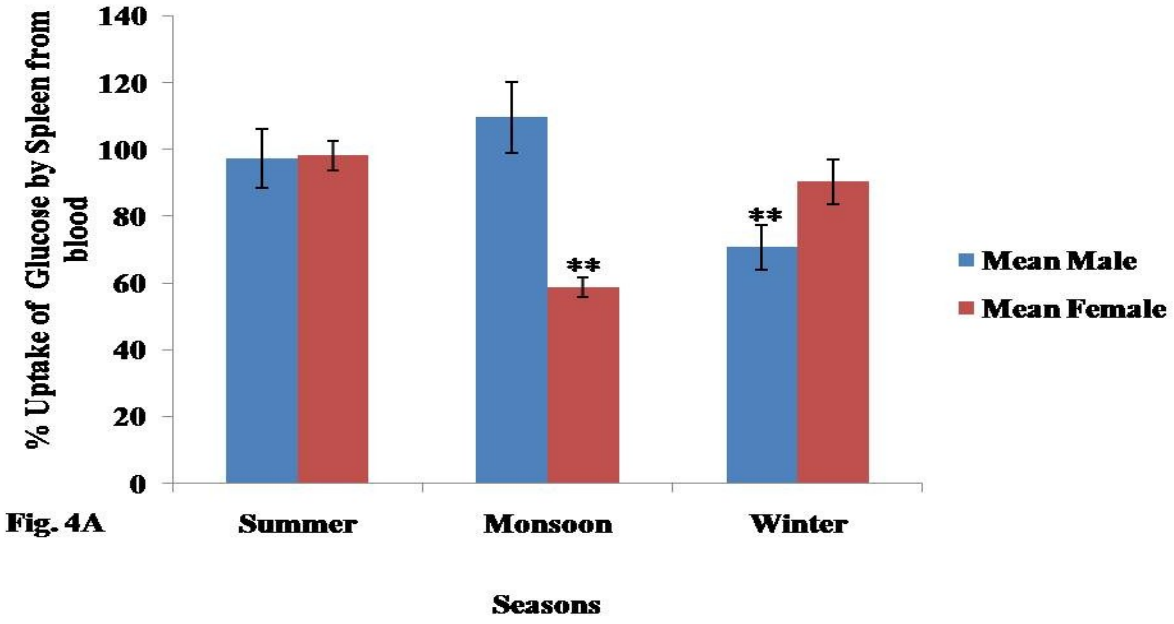

Fig. 4A

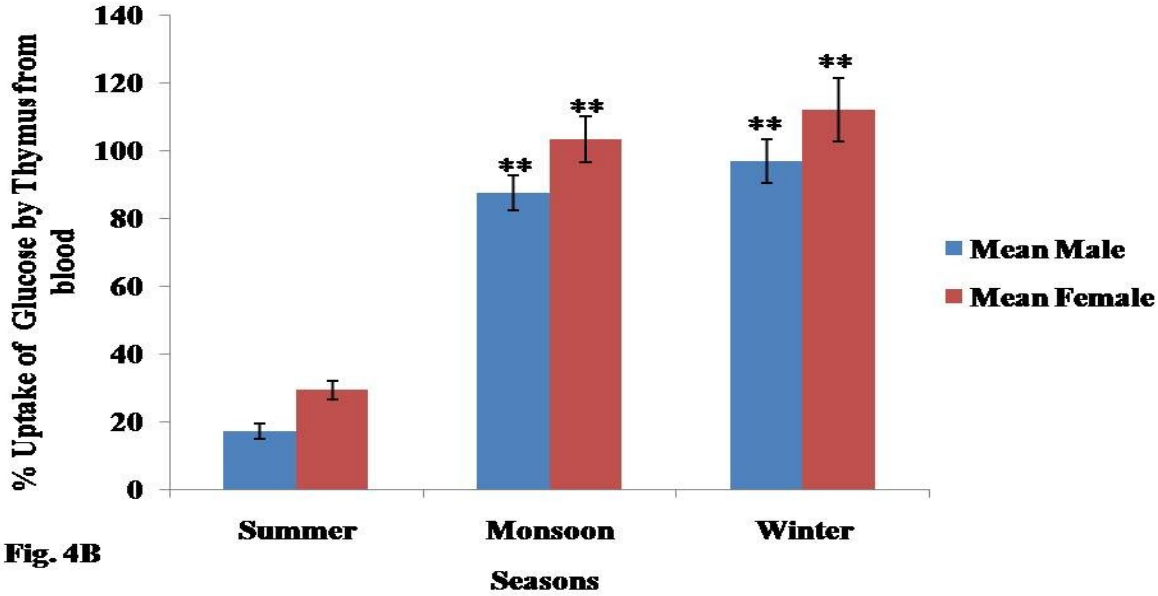

Fig. 4B

Fig.5A Season and sex dependent variations in % up take of cholesterol from blood by spleen 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 ap < 0.05, bp < 0.01; male vs. female. **B** Season and sex dependent variations in % up take of cholesterol from blood by thymus 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.

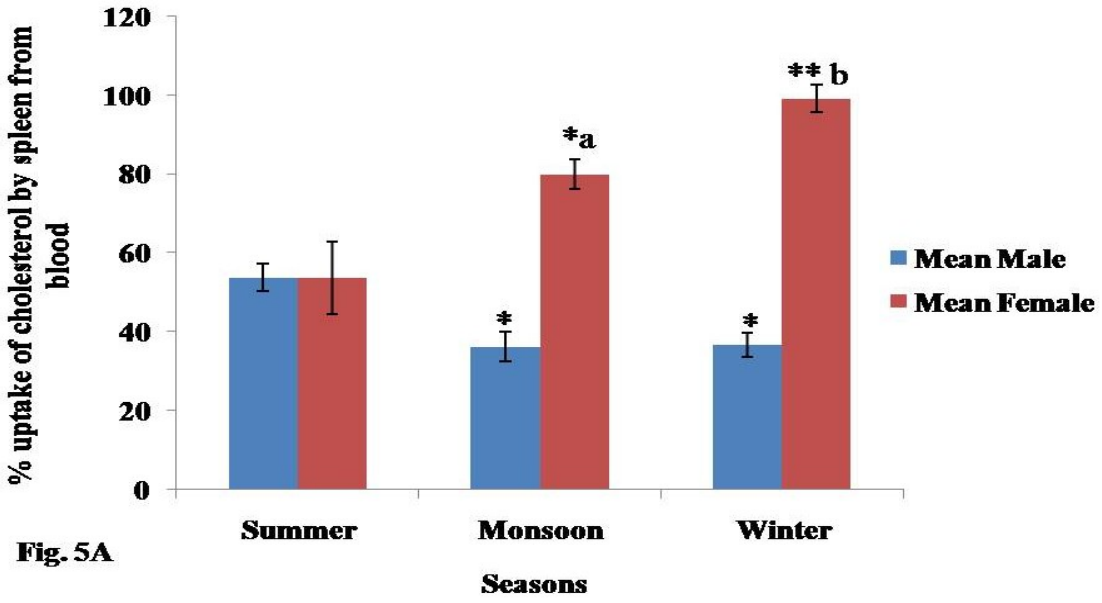

Fig. 5A

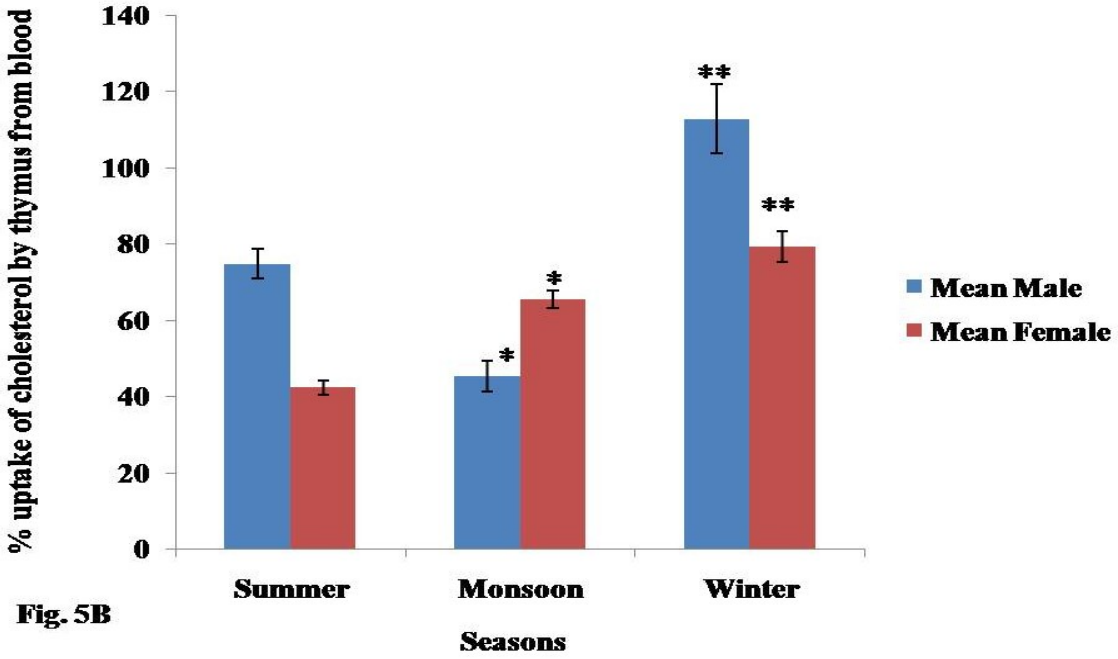

Fig. 5B

Fig.6A Season and sex dependent variations in phosphorylase activity in spleen 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; **B.** Season and sex dependent variations in phosphorylase activity in thymus 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; **C.** Season and sex dependent variations in phosphorylase 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.

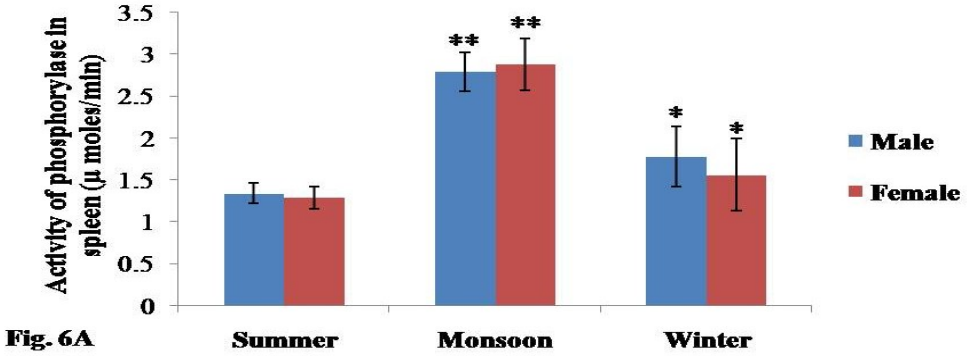

Fig. 6A

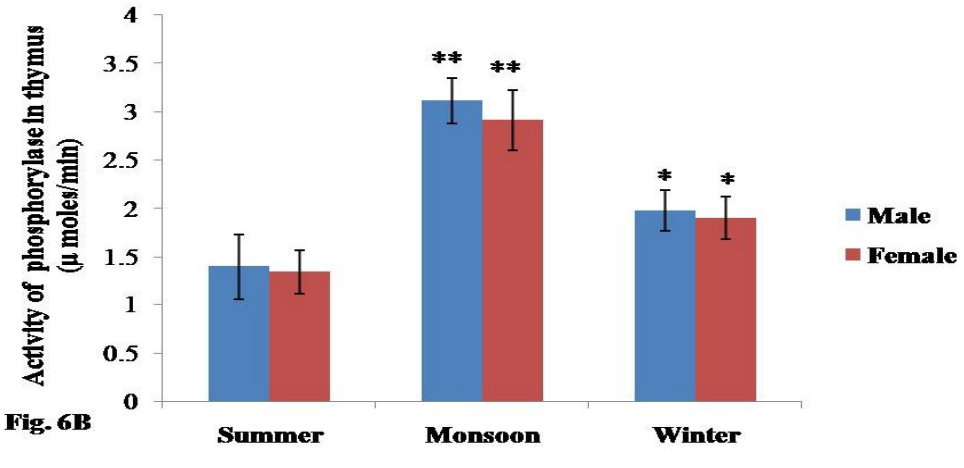

Fig. 6B

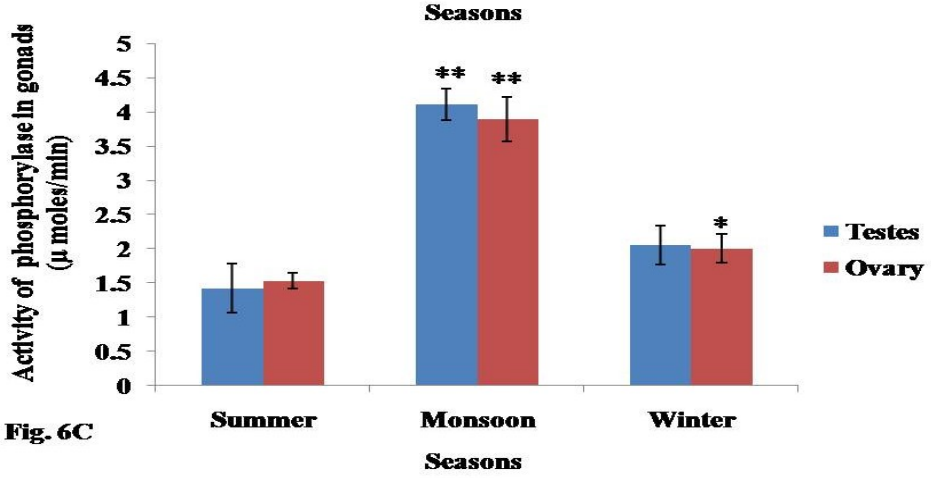

Fig. 6C

Fig.7 Season and sex dependent variations in tissue level of melatonin concentration in thymus 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< 0.01; summer vs. monsoon and winter.

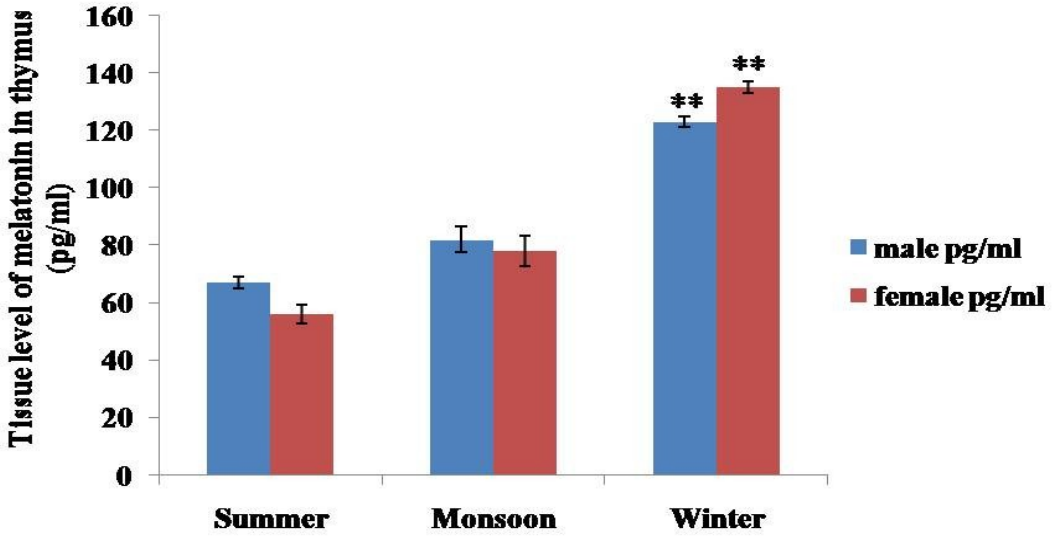

Fig. 7

We noted significantly higher level of glucose (the primary source of energy) in circulation of goats during monsoon only. But, in lymphoid organs of both the sexes of goats and in their gonads the tissue level of glucose was significantly high both during monsoon and winter. Both the gonads and lymphoid organs of females presented a high level of tissue glucose than males in year round manner, suggesting that females are in need of high energy to maintain immunity during monsoon and both immunity and gestation during winter.

We considered the circulatory level of glucose as the primary source of energy and tried to draw a relation between circulatory glucose level with that of tissue level i.e. in gonads and lymphoid organs. We noted that the thymus of both male and female goats had a higher level of % uptake of glucose which was even high in circulation. Thus, we looked for an alternate source of energy and our primary focus on glycogen level.

For glycogen we took liver of both males and females as internal positive control and we found that stored glycogen level was significantly high during summer. But, the stored glycogen level significantly goes down during monsoon and winter when compared with summer as the utilization rate (in the form of glucose by glycogenolysis) in those two season (summer and monsoon) might had increased. Further we noted tissue level glycogen storage in lymphoid organs and gonads as well. Our study clearly showed that, gonads, thymus and spleen were capable enough to store the glycogen as a source of energy and the level was substantial when compared to the liver (as an internal positive control). This result is in parallel with the previous report of Taira *et al.* (1982). But, the level of stored glycogen was significantly low particularly in lymphoid organs during monsoon and winter. This may be due to the fact that physiologically elevated energy demand was

not sufficiently quenched by circulatory level of glucose coming either from dietary sources or from glycogenolysis in liver. Thus, we may suggest that during the months of extreme stress i.e. in terms of immunity and reproduction the elevated energy consumption was counter balanced by the tissue level glycogenolysis. Hence, to check the level of glycogenolysis in both the lymphoid organs and gonads we studied the tissue level glycogen phosphorylase activity as a marker of glycogenolysis.

We noted significantly high level of glycogen phosphorylase activity in gonads, spleen and thymus of both the sexes of goats during monsoon and winter. Our, result clearly suggests that, the lymphoid organs and gonads are not only acting as the major action sites of immune modulation and reproduction respectively, but also, are the "local power house" of the body. When there is a need of high energy (for reproduction and immunity maintenance) then these power houses may act promptly by utilizing their own energy resources. This exceptional mechanism of energy allotment and utilization is a typical and particular phenomenon occurring in goats.

Cholesterol, either being in the circulation or in the tissue, may be used as secondary source of energy. We noted significantly high level of cholesterol in circulation, spleen and in gonads both during monsoon and winter months in both the sexes of goats. But, the level was significantly low during monsoon and winter in thymus of male and female goats. Further, % uptake level of cholesterol was significantly high in lymphoid organs of both the sexes during monsoon and winter months. In most of the cases females presented a higher level of cholesterol than males, may be due to the fact that monsoon being the reproductive preparatory phase for females (with high level of estrogen) while males are reproductively active throughout the year. Thus, during monsoon and winter the gonads needed higher cholesterol level for high level of steroidogenesis. Contrary to this, lymphoid organs (particularly the thymus) had low level of tissue cholesterol. This might be due to tissue level of steroidogenesis (Vacchio *et al.,* 1994), a process which is less common phenomenon but were reported particularly for some seasonal breeders like goats.

We recorded significantly high level of 3 βHSD activity during monsoon in thymus of both the sexes of goats with significantly high level of 3 βHSD activity in females only during winter months (Ghosh *et al.,* 2014). This unusual utilization of cholesterol is due to the fact that sometimes gonadal steroids may work as immune stimulator (particularly Estrogen) by increasing different pro-inflammatory cytokine particularly by PI3K/Akt pathway (Calippe *et al.,* 2008). Thus, it may be possible that during monsoon when plasma level of melatonin is moderately high, the gonadal steroids might have played some balancing role in goat immune modulation *via* the local inflammatory process. Further, to delineate the local inflammatory process under influence of melatonin (a known immune stimulator) we noted tissue level of melatonin which was significantly high only during winter. Cumulatively, all these results may suggest that the thymus is acting as most important organ in goat immune modulation either *via* gonadal steroids or *via* melatonin pathways.

Conclusion

Thus, in conclusion we may suggest that, to adjust with seasonal stress, the tissue level of melatonin in goats is not only acting as a buffer hormone to maintain the threshold

level of immune status but also playing an important role in regulating local "microcircuit" of melatonin and gonadal steroid hormones in lymphoid organs to modulate immunity. In this regard, the role of thymus is of highest importance (in terms of energetic), to maintain two energetically most important events i.e. immunity and reproduction simultaneously. It might also develop a functional synergism between the immune suppressive role of gonadal steroids and immune enhancing role of melatonin. Thus, it could be a special physiological adaptation of goats that made this ruminant short day breeder, a better survivor under different environmental odds as well as physiological stress.

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