RESEARCH ARTICLE

Changes in the thyroid gland of the male emballonurid bat, *Taphozous kacchensis* (Dobson) during the reproductive cycle.

Bansod DS[1*] and Dhamani AA [2]

[1]Department of Zoology, Shree G.M. Arts & Science College, Kurkheda- 441209
[2]Department of Zoology, N.H. College, Bramhapuri- 441206
**Corresponding author: email- bansodsd@gmail.com*

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ABSTRACT

The thyroid gland showed marked seasonal variations in weight, quantity of colloid and epithelial height during various phases of reproductive cycle. Suggesting the thyroid gland to be inactive during sexually quiescence period and active during breeding period similarly to the testicular cycle. The body weight, thyroid gland weight and testis weight in sexually active bats is higher as compare with sexually quiescence bats. It is suggested that in Taphozous kachhensis the positive correlation between thyroid gland and testicular cycle occurs only during the phases of the reproductive cycle when the body weight and testicular activity are also closely correlated.

Key words: Thyroid, testes, bats.

INTRODUCTION

In mammals, altered thyroid status is known to adversely affect many organs & tissues. Nevertheless, for many years, the impact of thyroid disorders on male reproduction remained controversial. However in the past two decades, several experimental and clinical studies have demonstrated that thyroid hormones play an important role in testicular development and function. (Wagner *et al*., 2008).

The male Emballonurid bats exhibits several unique features in the reproductive cycle. Thyroid gland showed marked seasonal variation in weight, quantity of colloid and follicular epithelial height, suggesting that it is to be inactive during quiescence and winter dormancy and active during the time of recrudescence and similarly to the testicular cycle in *Scotophilus heathi* (Krishna and Singh, 1998) and *Taphozous longimanus* (Singh *et al*. 2002).

In camel, *Camelus dromedarius*the thyroid follicles are of different sizes in both summer and winter. The large follicles were lined by very low cuboidal or semi-squamous follicular cells whereas the small ones were lined by high cuboidal or low columnar follicular cells (Abdel-Magied *et al*., 2000). The morphology of parafollicular cells (C) cells in the thyroid gland of bat was studied during different phases of reproductive cycle (Nerkar and Gadegone, 2008).

Since long, the thyroid gland has been recognized as an important modulator of reproductive function. Both hyper and hypo-thyroidism are associated with reproductive dysfunction and infertility (Kwiecinski and Damassa, 2000). This gland has been studied histologically in many seasonally breeding microchiropteran bats (Kwiecinski *et al*., 1991; Damassa *et al*., 1995). The thyroid gland performs highly specific functions such as synthesis, accumulation and release of hormones containing iodine and calcitonin. The hormonal profiles of the thyroid gland during various phases of reproductive cycle have been studied in goat (Bhattacharaya *et al.,* 1994), rat (Fakuda *et al.*1980), cow (Khurana and Madan, 1986) and bats *Macrotus waterhausii* (Burns *et al*., 1972), *Mollossus planceps* (Krutzsch and Crichton, 1985), *Epomops franqueti* (Ifuta *et al*., 1988).

Although the considerable information available on the histological structure of thyroid gland of bats (Hudson and Wang, 1979), literature related to the study of this gland during different phases of the reproductive cycle is scanty. The detailed study of the morphological, histological and functional characteristics of the thyroid gland of male *T. kachhensis* during different phases of the reproductive cycle has been undertaken because of its unusual reproductive behavior, and never the less to find out the probable role of thyroid gland in the control of reproduction in this seasonally breeding bat if any. Therefore, the pattern of seasonal changes in the thyroid gland of *Taphozous kachhensis* was studied in relation to the changes in its reproductive cycle.

MATERIALS AND METHODS

The male Emballonurid bat, *Taphozous kachhensis* (Dobson) was selected for the present study because of its unique reproductive habits. The specimens for present study were collected from Ambai-Nimbai, District- Chandrapur in Maharashtra (India) ($20^0$38'39.08"N and $79^0$35'30.99"E), throughout the year representing different reproductive cycle during (April 2006 to March 2009). They were brought to the laboratory alive with minimum stress and constant supply of food and glucose water. They were sexed after collection by their external genitalia. The males are easily identified by deep black or brownish beard on ventral side of the lower jaw. The females and small immature males were identified and released at the same place, while mature and large sized adult males were caged for the experiment. Body weight of each bat was recorded. Bats were sacrificed by decapitation using anesthesia.

For histological examination, testis and thyroid gland from sexually inactive and sexually active bats were removed out from the body cavity. The weight of thyroid gland was recorded on highly sensitive monopan electric balance. The tissues were fixed in alcoholic Bouin's fluid for 24 hrs, followed by preservation in 70 % ethyl alcohol and then dehydrated through the graded series of ethanol, cleared in xylol and embedded in paraffin wax. Tissues were cut at 5-6 µ with the help of BMT-9 craft Rotary Microtome. Sections were stained with Ehrlich's haematoxylin-eosin for histological observations (Pearse and Welsch, 1968). The microphotographs were taken with the help of a Lobamade camera attached to the microscope and enlarged to the required size.

For morphometric analysis, the sections were selected and measurements were taken with the help of an ocular micrometer. The mean diameter of various types of follicles and colloid during different phases of reproductive cycle were measured. The available data were analyzed by statistical method. Intergraph difference was statistically assessed using t-test; Pearson's product-moment correlation coefficient and Wilcoxon rank sum test (Non parametric test). Data is expressed as mean ± SEM.

RESULTS AND DISCUSSION

In *Taphozous kachhensis* the thyroid gland is bilobed structure. It lies in the neck embracing the superior part of trachea and inferior part of cartilage and larynx. Lobes are present on either side of trachea and are connected by isthmus.

The thyroid gland of bat shows variation in the weight during various phases of reproductive period. It is lowest (1.04mg) during May to August when the animals are inactive spermatogenetically. But there is gradual increase in the weight during the successive months. The weight of the gland is (1.12mg) in October and November, when the animals are sin pre-breeding phase and testis shows an initiation of spermatogenesis. There is further increase in the weight of the gland and shows highest value (1.46 mg) when the spermatogenesis is at peak level in the testis

during the months, December and January. The weight of the gland decreases in the following months of reproductive cycle (1.27mg) after the copulatory activities are over and testis and accessory sex glands are regressed (Table-1).

Table 1: Weight of the thyroid gland during different phases of reproductive cycle of male bat, Taphozous kachhensis.

Month of Collection	Reproduction Phase	Mean weight (mg)
May to August	Inactive	1.04
October to November	Pre-breading	1.12
December to April	Active-breading	1.46
February to April	Post breading	1.27

Light microscopic structure of thyroid gland

The thyroid gland lobes of *Taphozous kachhensis* contains many follicles covered by an outer capsule, which penetrates the glandular tissue as trabeculae and septa giving rise to incomplete partially enclosed areas, lobules. The follicles are varying in size and mostly spherical to oval to irregular in shape. The junctions of follicular cell represent the parafollicular cells. Inter-follicular stroma is mainly reticular and remarkably rich in vascular and capillary plexus. The histology of thyroid gland during different phases of reproductive cycle is as follows.

Thyroid gland during sexually quiescence period

The average weight of thyroid gland is 1.04 mg (SEM ± 0.005) (Table-1). It is roughly triangular to oval in shape. The thyroid gland is composed of large number of follicles of different shapes and sizes (Fig- 1).

The follicles are separated from one another by irregular masses of inter-follicular tissue. On the basis of size, shape, diameter and histological structure of different follicles in the thyroid gland, these are classified in to 3 types.

'A' type (Small sized follicle)-Diameter 20-40 µ
'B'type (Medium sized follicle)-Diameter 41-70 µ
'C' type (Large sized follicle)- Diameter 71-100 µ

The large and medium sized follicles are abundant and occupy middle or interior region of the gland while small sized follicles are less in number during the sexually quiescence period (Fig-1, 2).

***'A' type of follicles*:**

The follicles are small (25.74µ) in size occur predominantly in the periphery of the gland. These follicles are lined by low columnar epithelial cells (10.01µ). The follicles are secretary in nature and covered by connective tissue. The nucleus is spherical and darkly stained. Chromatin clumps are seen at the periphery of the nuclear membrane. Cytoplasm of these cells is basophilic. The lumina are narrow and filled with basophilic homogenous colloid material attached to the follicular epithelial cells having diameter (Table- 2). Few vacuoles are observed at the junction of colloid and epithelial cells (Fig-1, 2).

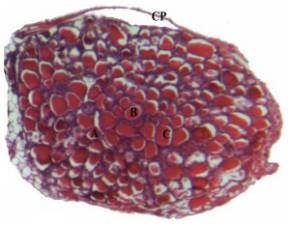


Fig.1

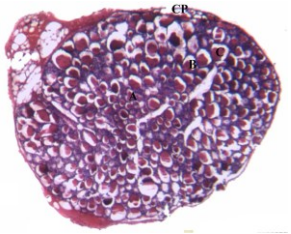
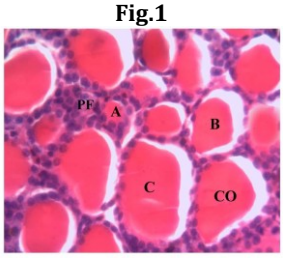


Fig.2



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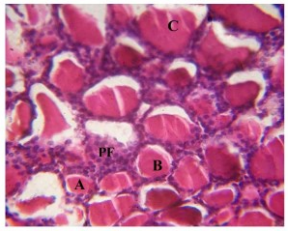

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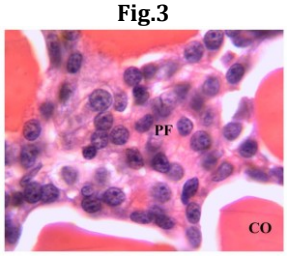

Fig.5

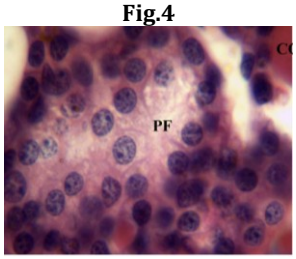

Fig.6

Fig.1: Light miscoscopic structure of thyroid gland during sexually quiescence period covered with capsule (**CP**) showing diffrenet types of follicles filled with colloid (CO)X100

Fig.2: Magnified part of thyroid gland during sexually quiescence period showing large (**C**), medium (**B**) and small (**A**)follicles with some parafollicular cells (**PF**) X400

Fig. 3: Magnified part of thyroid gland during sexually quiescence period showing parafollicular cells (**PF**)in the interfolllicular connective tissue with oval and eccentrically placed nucleus X 1000.

Fig.4: Light microscopic structure of thyroid gland during sexually active period covered with capsule (CP) showing different types of follicles filled with colloid (CO) X100.

Fig. 5: Magnified part of thyroid gland during sexually active period (**C**), medium (**B**) and small (**A**)follicles with some parafollicular cells (**PF**) X400.

Fig.6: Magnified part of thyroid gland during sexually active period showing parafollicular cells (**PF**)in the interfolllicular connective tissue with oval and eccentrically placed nucleus X 1000

***'B' type of follicles*:**

These are medium sized follicles but larger (42.90µ) than 'A' type of follicles. These are mostly observed in the middle region of the thyroid gland (Fig.-1). These follicles are more than that of 'A' type of follicles and lined by cuboidal epithelial cell (5.72µ) (Table- 2). The plasma membrane is not clearly visible. The cell is less secretary in nature. The centrally placed nuclei are large, darkly stained and spherical to oval in shape. Cytoplasm is basophilic. Chromatin material is observed at the periphery of the nuclear membrane. Homogeneous colloidal material attached to the follicular epithelial cells having diameter (34.s32µ) is observed in the follicular lumina. At the edge of the colloid, vacuolations are observed (Fig- 2).

'C' type of follicles:

These are largest follicle measuring (64.35µ) and mostly present in the centre of the thyroid gland (Fig-2). The follicles are more than 'B' type of follicles during the sexually quiescence period. Follicles are lined by squamous to low cuboidal epithelium (4.29µ) and compactly arranged by thin connective tissue. The nuclei are small, oval to elongated and darkly stained. Nuclear membrane is clearly visible, cytoplasm is eosinophilic. Numerous follicles having wide lumina are incompletely filled with homogenous colloid material, while in few follicles lumina are empty. The diameter of colloid in the lumen is 47.19µ. (Table- 2)

Parafollicular cells:

These cells are found singly or in groups or 2-3 cells in the follicular epithelium and inter-follicular connective tissue. These are close to the base of the epithelium. The nucleus is eccentric, darkly stained and oval in shape, nuclear membrane is clearly seen. Chromatin material is darkly stained while cytoplasm is lightly stained (Fig- 3).

Thyroid gland during sexually active period

The thyroid gland during sexually active period is highly vascular and weighing about 1.46 mg (± 0.007). It is composed of numerous follicles of different sizes. Smaller and medium sized follicles are predominant while large sized follicles are few (Fig- 4). These follicles are widely dispersed and separated by inter-follicular loose connective tissue.

'A' type of follicles:

These follicles dispersed throughout the gland but more in the centre and are larger in size (38.61µ) than the 'A' type of follicles during the sexually quiescence period. The number of follicles is higher than the sexually quiescence period. The height of epithelial cell of 'A' type of follicle is more (12.87µ) than 'B' and 'C' type follicular epithelial cells during sexually quiescence period and sexually active period of reproductive cycle (Fig-5). The follicles are lined by high columnar epithelium. The nucleus is spherical shaped and darkly stained. Nuclear membrane, chromatin material and nucleolus are clearly identified. Cytoplasm is basophilic in nature. The cells are highly secretary. The height of epithelial cells of these follicles is more than observed during sexually quiescence period. Lumen is very small with incompletely filled bluish-pink colloidal material attached to the follicular epithelial cells having diameter 17.16 µ (Table-2 & Histogram-1).

'B' type of follicles:

These are medium sized follicles observed throughout the gland. These follicles are larger in size (68.64µ) than 'B' type of follicles during the sexually quiescence period (Table-2 and Histogram-1). The follicles are moderate in number but more than 'B' type of follicles during the sexually quiescence period. (Fig.5). These are lined by low columnar epithelial cells (8.58µ) and

Table 2: Mean thyroid gland weight with SEM and diameter of thyroid follicles, colloid and epithelial cells height during the sexually quiescence and sexually active period of male bat, *Taphozous kachhensis.*

Sr. no.	Period	Mean Weight (mg)	SEM	Type of Follicle	Diameter in (µ)		Epithelial cell height (µ)
					Follicle	Colloid	
1	Sexually quiescence	1.04	±0.005	Small (A-Type)	25.74	17.16	10.01
				Medium ('B'Type)	42.90	34.32	5.72
				Large ('C' Type)	64.35	47.19	4.29
2	Sexually Active	**1.46**	±0.007	Small ('A' Type)	38.61	17.16	12.87
				Medium ('B' Type)	68.64	34.32	8.58
				Large ('C' Type)	97.24	51.48	6.43

are highly secretary 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 and lumina are filled with colloidal material attached to the follicular epithelial cells having diameter (34.32µ) (Fig- 5).

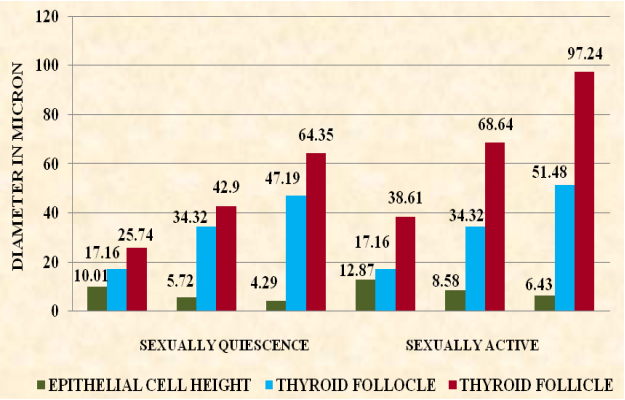

Fig.7: Relationship between measurements of epithelial cell height, thyroid colloid and follicle diameter of small, medium and large follicles during the sexually quiescence and sexually active period of male bat, *Taphozous kachhensis.*

'C' type of follicles:

These follicles are mainly seen at periphery of the gland. These are larger in size (97.24µ) than 'C' type of follicles during the sexually quiescence period (Table-2and Histogram-1). These follicles are lined by low columnar epithelial cells (6.43µ). These follicles are less in number than the sexually quiescence 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 colloidal material (51.48µ), while few follicular lumina are seen empty. (Fig - 5)

Parafollicular cells:

During the active breeding period, these cells are observed in follicular epithelium singly or groups of 4-6 cells or in inter-follicular connective tissue. The parafollicular cells are large with centrally or eccentrically placed, large, oval or irregular nucleus, with rim of chromatin material near the nuclear membrane. The nucleus possesses a centrally placed nucleolus in the nucleoplasm; cytoplasm is lightly stained. The number of these cells during the active period is more than those observed during sexually quiescence period (Fig-6).

In *Taphozous kachhensis* the thyroid gland is bilobed structure lies in the neck embracing the superior part of trachea and inferior part of cartilage and larynx. It consists of two symmetrical lobes on either side of trachea and is connected by isthmus (Dhamani and Chavhan, 2012). Similar structure were also found in other bats *Hipposideros lankadiva* (Dhamani, 2004), *Taphozous longimanus* (Shende, 2009) and mammals like horse where thyroid lobes are connected by an insubstantial isthmus; in cattle it is broad parenchymal tissue while in small ruminants it is inconstant or merely connective tissue (Dyce *et al*., 2002; Hájovská, 2002), in rat and mouse, an isthmus is located at the caudal end of the lobes (Ingbar, 1985) but in grass cutter (*Thryonomys swinderianus*) isthmus is absent (Igbokwe, 2010).

The thyroid gland of *Taphozous kachhensis* is composed of two different types of parenchymatous cells, follicular epithelial cells and parafollicular cells ('C' cells/ calcitonine secreting cells or basal granular cells). Similar observations are reported in *Myotis lucifugus lucifugus* where 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).

The medium and large sized follicles are found predominantly in the middle to peripheral region and small sized follicles are few in the centre of the gland during sexually quiescence period while during sexually active period, small and medium sized follicles are dispersed throughout the gland and large sized follicles are few towards the periphery. The average weight of the gland, the mean diameter of follicles, colloid and epithelial cell height and shows significant variation and is higher during sexually active period than during sexually quiescence period. During sexually quiescence period (May-August) of bat *Scotophilus heathi*, the thyroid gland is extremely heterogeneous in appearance showing great variability in the height of epithelium and also in the amount of colloid in various thyroid follicles. Some of the thyroid follicles were empty and other contain very small amount of colloid in their lumen. Numerous small follicles containing little colloid were found in the central part of the thyroid. These follicles are lined by cuboidal epithelial cells. During breeding phase (February -March), the thyroid gland contains follicles which are generally larger than those observed in the winter dormancy, (Krishna and Singh, 1998).

The thyroid gland of *Taphozous kachhensis* shows significant variation in weight during sexually quiescence and sexually active period of reproductive cycle. Same observations were noticed in the non hibernating bat, *Macrotus californius* (Burns *et al*., 1972). The weight of the thyroid gland is lowest in sexually quiescent bat. There is a gradual increase in the thyroid weight during recrudescence, when testis shows initiation of spermatogenesis. The weight of the thyroid gland is highest when spermatogenesis is at peak in the testis of sexually active bat. The weight of the thyroid gland decreases after the copulatory activities are over. The morphogenetic data indicate the thyroid activity increases during spermatogenesis and decreases when testes are regressed.

During sexually quiescence period the small sized thyroid follicle of bat, *Taphozous kachhensis* are lined by low columnar epithelial cell and are filled with homogenous colloid material. The spherical and darkly stained nucleus with peripheral chromatin clumps. Few vacuoles are observed at the junction of colloid and epithelial cells. The medium sized follicles are lined by cuboidal epithelial cells. The nucleus is spherical to oval shape and darkly stained with chromatin material at periphery of nuclear membrane. Colloidal material is homogenous attached to the follicular epithelium. Large sized follicles are lined by squamous to low cuboidal epithelium. The nucleus is darkly stained, oval, elongated or flattened. Colloid is incompletely filled and lumina of few follicles are empty.

During sexually active period the small sized follicles are lined by high columnar epithelial cells. Lumen is narrow with incompletely filled colloid. The nucleus is darkly stained and spherical with few chromatin materials. The medium sized follicles are lined by low columnar epithelial cells. The nucleus is spherical to oval and darkly stained. The lumina are wide containing colloid. In few follicles lumina are empty. Same findings were observed in bat *Scotophilus heathi* (Krishna and Singh, 1998), rat (Zaidi *et al*., 2004), Woodchuck (Krupp *et al*., 1976), cream hamster (Neve and Wollman, 1971) and pig (Wagi, 1969).

In bat, small follicles and high columnar follicular cells have been associated with a high rate of secretary activity (Nadler *et al*., 1954, Loewensteni and Wollman, 1967; Nunez, 1971). Therefore histological structure of follicular cells of sexually active bat indicates active metabolic state of the cells.

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