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# Histology and Immunohistochemical Localization of Different Hormone Receptors (MT1, MT2, AR, GR, Erα) in Various Organs (Spleen, Thymus, Ovary, Uterus and Testes) of Indian Goat *C. Hircus*.

**Somenath Ghosh, Chandana Haldar***

Pineal Research Laboratory, Department of Zoology,
Banaras Hindu University, Varanasi, India.
*chaldar2001@yahoo.com**

**Abstract:** *The histology of pineal, ovary and testis is partially documented in sheep and goats. But, detailed histo-architecture of pineal, gonads (testis, ovary and uterus) and lymphoid organs (thymus and spleen) had never been studied. We noted the seminiferous tubules of testes are laden with sperm throughout the year as because males are reproductively active throughout the year. The quiescent phase ovary presented cellular mass (both theca and granulosa cells) with large Graafian follicle suggesting that like other seasonal breeders (e.g. bats) the goat ovaries also present a prolonged oestrous. Histology of goat uterus shows zones of endometrium and myometrium. The endometrium is having uterine glands with secretory granules, however, the myometrium is having thick layer of non-voluntary muscles. The general histology of young goat pineal gland is having dark and light pinealocytes along with glial cells but, with advancement of age the pinealocytes are replaced with large concretions. The histology of spleen shows two different zones the red pulp and white pulp regions and the histology of thymus shows cortex and medulla. The medulla is having Hassel's Corpuscle which is regarded as maturation and priming site of T lymphocytes. Further, to delineate the role of different hormones in goat immune modulation and reproduction, we noted immunohistochemical localization of different hormone (melatonin, adrenal and gonadal steroids) receptors in lymphoid organs and gonads of male and female goats. High expression of melatonin receptors (MT1, MT2) were noted on the thymus, spleen, ovary, uterus and testes of the goats, suggesting the immunomodulatory and reproductive role of melatonin. Estrogen receptor (ERα) and androgen receptor (AR) expressions were noted on thymus of male and female goats respectively while glucocorticoid receptor expression was noted on the thymus of both the sexes. These results suggest that gonadal and adrenal steroids might be playing roles in goat immunomodulation.*

**Keywords:** *Goat, gonad, histology, hormone receptors, immunohistochemistry, lymphoid organs.*

## 1. INTRODUCTION

Reproduction in both males and females in every animals, particularly in vertebrates is tightly regulated by different extrinsic factors like different environmental stressors i.e. scorching heat of summer, humidity of monsoon and sizzling cold of winter [1] and intrinsic factors (hormones, chemokines, lymphokines and cytokines [2] in a very timely and well coordinated manner.

As a part of intrinsic factor, hormones are the common factor which regulate both the important biological mega events i.e. immunity and reproduction. Among the hormones, the gonadal steroids (testosterone and estradiol) play an important role in regulation of immunity as immune suppressor in most of the animal models [2], [3], [4]. Apart from gonadal steroids, there are adrenal steroids (cortisone/corticosterone) expressed in a species specific manner which is important to ameliorate any type of stress. By the "Flight or Fight" mechanism the adrenal steroids play important role in maintaining body homeostasis under spontaneous or induced stress [5].

Among the environmental cues, photoperiod is responsible for regulating different physiological functions in a season dependent manner and is chemically coded inside the body by a particular neurohormone, melatonin secreted from pineal gland [6]. This hormone is regarded as both "Clock and Calendar" [7] for the organisms. Melatonin is also regarded as an immune enhancer as documented in different animal models and systems [8], [9]. This hormone acts as pro-gonadotrophic [10], [11] or anti-gonadotrophic [12] for reproductive management as per need of species in different

ecological niches. Reports are available regarding the histoarchitecture of pineal (the main source of melatonin in circulation) and ovary in ruminants like sheep [13], [14].

Goat is a ruminant short day breeder and female goats reproduce during winter under the positive influence of melatonin [15] hence, melatonin acts as a pro-gonadotrophic hormone. Males are reproductively active throughout the year. Ovary and uterus are the most important and dynamic reproductive structures in females. The seasonality in reproduction and gestational efficacy in female goats are due to these two important reproductive structures and interestingly feature is that, in this animal maintenance of gestation and immunity is occurring side by side during winter.

The main immune organs of goats are the primary and secondary lymphoid organs i.e. thymus and spleen. However, being the most economically important free grazing animal, the health management of goat is neglected by the veterinarians and other basic researchers for a long period of time and till date no reports are available on basic histology of pineal, gonads (ovary, uterus and testes) and lymphoid organs (spleen and thymus) in Indian goat *Capra hircus*. In the present study we noted the general histology of pineal, ovary, uterus, spleen and thymus which were never reported in this ruminant short day breeder. Further, all the above mentioned organs are target for gonadal, adrenal steroids and melanin hormone hence, we also studied the immunohistochemical localization of different hormone receptors (membrane bound melatonin receptor MT1 and MT2, androgen receptor; AR, estrogen receptor α; ERα and glucocorticoid receptor; GR) to get a basic idea of areas of expression.

## 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), [16]. 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 (*Hordeumvulgare*, 1 part), crushed maize (*Zea mays*, 2 parts), linseed (*Linumusitatissimum*) 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 (Pennisetumglaucum*), sorghum (*Sorghum* sp.) and oat (*Avena sativa*). The concentrate contained oilseed cakes and soaked gram (*Cicerarietinum*) 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, $102.5^0$F–$103^0$F) 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), [17]. 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 $43.87^0$ ± $1.02^0$ 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 $28.68^0$ ± $2.76^0$ C, %RH 87.04 ± 3.50%, day length, light–dark cycle-12 hours:12 hours), and winter months (November–January, temperature $10.76^0$ ± $3.63^0$ 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 histological architecture of pineal, ovary, uterus, spleen and thymus 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.

### 2.3. General Histology of Tissues

Pineal gland, thymus, spleen and gonads (testis and ovary) were collected from opened abdomen of the goats and cleaned with 0.9% NaCl (normal saline). The tissues were preserved in neutral formalin (37% formaldehyde, distilled water, $Na_2HPO_4$ and $NaH_2PO_4$) up to 48 hrs. The preserved tissues in neutral formalin were washed overnight under running tap water. The tissues were dehydrated through serial dilution of ethyl alcohol (30%, 50% and in 70% alcohol) by giving 2-3 changes at the interval of 30 minutes. Some times to remove the yellow colour of picric acid, the washing was initiated with Lithium carbonate. Small sized (0.125 $cm^3$) tissues were cut and then once more dehydrated in alcohol grade 30%, 50% and 70% by giving single change of 30 minutes and two changes in 90% and 100% alcohol at the interval of 1 hour. The dehydrated tissues were then ready for embedding. The dehydrated tissues were cleared in absolute alcohol + xylene (1:1) and xylene (10 minutes) sequentially. The cleared tissues were kept in pre-warmed xylene + wax (1:1) solution for 30 minutes in an oven (maintained at 60-62 $^0$C). These tissues were transferred to wax I and wax II for 30 minutes each and then, in wax III for 45 minutes. The blocks were prepared in pre-seasoned melted wax with the help of L-piece. Tissues in blocks were then ready for sectioning. The block was fixed on the block-holder of the Leica semi-automated microtome (Leica Microsystems, RM2245) and was trimmed. The tissue was sectioned on a thickness of 6 µm. The sections were placed on gelatin pre-coated slide along with few drops of water. The slides were then placed on a mild hot plate and sections were allowed to spread. The slides were dried in air and allowed to attach firmly.

### 2.4. Permanent Slide Preparation by Haematoxylene-Eosine (Double-Staining) Method

Slides with spread tissue sections were de-paraffinized in xylene for 30 minutes. The sections were rehydrated by putting them sequentially into a battery of downgraded alcohol (100%, 90%, 70%, 50% and 30%) and in water for 10 minutes in each. The slides were kept in the haematoxylin stain for 15-20 minutes and then washed in running tap water for 45 minutes for differentiation. The slides were transferred to diluted acid water (1 mL of acetic acid in 500 mL of distilled water) for a single dip and again washed in running tap water. The slides were dehydrated by putting them sequentially into 30%, 50%, and 70% alcohol each for 5 minutes and transferred to alcoholic (70%) eosine stain for 5 minutes. The sections were differentiated in acid alcohol (1 mL of acetic acid in 500 mL of 70% alcohol, if desired), and transferred sequentially in 90% alcohol for 2 minutes followed by two changes of 100% alcohol each for 5 minutes. The dehydrated sections were kept in 100% alcohol + xylene (1:1) for 5 minutes, and then transferred sequentially to xylene-I and xylene-II for 10 minutes. The permanent slides were prepared by mounting with DPX (Distyrene Plasticizer Xylene, SRL, India), after 24 hrs were observed under microscope (Leitz MPV3 with photo-automat software) and were documented for general histology.

### 2.5. Morphometric Analysis

The area of cortex and medulla (for thymus), size of red pulp and white pulp (for spleen), the size of normal pinealocyte along with the aged ones (for pineal), the area of seminiferous tubule (individually for testis), area of theca and granulose cells (for ovary) and endometrium and myometrium (for uterus) were measured with the help of ocular micrometer (Webcon, India). Ten sections of pineal gland, thymus, spleen, ovary, uterus and testis sample tissue were randomly selected for morphometric analysis.

### 2.6. Immunohistochemical Localization of MT1, MT2, AR, GR and ERα in Lymphoid Organs (thymus and spleen) and Gonads (testes, ovary and uterus) of Goats

For immunohistochemical localization of melatonin receptor types (MT1 and MT2), androgen receptor (AR), estrogen receptor (ERα) and glucocorticoid receptor (GR) spleen, thymus, testes,

ovaries were fixed in neutral formalin and were processed (as described previously). Endogenous peroxidase activity was blocked by $H_2O_2$ in 80% methanol for 20 min at room temperature. Sections were washed three times with phosphate-buffered saline (PBS) and pre-incubated with 3% blocking serum (Vectastain, USA) in PBS for 40 min. Sections were then incubated with primary antibody MT1; Mel1aR, ab 96502, at a dilution of 1:250 and MT2; Mel1bR, ab128469, at a dilution of 1:250 from Abcam, England, AR; anti-AR, N-20, sc-1004, at a dilution of 1:200, ERα; anti-ERα, HC-20, sc-543, at a dilution of 1:200 and GR; anti-GR, N-20, sc-2045 at a dilution of 1:250 all from Santa Cruz Biotechnology, USA for overnight at 4 °C in a humified chamber. Sections were washed three times in PBS and were incubated with biotinylated secondary antibody (Vectastain ABC Universal kit; PK-6200, Vector laboratories, Burlinghame, CA, USA; dilution 1:10,000). Sections were washed with PBS and a pre-formed ABC reagent was conjugated to the free biotin of the secondary antibody. The antigens were visualized using the peroxidase substrate 3, 3-diaminobenzidine (DAB) [18]. Further, the prepared slides were observed and documented under microscope (Leitz MPV3 with photo-automat software) and were documented for immunohistochemical localization of different receptors.

## 3. RESULTS

### 3.1. General Histology of Goat Pineal

The pineal glands of goats are having three different cell types (Fig. 1A). They are glial cells having a large and prominent nucleus but without dendritic protrusions, light pinealocytes having small nucleus and dark pinealocytes having large nucleus almost covering the entire area of the pinealocyte. The entire histo-architecture is totally filled with matrix and calcified concretions are scattered throughout the structure if the goats are aged (Fig. 1B).

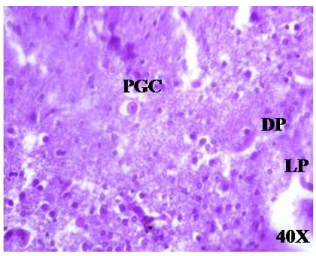


Fig 1A

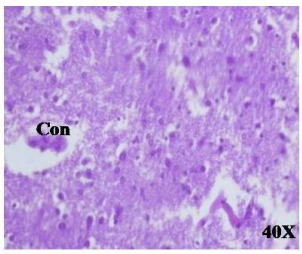


Fig 1B

**Fig1A.** *Histology of young goat pineal gland (40X).*

**Fig1B.** *Histology of old goat pineal gland (40X). Con: Pineal concretions, DP: Dark Pinealocytes, LP: Light Pinealocytes, PGC: Pineal Glial Cells.*

### 3.2. General Histology of Goat Ovary

The ovarian histology of goats shows a large Graafian follicle filled with huge amount of antral fluid. They are surrounded by thick layer of the cal cells and granulosa cells having secretory granules (Fig 2A and 2B).

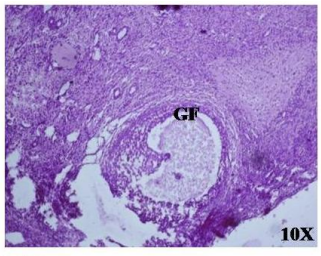


Fig 2A

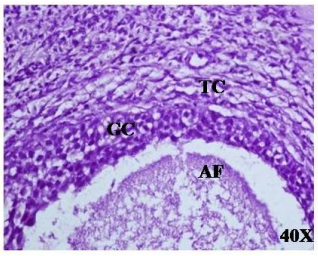


Fig 2B

**Fig2A.** *Histology of goat ovary (10X).*

**Fig2B.** *Histology of goat ovary (40X).AF: Antral fluid, GC: Granulosa cell GF: Graafianfollicle, TC: Theca cell.*

### 3.3. General Histology of Goat Testes

The general histology of goat testes shows the basic characteristics of a mammalian testis. The histology shows a number of seminiferous tubules surrounded by basal lamina. Just beneath this, there is the presence of germinal epithelium having large and active germinal cells with a large nucleus. The testes of goats are always in reproductively active phase; hence, the lumen of seminiferous tubule is filled with sperms. Adjacent to the seminiferous tubule the presence of large and triangular Leydig Cells are also evident (Fig. 3A and 3B).

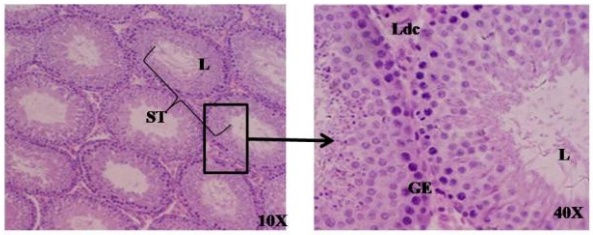


Fig 3A Fig 3B

**Fig3A.** *Histology of goat testis (10X).*

**Fig3B.** *Histology of goat testis (40X).GE- Germinal Epithelium, L- Lumen, Ldc- Leydig's cells, ST-Seminiferous Tubule.*

### 3.4. General Histology of Goat Uterus

The uterine histology of goats shows the uterus surrounded by basal lamina. Just beneath the basal lamina where the myometrium and endometrium structures were clearly demarcated. The myometrium is having multiple layers of non-voluntary muscle cells and the endometrium is having uterine gland cells filled with secretory granules (Fig. 4A, 4B and 4C). At higher magnification, the secretory glands with empty lumen and secretory granules were visible (Fig. 4C).

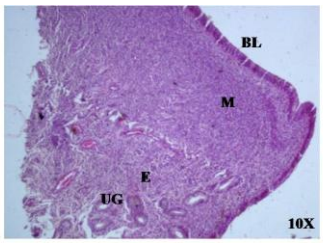


Fig 4A

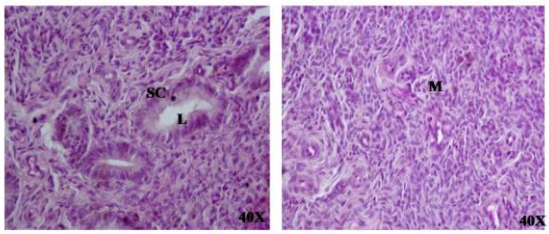


Fig 4B Fig 4C

**Fig4A.** *Histology of goat uterus (10X).* **Fig4B.** *Histology of goat uterus (40X).*

**Fig4C.** *Goat myometrium (40X). BL: Basal Lamina, E: Endometrium, L: Lumen, M: Myometrium, SC: Secretory Cells, UG: Uterine Gland.*

### 3.5. General Histology of Goat Thymus

Histologically the thymus of goats can be generally divided into 2 parts the cortex and the medulla. The cortex is the cell dense area of the tissue and medulla is cell translucent in nature well vascularised and having the characteristic Hassel's corpuscles both in the cortex and medulla. The Hassel's corpuscles are less in number in aged goats. This structure is regarded as the "playing ground" of T-cells and having a number of macrophages in them. The priming procedure of the T-cell is done here which is an important aspect of cell mediated immunity. Another important structure is PALS (Peri Arterial Lymphoid Sheath) which is regarded as the harbouring place for macrophages were also documented (Fig. 5A and 5B).

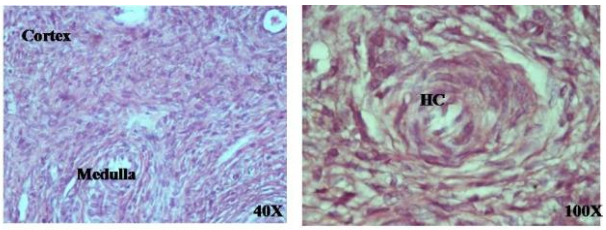


Fig 5A Fig 5B

**Fig5A.** *Histology of goat thymus (40X).* **Fig5B.** *Histology of goat thymus (100X). HC: Hassel's Corpuscle.*

### 3.6. General Histology of Goat Spleen

Histologically spleen is having the white pulp and red pulp areas (Fig. 6A and 6B). The red pulp is cell dense in nature and white pulp is cell translucent. The splenocytes which are immunologically important are located in the red pulp area.

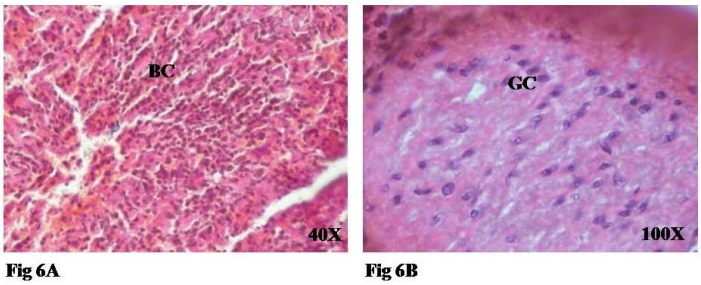


**Fig6A.** *Histology of goat spleen (40X).*
**Fig6B.** *Histology of goat spleen (100X). BC: Billroth's Cord and GC: Germinal Centre.*

### 3.7. Immunohistochemical Localization of MT1 and MT2 Receptors

*3.7.1. In Goat Ovary*

The goat ovary is having high expression for both MT1 and MT2 receptors. We found, high expression pattern for MT1 receptors in thecal and granulose cells (Fig. 7A and 7B) but the MT2 receptors were most abundantly present in thecal cells as well as on the cumulus oophorus layer of goat ovary surrounding the ovum (Fig. 7C and 7D).

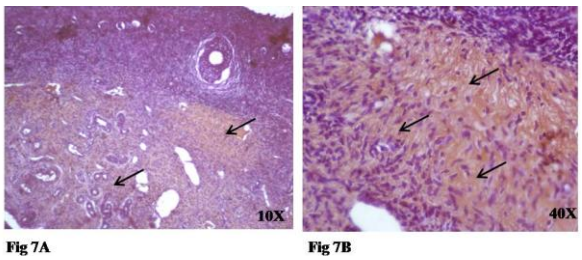


**Fig7A. & 7 B.** *Immunohistochemical localization of MT1 receptor in goat ovary (10X) and (40X). Arrows show immunoreactivity.*

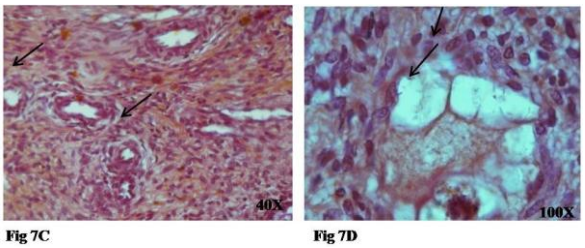


**Fig7C. & 7 D.** *Immunohistochemical localization of MT1 receptor in goat ovary (10X) and (100X). Arrows show immunoreactivity.*

*3.7.2. In Goat Uterus*

We noted high expression of both the membrane bound melatonin receptors (MT1 and MT2 receptor) in goat uterus. However, the MT1 was highly expressed only on the membranes of secretory cells of endometrium (Fig. 8A and 8B) but MT2 was highly expressed both on the non-voluntary muscle cells of myometrium and on the secretory cells of endometrium (Fig. 8C and 8D).

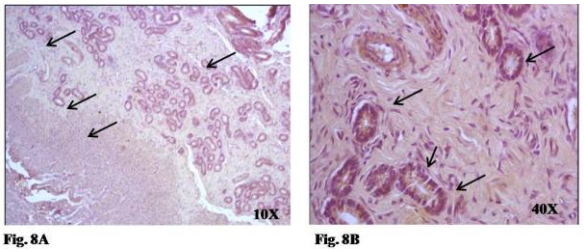


**Fig8A& 8B.** *Immunohistochemical localization of MT1 receptor in goat uterus at (10X) and 40X. Arrows show immunoreactivity.*

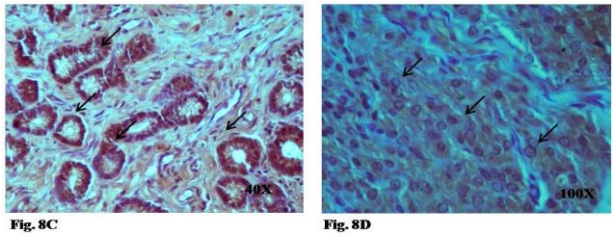


**Fig8C&8D.** *Immunohistochemical localization of MT2 receptor in goat uterus at (40X) and 100X. Arrows show immunoreactivity.*

### *3.7.3. In Goat Thymus*

The thymus was having a high expression of MT1 receptor and this receptor was cosmopolitan in distribution over the entire structure. The Hassel's corpuscles and the PALS were having more distribution of this receptor. Even though, the macrophages which were present in the Hassel's corpuscles were also having this receptor (Fig. 9A and 9B).

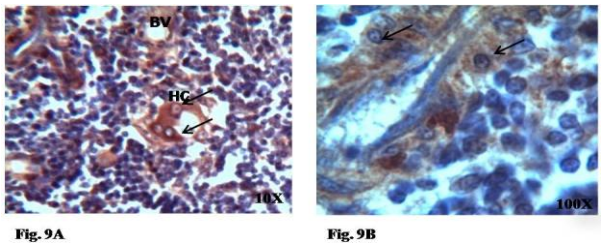


**Fig9A&9B.** *Immunohistochemical localization of MT1 receptor in goat thymus (10X) and (100X) magnifications. BV: Blood Vessel; HC: Hassel's Corpuscle. Arrows show immunoreactivity.*

### *3.7.4. In Goat Spleen*

The goat spleen is having higher expression of MT1 receptor on the splenocytes particularly in red pulp region. But, the white pulp region which is cell translucent is having less expression of MT1 or MT2 receptors (Fig. 10).

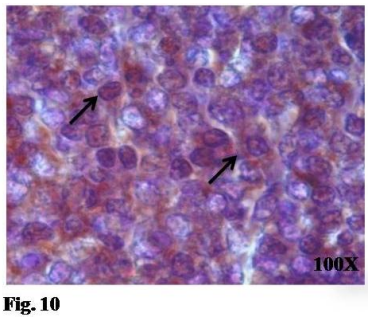


**Fig10.** *Immunohistochemical localization of MT1 receptor in goat spleen (100X) magnification. Arrows show immunoreactivity.*

### *3.7.5. In Goat Testes*

We noted high expression of MT1 receptor in the Leydig cells of testes of goats however; the MT2 expressions were less prominent in the Sertoli cells or in the Leydig cells of goat testes (Fig. 11).

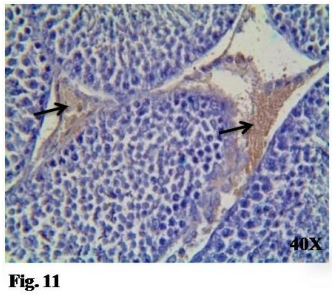


**Fig11.** *Immunohistochemical localization of MT1 receptor in goat testes higher (40X) magnification. Arrows show immunoreactivity.*

### 3.8. Immunohistochemical Localization of AR in Male Goat Thymus

The male goat thymus is having high localization of androgen receptor (AR). Both the cortex and the medulla are having the distribution of AR but the cortex is having more expression in comparison to the medulla (Fig 12).

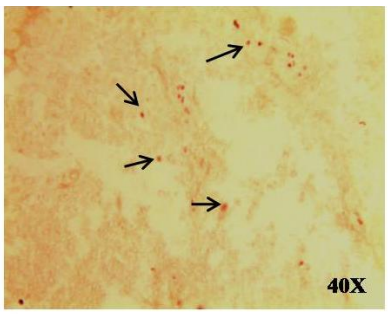


Fig. 12

**Fig12.** *Immonohistochemical localization of AR in male goat thymus (40X). Arrows show immunoreactivity.*

### 3.9. Immunohistochemical Localization of ERα in Female Goat Thymus

The female goat thymus is having localization of estrogen receptor (ERα). Both the cortex and the medulla are having the distribution of ERα. There was no zonal difference (cortex and medulla) in the estrogen receptor expression pattern (Fig 13) in the thymus of female goats.

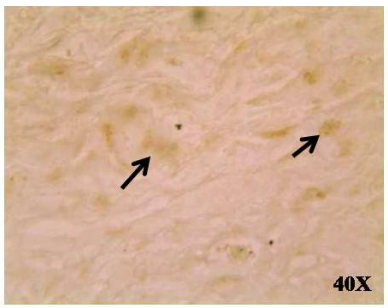


Fig. 13

**Fig13.** *Immunohistochemical localization of ERα in female goat thymus (40X). Arrows show immunoreactivity.*

### 3.10. Immunohistochemical Localization of GR in Goat Thymus

Glucocorticoid receptor (GR) was localized in thymus of both the sexes however the result of male thymus is presented over here. We noted higher expression pattern of GR in the periphery of thymus (i. e. the basal lamina and peri lymphoid zones) in both the sexes. The Hassel's corpuscle was having low expression of GR in both the sexes (Fig. 14).

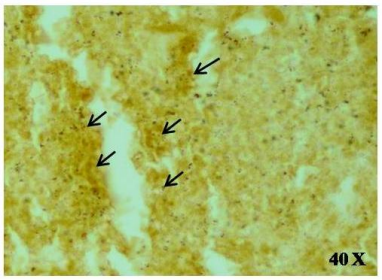


Fig. 14

**Fig14.** *Immunohistochemical localization of GR in male goat thymus (40X). Arrows show immunoreactivity.*

## 4. DISCUSSION

### 4.1. The Histomorphology of Goat Pineal

Pineal gland is one of the most important structures in brain as it is the primary source of melatonin [6] In the present study, for the first time we have discussed about the basic histo architecture of this gland. We noted high abundance of astrocytes, pineal glial cells, light and dark pinealocytes in young goats. But, the cells were degenerated with the advancement of age with huge pineal concretions

laden with calcareous depositions. Our present result in goats is equivocal with the earlier report of Singh et al., (2014)' [19] in human pineal gland where the degenerative effect of age is very much evident.

### 4.2. The Histomorphology of Gonads in Goats

*4.2.1. Testes*

Being reproductively active throughout the year, the histomorphological architecture of testes in goats is almost similar in a season round manner. However, depending upon the histology, the seminiferous tubules contain germ cells (embedded in Germinal epithelium), spermatogonia, primary sprematocyte, secondary spermatocytes and spermatids. In the germinal epithelium, the germ cells and particularly the primary spermatocytes were found in a mass of cells. This may be due to the fact that the germ cells and primary spermatocytes are connected with each other by inter cellular bridges (ICB). Thus, when they are under divisional stage, they are not separated from each other and finally form a horse shoe shaped cellular mass containing different types of germ cell layers in a linear manner but not in a tier. This interesting finding is in parallel with the previous report of Kojima (1992) [20]. Apart from this, the most interesting feature is in the Sertoli cells of goats, unlike other animals, they are having projections/processes. Morphologically, the Sertoli cell processes are classified into sheet-like and slender cord-like processes. The sheet-like process originated solely from the Sertoli cell column while the slender cord-like process projected either from the Sertoli cell column or the sheet-like process in a cumulative conjugation which is in equivocal with the previous report of Hess and Carnes, (2004) [21].

*4.2.2.* Ovary

The basic ovarian structure is similar to the other mammals as suggested by other workers [22]. Outermost layer of ovary is germinal epithelium. In the internal structure the ovary is primarily demarked as cortex and medulla. The cortical region contains ovarian follicles and stroma. The ovarian follicles are having cumulus oophorus, membrana granulosa (and the granulosa cells inside it), corona radiata, zona pellucida, and primary oocyte. The zona pellucida, theca of follicle, antrum and liquor folliculi is also contained in the follicle. Also in the cortex is the corpus luteum derived from the follicles. But, surprisingly we noted the presence of Graafian follicle for a prolonged time in histo architecture of ovary and our observation is similar to the previous reports of Sharma et al., (1996), [23]. Possible explanation may be that, also in goats, from the developing and to maturation of follicles there are seven different stages (from stage I to stage VII). This is the most important and remarkable feature of goat ovary which makes it different from others. The reason may be that in goats' seasonality in reproduction is very tightly regulated and well managed by different gonadal steroids. Thus, during the reproductive inactive phase, the ovary is under quiescent phase with no regular cyclical activity. But, during reproductively active phase, the ovary switches from non-cyclical stage to cyclic ones and high level of circulatory estrogen facilitates this process [24]. Thus, due to prolonged estrous, Graafian follicle was found in dormant stage like delayed ovulation condition in bats [25] which in turn may ovulate during reproductive active phase (i.e. winter; November to January) for a successful copulation.

*4.2.3. Uterus*

Uterus is a dynamic structure in terms of reproduction and hormonal regulation. Like other mammals, the goat uterus is also having the basal lamina and two clearly distinguishable zones i.e. myometrium and endometrium. In the myometrium, non voluntary muscle layers are found. It is the uterus of reproductively inactive or post partum phase hence, the myometrial layer is thin. In the endometrium the secretory gland are found with huge amount of secretory granules present in the secretory cells and a large lumen inside it. We have also noted high involutions in goat uterus during this particular phase, which may be due to the post partum changes occurring in goat uterus. This observation is in equivocal with the previous reports of Tielgy et al., (1982), [26] in Nigerian goats and Baru et al., (1983) [27] in Nubian goats.

### 4.3. The Histomorphology of Lymphoid Organs in Goats

*4.3.1. Spleen*

Spleen is the most important secondary lymphoid organ in mammals. The spleen is located just beneath the peritoneum associated with the stomach and small intestine. Both in adults and in neonate

pups they are having significant immunological importance and thus are not having age dependent degeneration. Like the basic splenic structure in other mammals, the goat spleen is also surrounded by outer capsule. In the internal structure it is mainly differentiated into white pulp and red pulp regions. Red pulp region contains sinusoids and splenic cords also called as Billroth's cords. In our present histological observation we found the presence of three-dimensional network of fibroblastic reticular cells located among branched sinuses. This result is in parallel with the reports of Omotainse and Anosa (2009), [28] in human spleen. The red pulp region is responsible for germination of red blood cells (at old age) and monocyte reserve. Further, the white pulp region is RBC translucent zone and contains Periarteriolar lymphoid sheaths (PALS; rich in T-lymphocytes) and lymphoid follicles. Thus, this region is most important for both cellular and humoral immunity.

*4.3.2. Thymus*

Thymus is the important primary lymphoid organs in all the mammals along with goats. The histomorphological observation of thymus in Indian goat *Capra hircus* is reported for the first time in our present study. The thymus being the most important lymphoid tissue for the neonates is highly developed in young ones but they gradually decrease in shape and size depending upon the age of the animal. The structure is distributed in a peculiar manner in goats. They are generally present just on the ventral side of the heart and two prolongations of the same are distributed on the two sides of trachea. In the histology, it is surrounded by parenchyumatus capsule and having outer cortex and inner medullary regions. In the medulla, there is presence of Hassel's corpuscles which is the playing ground of T-lymphocyte for priming and maturation. Particularly in the medullary region, there is presence of Periarteriolar lymphoid sheaths and it is rich with T-lymphocytes.

### 4.4. Immunohistochemical Localization of MT1 and MT2 Receptors in Lymphoid Organs and Gonads of Goats

There is a long established hypothesis about the role of melatonin in immunomodulation and handful literatures are available in this respect. Melatonin receptors have been localized in the circulating lymphocyte and on lymphoid organs [29], suggesting a direct effect of melatonin on the regulation of immune system [30]. High-affinity melatonin receptors have been localized on circulating lymphocytes from rodents, chickens, and humans [29], [31] and on thymocytes and splenocytes in humans [32] and a number of rodents [33]. Thus it is quite evident that the melatonin is modulating the immune function and in this respect the immunocytochemical localization of MT1 and MT2 receptor in lymphoid organs (spleen and thymus) is having noteworthy significance. This study not only for the first time demonstrates about the localization of melatonin receptors in spleen and thymus of Indian goat but also pinpoints towards the immunomodulatory role of this hormone again in Indian goat. Most importantly we found MT1 and MT2 receptor expressions in PALS region of thymus and spleen. The PALS region is area abundant with T-lymphocytes. Thus, presence of MT1 and MT2 receptors suggest the direct role of melatonin in goat immune modulation.

Further, we noted high expression of MT1 and MT2 receptors in theca and granulosa cells of goat ovary. This result clearly suggests the role of melatonin in goat reproduction and is in equivocal with the reports of Tamura et al., (2012), [34] in human. MT1 and MT2 receptors were also noted in the secretory glandular cells of goat uterus and particularly MT2 was abundantly present in the myometrium. Thus, we may conclude that melatonin might have some role in fetal development in goats (may be particularly by MT2) and the results are equivocal with the reports of Hafez et al., (2007), [35] suggesting pro-gonadotrophic action of melatonin along with its role in maintenance of gestation.

We found high expression of MT1 receptor in goat testes (particularly in Leydig cells). Thus, it may be speculated that melatonin is not only having a positive role in maintenance of male goat reproduction but also might have role in steroidogenesis (*via* androgen receptor AR) present on Leydig cells.

### 4.5. Immunohistochemical Localization of Gonadal Steroid Hormone Receptors (AR and ERα) and Glucocorticoid Receptor (GR) in Thymus of Goats

The immunosuppressive effects of different steroids (gonadal and adrenal steroids) are also evident and from time to time different authors have confirmed the same in different animal models [33], [36]. For example glucocorticoid induces DNA fragmentation in lymphocytes and thereby maintains

the morphological features of lymphoid organs [37]. However, melatonin can ameliorate the immunocompromising effect of glucocorticoids [36]. There are reports demonstrating the inhibitory roles of pineal indoles in steroidogenesis [38] and inhibitory role of gonadal steroids in immunomodulation [33]. Thus, the presence of Androgen Receptor (AR) in the thymus of male goats and presence of Estrogen Receptor α (ERα) in thymus of female goats are having significant roles in describing the immunomodulatory action of these two gonadal steroids. The spleen is regarded as the negative control for the androgen receptor and estrogen receptor. Thus, no androgen receptor and estrogen receptor were localized in the spleen. In addition we have also found high expression of glucocorticoid receptor (GR) in thymus of goats. This result also suggests an immunomodulatory role of GR in goats.

## 5. CONCLUSION

Finally we may suggest that MT/MT2, AR, ERα and GR are expressed at high level at one phase has made this study more interesting and further *in vivo* and *in vitro* studies are needed to put forward the correlation of the hormones (melatonin, testosterone, estrogen and cortisol) in goat.

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## AUTHOR'S BIOGRAPHY

**Prof. (Mrs.) Chandana Haldar, M.Sc. (Gold Medal), Ph.D. (Bhu)**

At present Head, Department of Zoology, BHU is a pioneer in Neuroendocrinology of pineal gland and Melatonin physiology with 30 years of research/training and teaching experience. She is the first Women scientist IBRO/UNESCO fellow to work on the pineal gland. She got INSA Young Scientist Award in 1983 (Gazetted Award); Alexander von Humboldt fellowship (Germany) in 1986-87 & 1990-91 and Career award, UGC, New Delhi, 1991-94 (Gazetted Award) along with Visiting Scientist - CSIR (India)-CNRS (France) 1996 Vigyan Ratna (UP-CST Gazetted award). President AOCP, Japan, SRBCE, Taramani India and Invited Speaker for Asia Pacific Pineal Meeting, Japan, 1997, FASEB, USA(2013), AOCP Japan, Sydney (2013), Korea (2015); Platinum Jublee lecture Ind Sci Cong.2012. Fellow and Gold medal (1999) by SRBCE for life time contribution for Pineal Study. Visiting Professor - Bilateral Exchange Programme - INSA (India)-JSPS (Japan) 1997-98; UGC-Egypt; Alexander von Humboldt Fellow (Germany) 1999 & 2004 and JSPS Japan Fellow. 2000.