Chapter 5

Summary And Conclusion
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The seasonal occurrence of breeding activity is the characteristic feature of nearly all the animals of temperate zone and majority of the vertebrates of the tropical zone. Reptiles are not exceptions to this.

Many detailed reports on the reproductive cycles of reptilian species belonging to the temperate zone have been published. A few tropical species such as *Leioposma rhombozalis*, *Javaneri* house gecko, *Cosymbotus platyrus*, *Hemidactylus frenatus* and *Paropus mutilatus* (Church, 1962) have been paid to the study of the possible factors controlling their cyclic rhythms. Bakers (1929) pointed out that the proximate causes of breeding seasons are of two types (1) external factors such as temperature, light and rain fall etc. and (2) some from internal rhythm.

Some earlier workers have carried out experiments to evaluate the effect of environmental temperature and photoperiodism on the reproductive functions in reptiles. However, the results of these workers are conflicting and the available literature is replete with controversial statement. It is, therefore desirable to study this problem in the greater details. The animal chosen for such a study is a common garden lizard, *Calotes versicolor* that is widely distributed in India. It is investigated that the following investigations may further add to our knowledge of reptilian reproduction in general. The following aspects of the problem have been studied:

1) The reproductive cycle of male *Calotes versicolor* in relation to biological aspects.
2) The reproductive cycle of male *Calotes versicolor* in relation to the environmental parameters.
3) The effect of some hormones like testosterone propionate and compound ICI 33,826.
4) The relation of reproductive cycle of male *Calotes versicolor* and Endocrine glands like pituitary, thyroid and adrenal.
5) The relation of reproductive cycle and biochemical changes in metabolism.

1) The reproductive cycle of male *Calotes versicolor* in relation to biological aspects:

The reproductive cycle of the male *Calotes versicolor* it was shown that there is relation of reproductive cycle with the seasonal variation there. On the basis of histological observation, the test of the lizard, *Calotes versicolor* undergo a definite seasonal cycle. The breeding takes place in summer (From the month of May to August) followed by a rapid regression of the testes and accessory sex organs. There is a definite arrest in the process of spermatogenesis till the following breeding season. The testes undergo rapid development during the month of April and attain complete sexual readiness at the beginning of May.

On the basis of the histochemical observations, seasonal variations are observed in the total, neutral and cholesterol positive lipids of the testes. Large quantities of lipids are stored in the interstitial cells during the non-breeding period. The amount of cholesterol positive lipids increases in the interstitial cells gradually prior to the onset of the breeding season. However, there sudden depletion immediately prior to the onset of the breeding season (April) and there complete disappearance during the
breeding period strongly indicates their utilization in the production of androgens. The simultaneous increase in the epithelial cell height of the ductus epididymis during the same period gives further support to this suggestion.

The testes of male lizard, *Calotes versicolor* show the changes occurring to the change in seasonal condition. The breeding and non-breeding cycle is occurs within one year in one time it may be from Month of May to the Month of October. Like other reptiles it also shows variation in the population of male. In breeding seasons of male the population of male increase but at the time of egg laying condition the female population increases. The size of the animals changes according to the maturity of the animals and at some stage of life it become mature. This phenomenon is used for calculation and relation to show the size and sex dependent maturity. The gonads changes their weight according to the breeding and non-breeding seasons and showing gonado-somatic index and they may be regressed or depressed.

Although it has been reported that the lizard, *Calotes versicolor* is a seasonal breeder (Asana, 1931); no attempt has been made so far to investigate the cyclic changes occurring in the gonads during the period of one complete year. In the present work, histological and histochemical observations on the male gonads have been carried over a period of one years. These observations are further supported by taking into account the weight of the gonads, diameter of seminiferous tubules and the number of cells per tubule. The seasonal changes in the total, neutral and the cholesterol-positive lipids have been noted histochemically.
2) **The reproductive cycle of male Calotes versicolor in relation to the environmental parameters**:

From the meteorological data on temperature, daylight, humidity and rainfall at Amravati, it appears that there is a correlation between these environmental factors and the testicular cycle. Temperature and daylight appear to operate conjointly in bringing the animals in sexual readiness.

An experimental attempt to evaluate the relative importance of daylight and temperature in the initiation of testicular recrudescence has resulted in the following conclusions: Sexually inactive animals were subjected to a gradual increase in the day length with the help of a day light fluorescent lamp for a period of 45 days. The gonadal histology of the lizard is studied after such a photoperiodic treatment. The additional seven hours of daylight during the period of sexual quiescence does not have any stimulatory effect on the testicular development even after a prolonged treatment of 45 days.

The elevation of the environmental temperature has a definite stimulatory effect on the testes. The maintenance of lizards at a temperature $39\pm1^\circ$C brings about complete spermatogenesis in the sexually quiescent animals in 30 days.

Since the stimulatory effect of the temperature is manifested in the absence of light, it is concluded that the temperature has a major role in the initiation of the sexual cycle of the lizard *Calotes versicolor* under study.

3) **The effect of hormones like testosteron propionate and compound like ICI 33,828**:

The effects of the rise in environmental temperature and the exogenous administration of testosterone propionate on thyroid
function has been studied. It is observed that environmental temperature does not have any stimulatory effect on the thyroid gland of *Calotes versicolor*. On the other hand, the injected androgen causes distinct stimulation of the gland.

In order to assess the role of hypophysis in reproduction surgical ablation of the gland is usually performed. Such an operation in *Calotes versicolor* was not successful as there was very high mortality in the operated animals. Recent literature on ICI compound 33,828 shows that it acts as an inhibitor of gonadal function. The changes in the testes and accessory sex organs have been recorded after the treatment of ICI compound 33,328 (a known inhibitor of pituitary gonadotrophs). Two different doses of the compound are used (200 ug. and 800 ug./daily). The treatment has resulted in significant regressive changes in the testes, blocking of the spermatogenic process and diminution in the amount of interstitial cell tissue. With a larger dose (800 ug.) of the compound the inhibitory effect on the spermatogenesis is more pronounced. The selective damage of the testicular function, by the compound administered, has given experimental confirmation of a direct relationship between the gonads and the pituitary. Therefore, the compound was administered in *Calotes versicolor* and its effects on the gonadal functions are studied.

4) **The relation of reproductive cycle of male calotes versicolor and Endocrine glands like pituitary, thyroid and adrenal:**

In the lizard, *Calotes versicolor* a period of sexual inactivity is known and during this period the gonads show regression to a great extent. The sexually quiescent animals were treated with bovine FSH for
different periods. The gonads responded to the mammalian gonadotrophins by showing hypertrophy and an increased rate of mitotic division. However, the stages related to reduction division were not observed. Moreover, in spite of a prolonged period of treatment extending over 30 days, sperm production was not observed.

A few sexually inactive animals were also treated with crude pituitary extract, prepared from acetone dried, deep freeze stored pituitaries taken out from sexually active animals. The results obtained were similar to those following FSH treatment. Pituitaries isolated from the sexually inactive animals when used for injections were ineffective, the results indicated that the pituitaries of the sexually active animals contained higher titers of gonadotrophins than those of the sexually quiescent animals.

The role of steroid hormones in reptilian reproduction is debatable. Forbes (1941) and Risley (1941) showed that apart from affecting the secondary sex organs the steroid hormone acted on germinal elements of the testes. In contrast to this observation, Ram swami and Jacob (1963) found no effect on the germinal elements. In view of this conflicting data sexually quiescent animals were treated with testosterone propionate and its effects on the germinal elements of testes.

On the basis of size, shape and tinctorial affinities, six cell types are identified in the adenohypophysis of Calotes versicolor. These are three types of basophiles, two types of acidophils and chromophobes. The cytological observations during one complete year reveal seasonal changes in the constituent cell types of the anterior lobe of the pituitary. On
the basis of similarities noticed in the morphology and the tinctorial affinities of the purple basophile (type 2 basophile) with those of the corresponding cell types described by earlier workers. The pituitary of *Calotes versicolor* has been studied in order to record seasonal changes in the number and staining ability of different cell type in it. Furthermore, by resorting to surgical operations such as thyroidectomy and consideration the alterations in their cytology.

It is widely held that the environment has an influence on the functions of various endocrine glands through the intermediation of hypothalamus in the brain. There are groups of specialized nerve cells (neurosecretory cells) in the hypothalamic region of the brain of vertebrates. The neurosecretory product is carried to the pituitary through the portal circulation. The cyclic changes have been observed in the amount of stainable neurosecretory material in the neurosecretory cells of both supraoptic and Para ventricular nuclei, in the axons of the hypothalamohypophysial tract and the median eminence. Prior to the breeding season there is an increase in the amount of NSS in the cytoplasm of the neurosecretory cells, in the axons of the hypothalamohypophysial tract and the median eminence. This neurosecretory material is gradually depleted from these sites during the breeding season. Thus, the accumulation of NSS in the neurosecretory cell and the median eminence during the non-breeding period and its depletion during the testicular growth and the period and of active spermatogenesis give circumstantial evidence in support of the participation of the hypothalamic neurosecretory system in the control of the gonadotrophic function of the
pituitary. Histological observations on the changes in the neurosecretory cells in the hypothalamus of the brain have been seen.

The cyclic changes in the histology of the thyroid have been correlated with various functions in different vertebrate classes. In fishes the thyroid activity has been associated with seasons of rapid growth, sexual development. Changes in the environmental temperature. In amphibian, it is related to metamorphosis. In birds thyroid plays significant role in migration and reproduction. However, Knowledge regarding the role of thyroid in reptiles is scarce and the earlier reports are conflictingly. Some workers have related increased thyroid activity to reproduction, while others have observed that higher temperature causes thyroid hypertrophy. Histological studies on the thyroid reveal seasonal changes. The increased thyroid activity is observed during the period ranging from April to August. However, the maximum activity is recorded in the month of June. From July onwards there is a progressive fall in the activity of the thyroid, as judged by the reduction in the cell height and increase in the follicular diameter, till a minimum thyroidal activity is reached in December. On comparing the trends in activity of the thyroid and the testes it is seen that the maximum activity of the thyroid gland coincides with the period of maximal spermatogenic activity of the testes. This is period of maximal spermatogenic activity of the testes. This parallelism between the activities of the thyroid and the testes affords persuasive evidence, in support of an existence of a direct relationship between the two glands.

It is observed that environmental temperature does not have any stimulatory effect on the
thyroid gland of *Calotes versicolor*. On the other hand, the injected androgen causes distinct stimulation of the gland.

The findings are based on the quantitative measurements of the follicular epithelial cell height follicular diameter and the number of cells in each follicle during one complete year. From these observations it was seen that there is a parallelism between the activities of the thyroid and gonads. The higher gonadal activity is accompanied by an increased thyroid activity. However, it is likely that the greater production of the steroid hormone by the gonads might have a stimulatory effect on the thyroid. Such a stimulatory effect of the steroid hormones on the thyroid function was observed by earlier workers (Evans and Hegre, 1938; Kumarson and Turner, 1967). It is, therefore, desirable to assess the effects of test steroids on the functional activity of the thyroid.

The adrenal gland of calotes will play a roll in the reproductive cycle but there are a seasonal change that shows the adreno-somatic index and relation of adrenal gland.

5) **The relation of reproductive cycle and biochemical changes in metabolism of lizard, *Calotes versicolor***:

The relation of Carbohydrate metabolism and reproductive cycle has been studied. The changes in seasonal condition affect on the metabolism. The breeding and non-breeding season also affect on the metabolism of carbohydrate. The increase in metabolism and deposition is up to maturation of egg or sperm is seen but at the time of breeding the percentage of carbohydrate goes down. This shows that the body as a
source of stored energy utilizes carbohydrate.

The relation of Fat metabolism and reproduction is shown in this work. The body content of the fat increases during ripening of gonads and in non-breeding season. There also the seasonal variation occurs in fat percentage of the body. The fat appear to be one of the most important energy sources of metabolism. During breeding season it lowers down the percentage of fat in the body and it become lowest at the time of breeding in male. Testes also uses the fat and lipid cycle is also seen in the *Calotes versicolor*.

Protein levels in *Calotes versicolor* show the relation with reproductive activity. High protein values prior to the breeding peak suggest that it might be concern by the gonad as an important source of metabolite & show decline following breeding peak may be because of the loss of gametes. It start increasing as gametes start maturing, the highest values are seen at time of full maturation of gametes and decrease at the time breeding.