EXECUTIVE SUMMARY

OF

UGC SANCTIONED MINOR RESEARCH PROJECT

(F-NO: 47-1075/09 (WRO)

PROJECT ENTITLED

ELECTRON MICROSCOPIC STUDIES ON THE ENDOCRINE GLANDS OF THE INDIAN MALE FRUIT BAT *PTEROPUS GIGANTEUS, GIGANTEUS* (Brunnich) DURING REPRODUCTIVE CYCLE

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BY

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INTRODUCTION: - Bat considered as highly successful mammals. Considering the worldwide distribution and immense diversity exhibited by member of order Chiroptera. Reproduction in bats is of special interest due to numerous specializations exhibited by this diverse and successful group of mammals. This order exhibit several remarkable facts concerning its breeding habits, but limited attention have been given to reproduction and endocrine study in male bats. Although the endocrine glands play a significant role in regulating reproductive cycle of mammals. Male bats also exhibit diversity in the timing and frequency of their reproductive cycles annually.

Considering these facts I sent a proposal through college to UGC on 12 March 2009 with specific purpose for the detailed study entitled Electron Microscopic studies on the endocrine glands of the Indian male fruit bat, Pteropus giganteus giganteus during reproductive cycle. The UGC accepted the proposal on 2 April 2009.

The project was in two phases. The first phase consists of collection of the specimens *Pteropus giganteus giganteus* in every calendar month of 2009 and 2010. For light microscopy endocrine glands fixed in Bouin's fixative and stain with haematoxylene and eosin and cut tissues 5 & 6 µ with Rotatory microtome and stained with double staining technique. Simuulteniously hormonal regulation of testicular cycle, breeding and regressed investigated by measuring serum testosterone and pituitary gonadotropin. Result evaluated by RIA Kit by PITALE Pathology, Nagpur.

For electron microscopy tissues, Thyroid, Adrenal, Testis, Pituitary, are sliced and immersed in fresh 3 % glutaraldehyde solution and changed to buffer and send to Jaslok Hospital Mumbai for further observation. The first annual report was sent to UGC on 15 November 2011.

Second phase consist of photographs of light microscopy and electron microscopy and hormonal analysis was done while final report of minor research project was prepared.

SCOPE OF STUDY: - Endocrinology is a scientific discipline focused on study of endocrine glands, endocrinology is the study of body function, that is, how cell, tissues and organs work

and how they are integrated together in the whole individual. The study of basic endocrinology is of vital importance in medicine and health science in providing a thorough understanding of normal functioning that allows for a more effective treatment of abnormal or diseased state. It also relates to molecular biology and physiology.

MATERIALS AND METHODS:- The specimens of *Pteropus giganteus giganteus* were collected from Armori (Padmapur and Vairagad) about 110 km from Nagpur during every calendar month. Male bats, *Pteropus giganteus giganteus* collected from the fruiting site by making trap net and bats were brought alive to the laboratory with minimum stress. Specimen was weighted on a sensitive spring balance. Bat was at once anesthetized by ether and the thoracic cavity was opened, blood was drawn from the left ventricle for hormonal assay.

The pituitary gland of *Pteropus giganteus giganteus* was dissected out after removing the lower jaw and weighted on sensitive electric balance and fixed in formal sublimate fixative. Meantime reproductive tract, testis, thyroid and adrenal glands were taken out one by one gently rolled on the filter paper, quickly weighed and immersed in alcoholic Bouin's fixative for light microscopic studies.

For electron microscopic studies, pituitary, testis, thyroid, adrenal tissues were immediately dissected out from animal body were immersed in glutaraldehyde fixative.

Light Microscopy

The thyroid, adrenal and testis were fixed in alcoholic Bouin's and washed with 70% ethanol. These glands were weighted on highly sensitive electric balance For histological examination the tissues were dehydrated through the graded series of ethanol cleared in xylene and embedded in paraffin wax at 60-62^{o}c. Blocks were prepared, trimmed and cut into thin sections of 5 µ to 6 µ with the help of BMT-9 craft Rocking Microtome. The ribbon containing sections were spread over the slides smeared with Mayer's albumen. Thyroid, adrenal and testes was stained with Ehrlich's haematoxylin and eosin for histological study (Pearse, 1968).Then slides were cleared in xylene and mounted in DPX.

The desired stained sections were micro photographed at different planes. The micro-measurements were taken with ocular micrometer. The diameter of seminiferous tubules, interstitial cell of Leydig, thyroid follicles and the adrenal zones, cortex and medulla during

different phases of reproductive cycle were micro measured with the help of the ocular micrometer.

Transmission Electron Microscopy

Fixation

Thyroid, adrenal glands, pituitary, testis were selected for electron microscopic studies during the sexually breeding and sexually quiescence period. Pituitary, thyroid, adrenal and testis glands were removed from the animal. The pituitary of the animal was dissected out by removing lower jaw and weighed on electric balance. Thyroid, pituitary, adrenal, testis were sliced into 2 mm pieces and immersed in fresh, ice-cold 3% glutaraldehyde solution. The fixation was carried out over a period of 2 to 3 hours at 4^{o}C temperature. A fresh change of cold glutaraldehyde was given at the end of fixation. The tissues were then washed in cold 0.1 M sodium cacodylate buffer over half an hour with 3 to 4 changes to ensure removal of excess glutaraldehyde. Post fixation with OsO_4, osmification with 1% OsO_4 in sodium cacodylate buffer was carried out for 2 hours at 4^{O}C temperature.

Dehydration

Dehydration of tissues was carried out by passing the fixed tissues through a series of graded alcohols of increasing concentration of the dehydrating agent ethyl alcohol in water ending with absolute dehydrating agent. Most epoxy resins are soluble in ethyl alcohol and acetone but they mix much more readily with propylene oxide. Thus prior to embedding, the tissues are passed through two changes of a transitional or intermediate solvent, propylene oxide over a period of half hour.

Infiltration and Embedding

Infiltration and embedding process accomplished the complete and uniform penetration of tissue specimens by the embedding medium. Embedding consists of a complete impregnation of the interstices of a tissue specimen with the medium. This was done as follows.

i) Propylene oxide: Araldite 'A' solution 1:1 for one hour at room temperature.

ii) Fresh Araldite: 'A' solution - kept at room temperature in desiccators overnight.

iii) Araldite 'B' solution - is kept for one hours at room temperature.

Embedding of tissue blocks was done in BEEM capsule with fresh araldite 'B' solution; the capsules were kept in an oven maintained at 60^{o}C for 24 to 48 hours to complete polymerization.

Blocks are freed from the sample by cutting away the plastic; the trimmed with a safety razor blade under a stereomicroscope. Semi thin sections of 1 to 2µ in thickness were cut on an LKB Ultra tome V, with glass knives, prepared on an LKB 7800 B knife markers. These sections were dried on a hot plate (60^{o}C) and stained with 1% toludene blue for 20-30 seconds and observed on the light microscope. The selected areas for ultrathin sections were marked out.

The blocks were further trimmed and ultrathin sections or 'thin sections' 600-900 A^{o} thick corresponding to pale gold colour were cut and sections were collected on 300 mesh copper grids. To enhance the contrast a double staining technique is employed. The grids were subjected to 10% alcoholic uranyl acetate for half an hour followed by lead citrate for 10 minutes. All grids were observed on a JEOL -100S Electron microscope at 80KV accelerating voltage. Microphotographs were taken of the desired samples at different desired planes and different magnifications. All these electron microscopic processes were done in electron microscopy unit at Jaslok Hospital, Mumbai.

Radio immunoassay (RIA)

Serum T_3, T_4, TSH, FSH, LH and Testosterone were assayed by antibody coated test tube assay kits which were obtained from commercial manufacturers. The RIA system is involved the competitive bindings between specific set of antigen to that of the antibodies. Serum T_3, T_4, TSH, FSH, LH and Testosterone were assayed in PITALE DIABETES And HORMONE LABORATORY, NAGPUR.

RESULT AND DISCUSSION:-

Pteropus giganteus giganteus (Brünnich) is an exclusive Indian Pteropodidae fruit bat; this is a seasonally monoestrous species. Microscopic examination of the male reproductive organs revealed that as animal grows there is a progressive increase in the number of layers of spermatogonial cells and the diameter of tubule also increases. The body weight also doubled along with testis weight, during September, October. After this period, the diameter of the seminiferous tubules increases, thus reducing the intertubular area containing groups of interstitial Leydig cells. Each tubule is lined by 2 to 3 layers of cells consisting of peripheral spermatogonia followed by spermatocyte from July onwards the activities in the testis are increased leading to the production of spermatozoa. Body weight is increased during September and October. During this period vigorous spermatogenesis is observed in the testis.

From November onwards spermatogenesis in testis ceases and rapid decrease in testicular size and weight is also noticed. The exhausted tubules start shrinking as the tubular lamina is cleared off gametic stages and residual cytoplasmic material. However some partly collapsed seminiferous tubules with few spermatozoa and debris in the tubular lamina are observed during November onwards. After this period testis are regressed and remain sexually quiescence.

The number of Leydig cells, the diameter of nuclei and appearance of cytoplasm varies during the quiescence breeding cycle.

During the non-breeding season when circulating levels of FSH, LH and testosterone are low, Sertoli cells of spermatogenically inactive testis of bat, do not contain conspicuous stacks of granular reticulum, suggesting reduced protein secretory function, when circulating levels of hormones are very high and testis showing vigorous spermatogenesis, the Sertoli cells show well developed granular and agranular reticulum, large number of mitochondria, suggesting active production of proteins and steroids.

Ultrastructure features of Leydig cells suggest that there is a maximum development of these cells in relation to steroidogenic capability during spermatogenic period, when there is a hypertrophy of accessory sex glands, whereas involution of these cells occurs, when testis is aspermatogenic and accessory glands are hyposecretory.

Thus, seasonally monoestrous non-hibernating Pteropodidae bat shows complete synchrony in spermatogenic and endocrine function of testis.

The pituitary gland plays a major role in regulating the sexual cycle in bats. The weight of pituitary gland is highest when testis shows vigorous spermatogenesis and is lowest when testis is spermatogenically inactive.

The serum PRL, LH and FSH levels are high in sexually active males whereas low levels are observed in sexually inactive males. The large number of prolactin cells, high levels of plasma prolactin and testosterone are responsible for enhancement of spermatogenesis and growth and activity of the accessory sex organs of Pteropodidae bat.

STH cells of male bat are associated with high rate of secretory activity. TSH cells are actively participated in secretory activity and exhibit a high rate of oxidative metabolism. Ultrastructural characteristics of ACTH cells during the sexually active period suggest that these cells regulate the synthesis and release of corticoids and androgens from the adrenal cortical cells as judged from the ultrastructural studies of adrenal cortex which are involved in reproductive processes of male *Pteropus giganteus giganteus* bat.

The large number of gonadotrophs, increased secretory activity of gonadotrophs, high levels of FSH and LH and Testosterone are observed during the active breeding period. Thus in *Pteropus giganteus giganteus*, male exhibits complete synchrony of spermatogenesis, testicular steroidogenesis, mating behaviour and accessory gland functions under the stimulation of gonadotrophs.

Males of *Pteropus giganteus giganteus* exhibit a well defined seasonal cycle of plasma T3, T4 and TSH concentration. The hormonal levels are high during the peak of spermatogenesis and are low during the aspermatogenic period. The weight of the thyroid gland is highest when spermatogenesis is at peak in the testis and is lowest when testis is regressed.

The ultrastructural features of follicular cells suggest the low rate of thyroid activity during aspermatogenic period and high metabolic activity during spermatogenesis.

The ultrastructural characteristics of parafollicular cells of thyroid suggest that these cells are the sites of synthesis and storage of serotonin and calcitonin. Thus, homeostasis would be maintained through fine regulation of the balance between blood and bone calcium. The thyroid gland has long been recognized as an important modulator of reproductive function and both hyper and hypothyroidisms are associated with reproductive dysfunctions and infertility. The metabolically active follicular cells and high levels of plasma, T3, T4 and TSH in

spermatogenically active bats suggest that thyroid hormones acting directly or by metabolic effects can influence reproductive processes in male bat.

The left Adrenal gland of male bat is always larger in size, weight and vasculature than the right. Adrenal of the sexually active bat shows significant differences of histoarchitecture over the sexually quiescent bat adrenal. Ultrastructural characteristics of cells of adrenal cortex suggest that these cells are participated in steroid biosynthesis and interconversion as well as synthesis of proteins.

The primary function of the adrenal gland is to protect the organism against acute and chronic stress. Stress as well as hyper and hypofunction of the adrenal gland is known to suppress reproduction.

The ultrastructural studies of adrenal gland suggest that there are both seasonal and stress related changes in adrenocortical and medullary cells of *Pteropus giganteus giganteus*. The well developed zona glomerulosa and fasciculata and enlarged medulla confirm an active participation of adrenal gland during reproductive processes of this bat.

The medullary cells of *Pteropus giganteus giganteus* during the sexually quiescence breeding phases are loaded with epinephrine and norepinephrine secretory granules. These catecholamines of medulla of this bat may mobilize glucose, fatty acids for energy production and prepare that heart, lung and muscles of bat for action against stress.

The above reports make this *Pteropus giganteus giganteus* bat an interesting model for further studies to find physiological role of endocrine gland on reproductive timing. This bat can be used as a model for study the complex relations of Leydig cells and male accessory sex glands which are normally synchronized.