

Interactive seasonal changes in the testis and thymus of the lizard *Calotes versicolor* Daudin

R. Lal,¹ B.K. Nirmal¹ and Ajit K. Saxena²

¹Department of Zoology, Harish Chandra PG College

²Centre of Experimental Medicine and Surgery, Institute of Medical Sciences
Banaras Hindu University, Varanasi 221005, India

SUMMARY

The development and secretory activity of testis of lizards is modulated by environmental factors, especially temperature, and it follows a seasonal pattern. Testosterone, secreted by the testis, modulates the functioning of the thymus which governs the immunity of animals. Thymosin is synthesized and secreted by the thymus and governs the development of immunity. At the time when the thymus is hypoactive the other lymphoid tissues take care of the maintenance of immunity. In the present study, the testis and thymus of *Calotes versicolor* were studied during the different months of the breeding as well as non-breeding phases, and the specific variations were observed. The recrudescence of testis begins in the month of March and attains the peak in June, which coincides with the beginning of breeding phase. During this period, significant increase in the weight of testis was found. The fully developed testis is maintained during July to September. During the breeding phase the thymus was regressed and had shrunken thymocytes indicating poor secretion of thymosin. From October onwards, the weight of the testis decreased, indicating regression. During the non-breeding phase, from November to February, the testis was fully regressed, when testis was devoid of spermatozoa. During this phase the thymus was well-developed, and the thymocytes were enlarged indicating substantial activity.

Key words: *Calotes versicolor*, testis, thymosin, thymus, seasonal variation

Introduction

The study of interaction between endocrine glands and environment in relation to vertebrate reproduction and immunity has been subject of intensive investigation but several issues here still remain unattended to. Seasonal changes in aspects of reproduction and plasma steroid levels are well documented in several species of lizards (Arslan et al., 1978; Bona-Gallo et al., 1980; Ando et al., 1991; Amey and Whittier, 2000; Radder et al., 2001). The seasonal changes in size and histology of testis, accessory reproductive organs and thymus are well known in many reptiles (Callard et al., 1976; Singh and Kar, 1888). The seasonal pattern of reproduction of reptiles is greatly determined by the interactive relationship between hormones. Monthly changes in androgen level in peripheral plasma and in testis of *Uromastix hardwickii* have been observed and the annual androgen rhythm is correlated with changes in testicular weight and spermatogenic cycle in this lizard (Arslan et al., 1978).

The lizard *Calotes versicolor*, widely distributed in India, is a seasonal breeder (Shanbhag and Prasad, 1993). The males of this species are spermatogenically active during April to September (Gouder and Nadkarni,

1979). Gravid lizards are encountered during May-October (Shanbhag and Prasad, 1993; Shanbhag et al., 2000). According to Al Sadoon et al. (1990), cold-acclimated gonadectomized male and female *Chalcides ocellatus* show significant increase in whole body rate of oxygen consumption, which results in the development of the other organs due to increase in testosterone and estradiol. It has been reported by Munoz et al. (2005) that the reptilian immune system is strikingly affected by seasonal variations, which induce changes in the structure of the lymphoid organs and the function of leucocytes. The effect of sex hormones on the structure and function of thymus in reptiles has been poorly studied. The present study was therefore undertaken to determine the pattern of changes in the structure and function of testis in relation to thymus during the breeding and non-breeding phases in *Calotes versicolor*. Histomorphology of thymus of some species of Squamata was studied by Singh and Kar (1988).

Materials and Methods

Indian garden lizard *Calotes versicolor* is found through out the year at Varanasi (Latitude, 25° 18' N; Longitude 83° 01' E). The thymus and testis of this lizard were studied during breeding as well as non-breeding phases.

Adult male *C. versicolor* (mean body weight 23.56 ± 67.2 (?) g; snout-vent length 8.67 ± 0.48 cm) were collected locally during the second week of every month and maintained in wire-netted wooden cages and acclimated to natural day length, temperature and other environmental factors. The lizards were fed with what ? *ad libitum*. After 5 days of laboratory acclimation the lizards were anesthetized to remove the testicles and thymus lobes. The testicles were kept in 7% saline and weighed and also the length and diameters were measured. After measurement, testicles and thymus lobes were fixed in Bouin's fluid, embedded in paraffin wax and sectioned serially at $5\mu\text{m}$ thickness. The sections were stained with hematoxylin and eosin. Samples were collected each month of the year to study the interactive development of testis and thymus.

Results

The body weight and testis weight of *Calotes versicolor* during the one year period of study are presented in the table 1. The weight of the testis began to increase significantly in March and reached the peak in June, which coincided with the onset of breeding phase. The weight was maintained closer to this level throughout the breeding phase, which extends from July to September, when the seminiferous tubules were well developed and produced spermatozoa. The interstitial cells were prominent. From October onwards the weight of the testis decreased (Fig. 1a, 2a)



Fig. 1a. Inactive testis during non-breeding phase showing regressed seminiferous tubules. x400.

The appearance of the thymus also differed between breeding and non-breeding phases. The bi-lobed thymus increased in size from October onwards, which continued up to March. The epithelial cells in the cortex were well developed and provided for great many thymocytes during December and January. During this period, the corpuscles

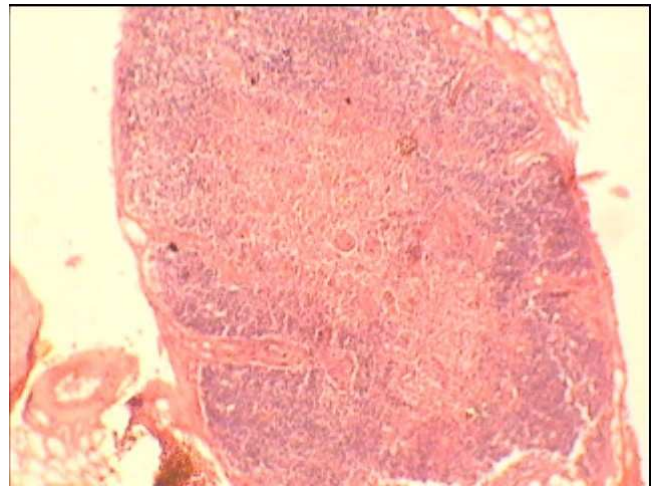


Fig 1b. x100. Well-developed thymus during non-breeding phase. x100.



Fig 2 a. Active testis with well-developed seminiferous tubules and other tissues during breeding phase. x400

of Hassall in the medullary lobules were distinct. The lymphocytes were densely packed in the inner medulla. The thymus regressed during April to September and the cells were cord-like during peak of the breeding phase. From October onwards the thymus increased in size and remained well-developed up to February (Fig. 1b, 2b). The histological observations were thus indicative of an opposing trend between testis and thymus.

Discussion

The thymic functions, androgen level and testicular activities as studied in the present investigation were observed in other species of lizards. Some reports are also available, which discuss the thymic regulation of testicular development but period-specific variations were investigated for the first time in this study. The annual



Fig. 2b. Regressed and inactive thymus during breeding phase. x100.

androgen rhythm in the spiny-tailed lizard was studied by Arslan et al. (1978). According to Bourne et al. (1986), an annual cycle of plasma and testicular androgens exists in the lizard *Tiliqua rogosa*. According to Lofts et al. (1966), there is a seasonal pattern in the testis of cobra *Naja naja*. The present data indicate that in *Calotes versicolor* the plasma testosterone level begins to rise in March and reaches the highest in June. This rise in plasma testosterone concentration coincides with enhanced testicular weight, active spermatogenesis and appearance of mature spermatozoa in the testis. Arslan et al. (1978) observed a similar annual androgen rhythm, which affects the testicular weight and spermatogenic cycle. According to Callard et al. (1976), peripheral testosterone level influences the development of interstitial cells and the seminiferous epithelium.

The sharp decrease in testicular weight and the regression of seminiferous tubules during the month of October can be correlated with the decrease in plasma as well as testicular androgen levels. The low level of testosterone evidences reduced activities of the steroidogenic tissue that secretes the androgen, which is in turn influenced by the levels of FSH and LH. The effect FSH and LH on testis during non-breeding phase in *Calotes versicolor*, based on histological and biochemical

studies, was investigated by Vijaykumar et al. (2002). The pattern of variation within populations of some species depends on geographic distribution, which influences reproductive cycle, reproductive physiology and life history in reptiles. An overview of influence of geographic variation on life history traits of *Calotes versicolor* was presented by Radder (2006). According to Al-Sadoon et al. (1990) the gonadal development in reptiles is regulated by seasonal variations, an inference based on a study in cold-acclimated gonadectomized male and female *Chalcides ocellatus* (Forsk.) The ethogram of courtship and mating behavior of *Calotes versicolor* provide evidence in support of variations in gonadal activities (Pandav et al., 2007). It was suggested by Amey and Whittier (2000) that seasonal pattern of plasma steroid hormones in male bearded dragon lizard *Pogona barbata* is responsible for the maximum spermatogenic activity in spring, followed by cessation of spermatogenesis after the breeding period and testicular recrudescence in late summer.

The immune system of reptiles also is affected by seasonal variations, as reflected in changes in the structure of the lymphoid organs and function of the leucocytes. The endocrinology of thymus is characterized by the influence of various hormones on the thymus, consisting of thymocytes, thymic epithelial cells and thymic stromal cells (Hadden, 1992). The thymic activities are regulated by the action of gonadal hormones such as androgens and estrogens. Nikolaevich et al. (1991) suggested that major reproductive hormones are regulators of cell-to-cell interaction in humoral immune responses. *Calotes versicolor* shows seasonal breeding as other reptiles and has controlled gonadal hormone synthesis and secretion. Thymic growth and development of thymic epithelial cells and thymocytes are regulated by the androgens during breeding phase and non-breeding phase. In the present investigation the epithelial cells and thymocytes were found densely packed during the non-breeding phase, and sparse during the breeding phase. The thymic involution occurs when androgen is at peak during the breeding phase, as was suggested by Min et al. (2005).

Table 1. Body weight and testis weight of male *Calotes versicolor* during different months of a year.

Month	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Body wt (g)	20.34 ±2.01	15.99 ±1.98	16.71 ±2.81	28.16 ±2.46	26.67 ±6.58	26.46 ±4.66	32.80 ±3.43	32.55 ±3.63	33.38 ±6.40	18.79 ±2.97	16.76 ±3.15	14.12 ±2.42
Testis wt (g)	0.061 ±.006	0.006 ±.003	0.022 ±.007	0.062 ±.004	0.235 ±.067	0.318 ±.123	0.284 ±.076	0.143 ±.039	0.114 ±.006	0.073 ±.009	0.072 ±.003	0.062 ±.005

Thus, this study of seasonal variations in *Calotes versicolor* provides evidence for environmental influence on male reproduction and for an antagonistic relationship between testis and thymus.

References

- Al-Sadoon MK, El-Banna AA, Ibrahim MMM, Abdo NM, Al -Rasheid KA. (1990). Effect of gonadal steroid hormones on the metabolic rate of the cold-acclimated gonadectomized male and female *Chalcides ocellatus* (Forsk.) *Gen Comp Endocrinol.* **80**: 345-348.
- Amey AP, Whittier JM. (2000). Seasonal patterns of plasma steroid hormones in males and females of the bearded dragon lizard, *Pogona barbata*. *Gen Comp Endocrinol.* **117**: 335-342.
- Ando S, Ciarcia G, Panno ML, Imbrogno E, Tarantino G, Buffone M., Angelini BF, Botte V. (1992). Sex steroids levels in the plasma and testis during the reproductive cycle of the lizard *Podarcis s. sicula* Raf. *Gen Comp Endocrinol.* **85**: 1-7.
- Arsalan M., Lobo J, Zaidi AA, Jalali S, Qazi MH. (1978). Annual androgen rhythm in the spiny-tailed lizard, *Uromastix hardwickii*. *Gen Comp Endocrinol.* **36**: 16-22.
- Bona-Gallo A, Light P, Duncan MS, Lofts B. (1980). Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steroids and thyroid activity in the Chinese cobra (*Naja naja*). *Gen Comp Endocrinol.* **42**: 477-493
- Bourne AR, Taylor JL, Watson TG. (1986). Annual cycles of plasma and testicular androgens in the lizard *Tiliqua (Trachydosaurus) rugosa*. *Gen Comp Endocrinol.* **61**: 278-286.
- Callard IP, Callard GV, Lance V, Eccles S. (1976). Seasonal changes in testicular structure and function and the effects of gonadotropins in freshwater turtle. *Gen Comp Endocrinol.* **30**: 347-356.
- Francisco J, Monica De IA. (2001). The effect of the seasonal cycle on the splenic leukocyte functions in the turtle *Mauremys caspica* *Physiol Bbiochem Zool.* **74**: 660-667. Where cited?
- Gouder BYM, Nadkarni VB (1979) Histometric and histochemical changes in the seminiferous epithelium, Leydig cells and Sertoli cells in the testis of *Calotes versicolor*. *Biol Bull India* **1**: 15-22
- Hadden JW. (1992). Thymic endocrinology. *Int J Immunopharmacol.* **14**: 345-352.
- Lofts B, Phillips JG, Tom WH. (1966). Seasonal changes in testis of cobra, *Naja naja* (Linn.). *Gen Comp Endocrinol.* **6**: 466-475.
- Lofts B, Murton RK, Thearle RJP. (1973). The effect of testosterone propionate and gonadotropins on the bill pigmentation and testes of the house sparrow (*Passer domesticus*). *Gen Comp Endocrinol.* **21**: 202-209. Where cited?
- Min H, Montecino ER, Dorshkind K. (2005). Reassessing the role of the growth hormone and sex steroids in thymic involution. *Clin Immunol.* (Epub Ahead of Print). Not yet printed?
- Nikolaevich KN, Ivanovich S J, Victorovich SS. (1991). Major reproduction hormones as regulators of cell-to-cell interaction in humoral immune responses. *Brain Behav Immunol.* **5**: 149-161.
- Pandav BN, Shambhag BA, Saidpur SK (2007). Ethogram of courtship and mating behavior of garden lizard, *Calotes versicolor*. *Curr Sci.* **93**: 1164-1167.
- Radder RS. (2006). An overview of geographic variation in the life history traits of the tropical agamid lizard, *Calotes versicolor*. *Curr Sci.* **91**: 1354 – 1363.
- Radder RS, Shanbhag BA, Saidpur SK. (2001). Pattern of plasma sex steroid hormone levels during reproductive cycle of male and female tropical lizard, *Calotes versicolor*. *Gen Comp Endocrinol.* **124**: 285-292.
- Shanbhag BA, Prasad BSK. (1993) Follicular dynamics and germinal bed activity during the annual ovarian cycle of the lizard, *Calotes versicolor*. *J Morphol.* **216**: 1-7.
- Singh R, Kar I. (1988). Histomorphology of thymus in some species of Squamata. *Zool J Anat.* **117**: 245 – 253 .
- Vijaykumar B, Ramjaneyulu T, Sharanabasappa A, Patil SB. (2002). Effect of FSH and LH on testis during non-breeding season in *Calotes versicolor* (Daud.). *J. Environ Biol.* **1**: 43-46.