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# CHANGES IN THE THYROID GLAND DURING THE REPRODUCTIVE CYCLE OF THE MALE IN PTEROPUS GIGANTEUS GIGANTEUS DURING BREEDING SEASON.



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## Abstract:

The present investigation aimed to study the histological characterizations of the thyroid gland of the *Pteropus giganteus giganteus*. The gland consists of pear shaped randomly distributed micro and macrofollicles with very little interstitial tissues between them. Histologically, thyroid gland consisted of a connective tissue capsule and trabeculae were found extending from the capsule into the substance of the gland, which divided it into lobules. Each lobule consisted of three sized follicles in variable numbers, the large, medium and small. The large follicles were lined by low cuboidal epithelium, while the medium size follicles are lined by cuboidal epithelium cells and the small follicles were lined by high cuboidal to columnar epithelium. The follicles had colloid material in their lumen. The features and sizes of the follicular epithelial cells and the presence of parafollicular cells suggest activity in the thyroid gland of the bat *Pteropus giganteus giganteus*. Plasma thyroxin (T4) concentration showed a significant seasonal changes with high concentration during breeding and post breeding while low concentration during inactive period. However, the T4 concentration increased from breeding to post breeding phase and decreased in regressed phase. It is suggested that in *Pteropus giganteus giganteus* positive correlation between thyroid and testicular cycle occurs during active phase of the reproductive cycle when the body weight and testicular activity are also closely correlate.



**KEY WORDS -**

Anatomy, Thyroid gland, Bat.

**INTRODUCTION**

The present study was undertaken to correlate changes in thyroid gland of *Pteropus giganteus* with the reproductive cycle. Reproduction in Chiroptera is of special interest due to numerous adaptive specializations exhibited by this diverse group of mammals. The thyroid gland has long been recognized as an important modulator of reproductive function. Both hyper- and hypo-thyroidism are associated with reproductive dysfunction and infertility. There is direct effects of thyroid hormones on cellular metabolism and hormone responsiveness have been demonstrated in the gonads and the sex accessory organs. In addition, thyroid hormones appear to play a important role in the expression of stages that occur seasonal reproductive cycles. The thyroid gland of *Pteropus giganteus* is composed of two types of parenchymatous cells, follicular epithelial cells (releases T4 hormones) and parafollicular cells (C cells or calcitonin-secreting cells or basal granular cells). Similar observations are reported in *Myotis lucifugus* as the thyroid follicle is made up of three principle components: the lining follicular cells, the luminal colloid and the basal parafollicular cells (Nunez et al., 1969; Kwiechinski et al., 1991). Morphological studies indicate that the thyroid increases activity rainy season, is active throughout the winter, and regresses by summer. Such an activity cycle is commonly found in most seasonally breeding mammals.

**MATERIALS AND METHODS**

Indian male fruit bat, *Pteropus giganteus giganteus* (Brünnich) found on trees hanging upside down near water sources. This bat was selected for the present study because of its unique reproductive habits. The specimens were collected from Padmapur 40 Kms. from Bramhapuri, District- Chandrapur in Maharashtra (India), throughout the year representing different cycle during (December 2007 to November 2009). They were brought to laboratory alive with minimum stress. The males are easily identified by their external genitalia and golden-brown fur around neck. Mature and large sized adult males were caged for the experiment weight recorded with sensitive balance before they sacrificed. Bats were sacrificed by decapitation using anesthesia.

For histological examination thyroid gland from sexually active bats excised out from the body cavity. The weight of thyroid was recorded separately by using highly sensitive monopan electric balance. The tissue fixed in alcoholic Bouin's fixative for 24 hrs, followed by preservation in 70% ethyl alcohol. The tissues were dehydrated through the graded series of ethanol, cleared in xylol and embedded in paraffin wax. The tissues were cut at 5 to 6  $\mu$  with the help of Rotary microtome. The sections were stained with haematoxyline and eosin for routine histological examination. The microphotographs were taken with the help of a Lobamade camera attached to the microscope and enlarged to the required size.

For histological analysis, sections were selected and measurements were taken with the help of an ocular micrometer. The diameter of the whole thyroid gland, diameter and width of small medium and large follicles during different phases of reproductive cycle were measured. The available data was analyzed by statistical method.

**RESULTS :**

Studies of thyroid gland of *Pteropus giganteus giganteus* revealed that the gland was located near the first ring of trachea and consisted of two lobes. An isthmus connected these lobes to each other. The colour of the gland was reddish brown. Thyroid gland of *Pteropus giganteus giganteus* shows marked seasonal variations in histology. The thyroid gland consists of follicles of different size during various phases of reproductive cycle.

**THYROID GLAND DURING SEXUALLY ACTIVE PERIOD**

Bats collected in the month of September and October shows vigorous spermatogenesis in their testis. During this period the thyroid gland appears almost oval in shape and is highly vascularized. In this period gland is composed of numerous follicles of different sizes, smaller and medium sized follicles are predominant while large size follicles are few. These follicles are widely dispersed and separated by inter-follicular loose connective tissue.

**'A' type of follicles:**

These are present in the thyroid gland of *Pteropus giganteus giganteus* mainly distributed in the middle and lower part of the gland. Number of these follicles are more than those observed in the thyroid gland of in sexually inactive bat. The 'A' type of follicle is (48u to 50u). The height of epithelial cell of 'A' type of follicle is (10u). The follicles are lined by high columnar epithelium. The spherical shaped and darkly stained nucleus is observed. Nuclear membrane, chromatin material and nucleolus are clearly identified. Cytoplasm is basophilic in nature. The cells are highly secretory. The height of epithelial cells of these follicles is more than the height during sexually (inactive) period. Lumen is very small with incompletely filled bluish-pink colloidal material attached to the follicular epithelial cells having diameter (42u).

**'B' type of follicles :**

These are medium sized follicles observed throughout the gland. These follicles are larger in size (145u) than 'B' type of follicles during the sexually inactive (quiescence) period. The follicles are moderate in number but more than 'B' type of follicles during the sexually quiescence period. These are lined by low columnar epithelial cells (8u) and are highly secretory in nature. Nucleus is large and spherical to oval in shape. Chromatin material is darkly stained and eccentric nucleolus is present in nucleoplasm. Cytoplasm is eosinophilic in nature. Lumina are filled with homogenous, colloidal material attached to the follicular epithelial cells having diameter (135u).

**'C' type of follicles :**

These follicles are mainly seen at periphery of the gland. These are larger in size (215u) than 'C' type of follicles during the sexually inactive period. These follicles are lined by low columnar epithelial cells (6u). These follicles are less in number than the sexually inactive period follicles. Nucleus is small and spherical to oval in shape and darkly stained. Nuclear membrane, chromatin material and nucleolus is clearly visible. Cytoplasm is lightly stained and eosinophilic. The large lumina of these follicles are filled with an eosinophilic pink coloured material (210u). In few follicles lumina is empty.

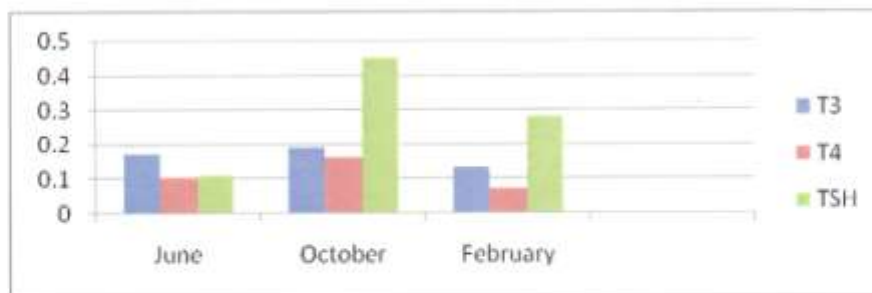
**Parafollicular Cells :**

During the active breeding period in the month of September, the parafollicular cells are found singly or groups of 4-6 cells within the follicular epithelium and in the interfollicular connective tissue. The parafollicular cells are large with centrally placed large, oval nucleus with rim of chromatin material near the nuclear membrane. The nucleus possesses a centrally placed single nucleolus in the nucleoplasm. Cytoplasm is lightly stained. The number of these cells during the active period are always more than those observed during the sexually inactive period.

**Table No. 1**

Month of sample	Reproductive Phase	Concentration of Hormones		
		T3	T4	TSH
June	Prebreeding	0.17 ng/ml	0.10 ng/ml	0.11 ng/ml
October	Breeding	0.19 ng/ml	0.16 ng/ml	0.45 ng/ml
February	Regressed	0.13 ng/ml	0.07 ng/ml	0.28 ng/ml





The seasonal changes of TSH, T3 and T4 have been studied in *Pteropus giganteus giganteus*. Males exhibit a well defined seasonal cycle of T3 and T4 concentrations. When spermatogenesis begins in the testis the T3 and T4 level was measured as 0.17 ng/ml and 0.10 ng/ml respectively which is prebreeding which reaches to peak when vigorous spermatogenesis is noticed Breeding T3(0.19 ng/ml) and T4(0.16 ng/ml). Later on during regressed phase the level of T3 and T4 becomes moderate measuring 0.13 ng/ml and 0.07 ng/ml respectively. Similarly TSH level were also measured during the different phases of the reproductive cycle. During February when the animals were sexually inactive regressed TSH level was 0.28 ng/ml and there was a increase in the level during pre-breeding period in the month of June (0.11 ng/ml) further it is increased significantly during active breeding period in the month October was measured (0.45 ng/ml) Table no 1.

#### DISCUSSION

Thyroid is an endocrine gland that secretes hormones including thyroglobulin, triiodothyronine and thyroxin. Thyroxin hormone secreted by this gland plays an important role in metabolism of the body (TURNER, 1966). In *Pteropus giganteus giganteus*, the location of thyroid gland in the body is similar to other bats like *Hipposideros lankadiva* (SERAPHIM, E.R, 2004), *Taphozous longimanus* (NERKAR, 2007) i.e., with the first ring of trachea and consisted of two lobes on both side and an isthmus connecting these lobes. In *Pteropus giganteus giganteus* the gland appeared reddish brown in colour which is in concordance with the findings of *Megaderma lyra lyra*, (SONWANE, D.P, 2010). In *Pteropus giganteus giganteus* the thyroid follicles, colloid and the epithelial cell height show significant variation during the reproductive cycle. During active breeding thyroid gland shows heterogeneous population of follicles. It is mainly composed of medium size follicles and few large and small follicles. The medium size follicles are lined by cuboidal epithelium cells and are synthetically active follicles. The small size follicles are lined low columnar epithelium and synthetically most active follicles. The large size follicles are lined by low cuboidal to squamous epithelium and are synthetically inactive follicles.

Histological changes in the thyroid microstructure showing seasonal variation have been reported in bats, *Megaderma lyra lyra* (SONWANE, D.P, 2010), *Taphozous longimanus* (SINGH et. al. 2002., NERKAR, 2007), *Hipposideros lankadiva* (SERAPHIM, 2004).

In *Hipposideros lankadiva* (SERAPHIM, E.R, 2004) thyroid gland is active during active breeding similar to that are reported in *Pteropus giganteus giganteus*. In present study the thyroid gland is active during breeding period *Pteropus giganteus giganteus*. After that thyroid gland become active and remains active till early regressed period (SERAPHIM, 2004). In *Pteropus giganteus giganteus*, thyroid activity decreases in regressed period as indicated by large follicle in the thyroid gland of the regressed bat.

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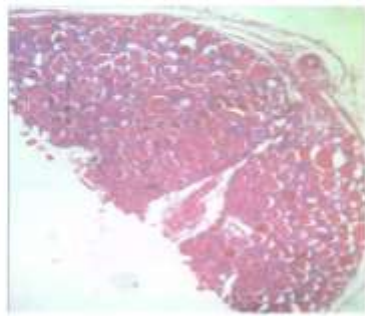


Fig 1

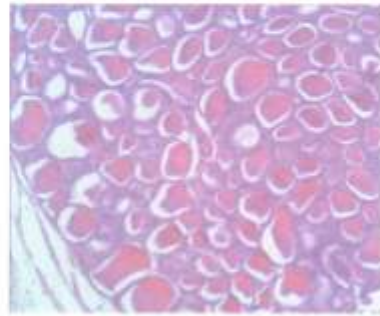


Fig 2

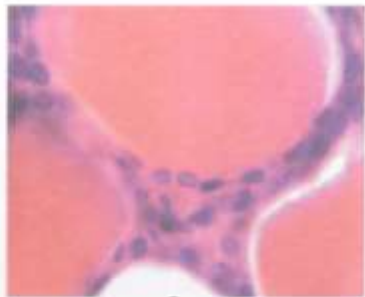


Fig 3

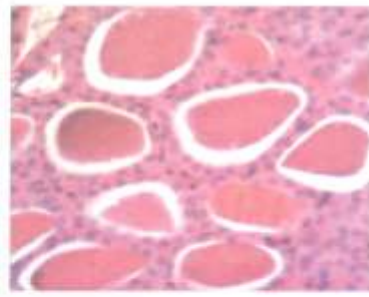


Fig 4

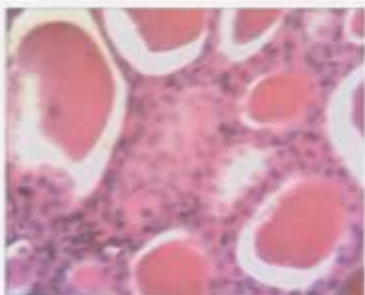


Fig 5

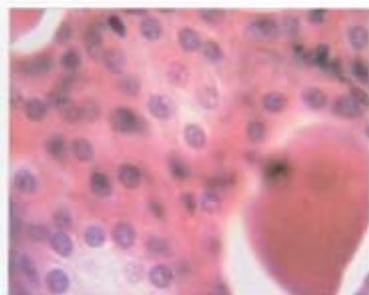


Fig 6

Fig 1

Fig. 1 T.S of thyroid gland during active breeding. Gland is enclosed in a capsule. Note the presence of large number of medium size follicle ('B' type) and some large size follicle('C' type). X 100

Fig. 2,3,4 Magnified part of thyroid gland to show large follicle ('C' type) lined with low cuboidal epithelium and filled with colloid material. Colloid material is retrieved to the center of some follicles. X 400

Fig. 5 & 6 Magnified part of thyroid gland to show medium size follicles ('B' type) lined by cuboidal epithelium and filled with colloid material. Parafollicular cells are also seen in the interfollicular space. X 400