CASTRATION RELATED HISTOMORPHOLOGICAL CHANGES IN THYMUS OF MALE GARDEN LIZARD; CALOTES VERSICOLOR

[1] B. K. Nirmal, [2] R. Lal,[3] A. K. Saxena
[1,2] Department of Zoology, Harish Chandra P. G. College, Varanasi - 221 001, India.
[3]Department of Pathology and Lab Medicine ,All India Institute of Medical Sciences (AIIMS) Patna, India.

ABSTRACT

Environmental factors influence different glands during developmental as well as developed stages Thymus of Calotes versicolor shows the variations in different phases influenced by environmental and internal factors. In the present study, thymus of Calotes versicolor was taken in different reproductive phases to observe the developmental changes due to different doses of testosterone. These changes were observed in developed thymocytes and other histological tissues. During non-breeding phase thymus can be observed well developed which is involuted due to administration of different doses of testosterone, it was highly involuted with high dose. During breeding phase involuted thymus further detoriated due to administration of different doses of testosterone administration. In this phase thymocytes exhibits testosterone related morphological changes.

KEY WORDS: Thymus, Castration, Histology

INTRODUCTION

The thymus acts a vital organ for homeostatic maintenance of peripheral immune system. Several possible mechanisms for age related thymic involution has been suggested by Heather E Lynch et.al.,2009. The Lizard *Calotes versicolor* is a seasonal breeder , where males are spermatically active during April to September (Gouder and Nadkarni; 1979). Gravid lizards are encountered during May-October (Shambhag and Prashad, 1993; Shambhag et.al.,2000a). According to Al Sadoon M.K., El Banna A.A. et.al.,1990, a cold acclimated gonadectomized male and female *Calcideso cellatus* shows significant increase in whole body rate of oxygen consumptions which results in to the development of

the other organs of the animals due to increase in testosterone and estradiol activity. It has been reported by Munoz Francisco J.et.al., 2001, that the reptilian immune system is strikingly affected by seasonal variations, which induce changes in the structure of the lymphoid organs and in the function of the leucocytes. In spleen lymphoid organs are depend on thymus in *Calotes vesicolor* suggested by Pitchappam R.M., 1977. Munickasundari M.et.al; 1988 has developed relationship of derived cell of lizard in its structure and function. The structure and function of thymus of reptiles in response to Sex hormones are less studied. The present study was therefore undertaken to determine the pattern of changes in histology of thymus and its development during breeding and non-breeding phase in castrated garden lizard. The gonadal hormones are synthesized and secreted by gonads which are regulated by gonadotropic releasing hormone (GnRH) and sex steroids. The role of thymic peptides and it's transmittion between neuroendocrine and immune system has been studied by Dardenne M., 1999. We have taken a model animal *(Calotes versicolor)* for the study of androgenic effects on histomorphology of thymus under influence of gonads. Histomorphology of thymus was studied by Singh R. and Kar I., 1988 in some species of squamata. There are also some researchers who have observed its microscopic, ultra structural and seasonal variation in thymus (Saad and Zapata., 1992). According to Murrey F.W.et.al., 2000, progesterone inhibits glucocorticoid-inducedmarine thymocytes apoptosis and supports the histological development of thymus. Thymus also has effect of hormones in their immune system. The involvement of gonadotropin releasing hormone (GnRH) in differentiation of lymphocytes and regulation of immune response leads to lifelong (Zakharova L.A.2009).

The vertebrate immune system is a vastly complex network of circulating cells and molecules as well as tissues and organs. Thymus is an organ which shows all activities like other organs along with under control of GnRH via the hypothalmo-pituitary axis and is involved in autocrine or paracrine regulation of the immune response during some phases of life (Nicholas R.S. et.al., 1992). Castration leads in the increased number of pre-B cells in the bone marrow and mature B cell in peripheral organs with spleen growing in size (Olsen et. al., 1996).Many researchers had suggested that the development of GnRh neurons and hypothalmopituitary-gonadal system as a whole is apparently subject to dramatic changes upon activation of immune system (Summer C.H., 1995 and Sutherland J.S.et.al., 2005). Immune functions in vertebrates especially in reptilians are well studied by Zimmerman L.M.et. al.,2010 with different aspects of immune system. More review has been developed on reproductive immune interaction by Bram Luttonet. al., 2006.

MATERIAL AND METHODS

To observe the castration related histological changes in thymus , adult male *Calotes versicolor* (Average wt 23.56±7.2 gm; SVL 8.67± 0.48 cm) were procured during non-breeding phase in February month and breeding phase in August month and maintained in wire netted wooden cages and acclimated to natural day length , temperature and other environmental factors . Live cockroaches, grasshoppers, crickets, and maggots were provided a mixed diet to the lizard every day along with water ad libitum. A group of lizards were kept as control and their testes were intact. During breeding phase other groups of Lizards were anesthetized by ethyl ether and kept ventrally on an operating board, minor incision made at both side of abdomen to remove the testes with the help of a pair of fine forceps and scissor and same was repeated during non-breeding phase. After sprinkling streptomycin powder over the exposed area, wound closed by medicated bandages. Lizards were kept for 15 days in breeding as well as non-breeding phase. After aforesaid period thymus was removed by scarifying the lizards and fixed in Bouins solution.

The histological slides of tissues (thymus) were prepared by washing, dehydration, embedding in paraffin and sectioned serially at 5µ thickness. The sectioned materials were stained with haematoxylin and eosin. Slides were observed in **Dewinter Image Microscope** at different magnification to find the castration related histological changes in thymus during breeding and nonbreeding phase.

RESULTS

Present experiment revealed significant castration related changes in thymus.These changes observed in morphological as well as cellular level of thymus. Testes were removed to observe the effect of castration on thymus. The gonadal hormones influence the thymus during breeding and non-breeding phase. Histological slides were observed and great variations were noticed. During breeding months (March-August) involuted thymus showed its inactive nature as well as histologically poorly developed. Thymus was packed with connective tissue with indistinct cortex and medulla.

In breeding phase ,**control** group of lizard showed atrophied thymus having less distinct corticomedullary differentiations ,where significantly reduced number of thymocytes, lymphocytes, macrophages and lack of Hassall's corpuscles observed (Fig.1a,b).In the **experimental group,** after removal of testes, in breeding phase castration related changes in thymus were observed and showed

the resemblance with non-breeding of the thymus as shown in fig.2a, b. A well marked distinction between cortex & medulla, enhanced amount of reticular cells and thymocytes were observed. Interestingly, distinct corticomedullary regions with increased number of thymocytes, phagocytes and Hassall`s corpuscles were observed as shown in Fig.3a, b. Large number of macrophases, thymocytes and Hassall`s corpuscles were observed in cortex and medulla regions (Fig.4a, b). During non-breeding phase the increased structural and functional an activity of thymus was noticed which paralleled with the diminished size of testis. The large number of lymphocytes, macrophages, thymocytes and presence of Hassall`s corpuscles were observed with clear differentiation between cortex and medulla as in **control group** of lizards (Fig.5a, b). After removal of testes, in non-breeding phase no marked changes could be observed and activity of thymus remained same as control group of lizards (without castration) as shown in fig.6a to 8b.The present study has confirmed the role of steroidogenesis after castration of testis on thymus against significant cellular changes in development of thymus in *Calotes versicolor*.

CASTRATION RELATED HISTOLOGICAL CHANGES IN THYMUS DURING BREEDING PHASE

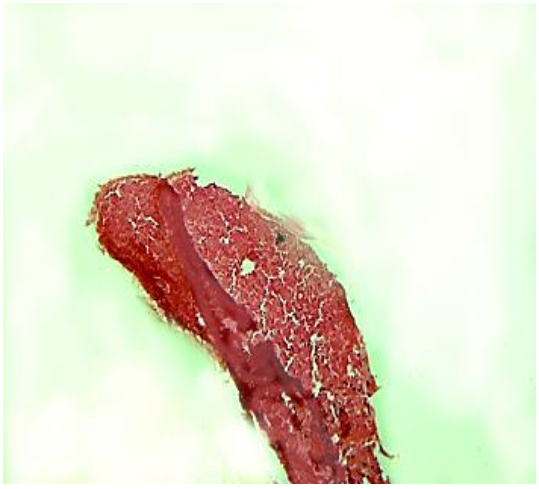


Fig.1a; Thymus (control) 100X, showing involuted thymus.

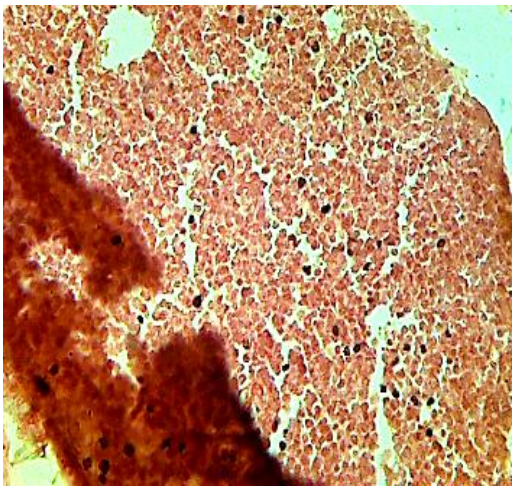


Fig.1b; Thymus (control) Enlarge view (400X) showing less number of thymocytes.

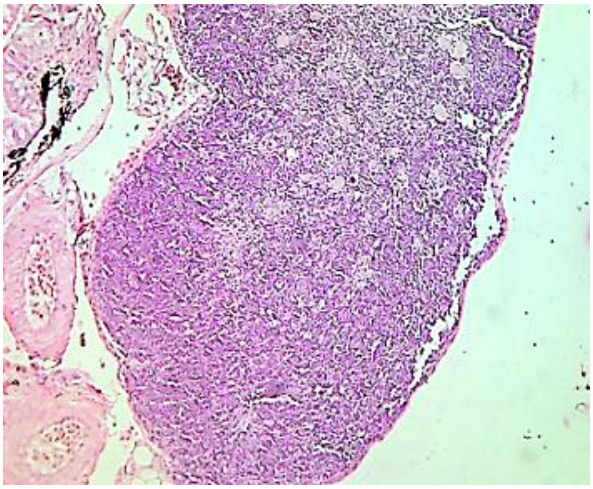


Fig 2a; 100X, showing distinct corticomedullary region

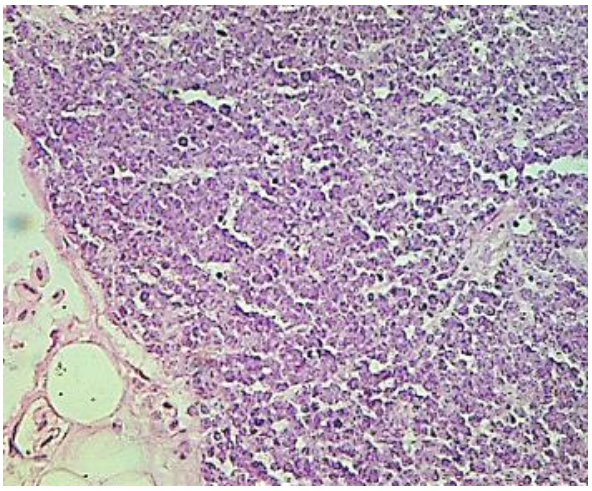


Fig.2b; Thymus 400X, showing thymocytes and reticular cells

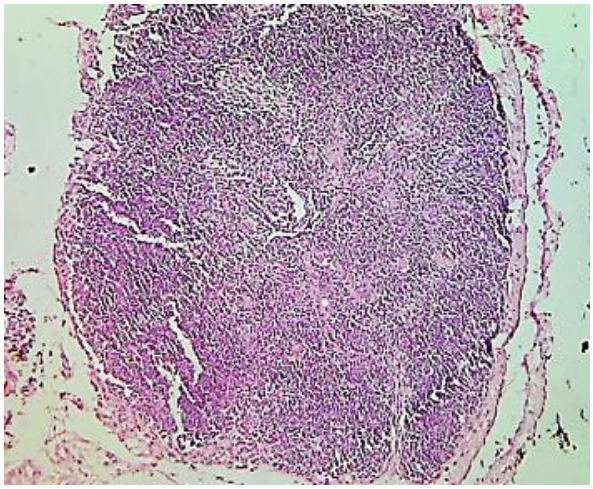


Fig.3 a; Thymus (100X) showing distinct corticomedullary region.

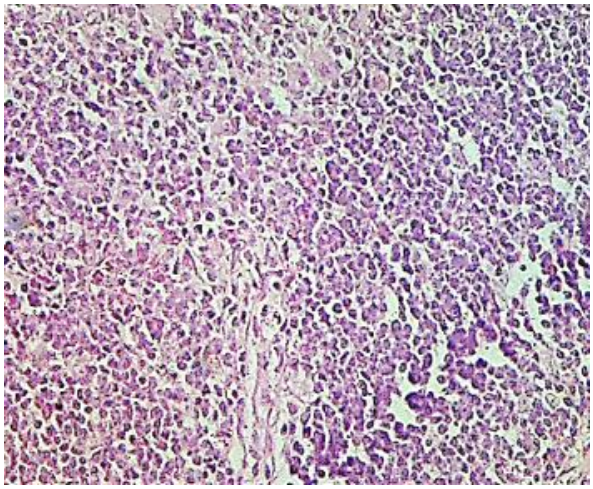


Fig.3b; Thymus, Enlarged view (400X) showing all cells are well developed and distinct.

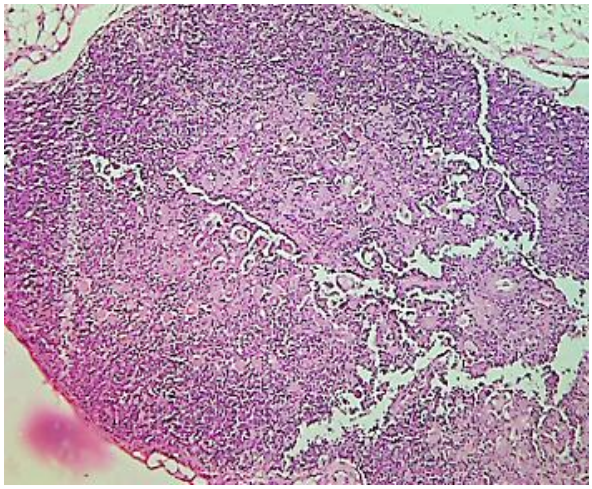


Fig.4a; Thymus (100X) showing distinct cortex and medulla.

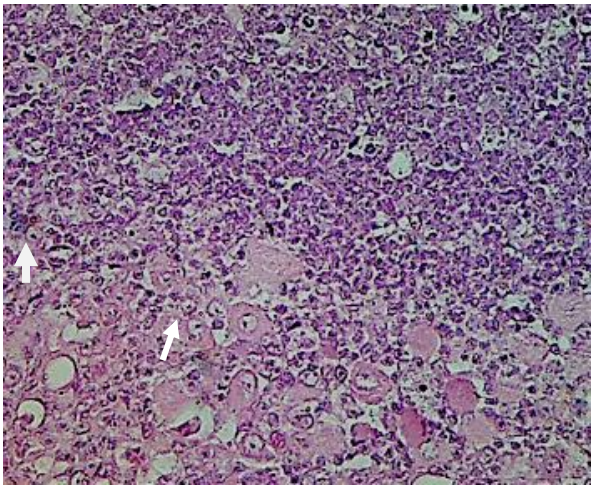


Fig.4b; Thymus, Enlarged view (400X) showing large No. of thymocytes, macrophages and reticular cells .

CASTRATION RELATED HISTOLOGICAL CHANGES IN THYMUS DURING NON-BREEDING PHASE

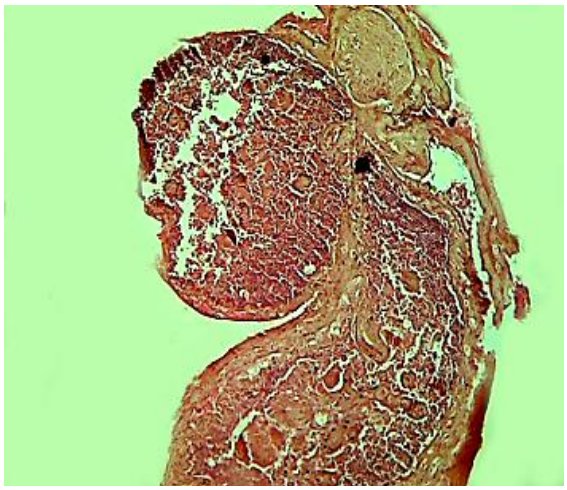


Fig.5a; Thymus (control) 100X showing slightly distinct cortex and medulla.

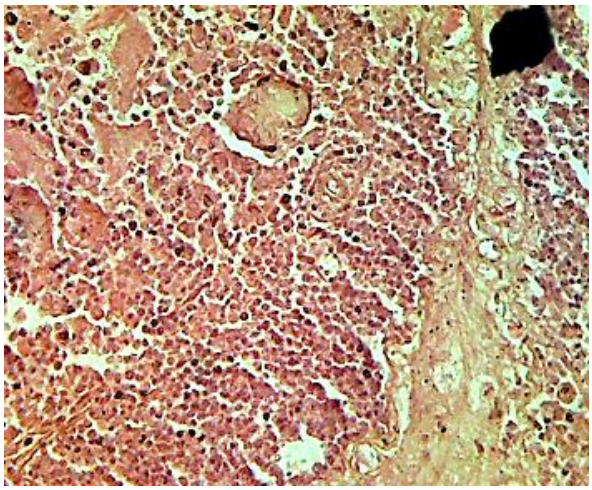


Fig.5b; Thymus (control) Enlarged view (400X) showing reticular cells and thymocytes.

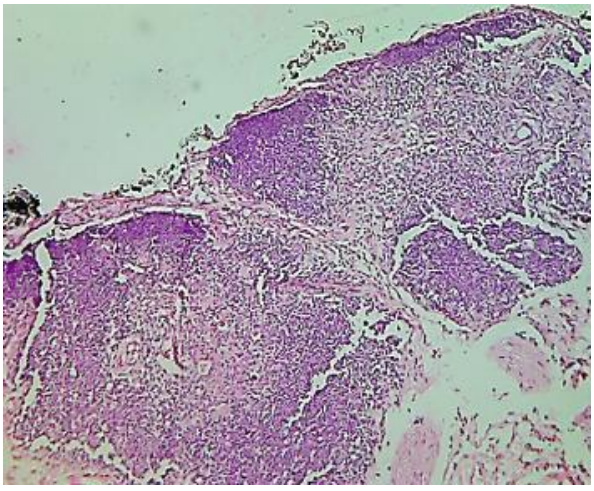


Fig.6a; Thymus (100X) showing cortex & medulla .

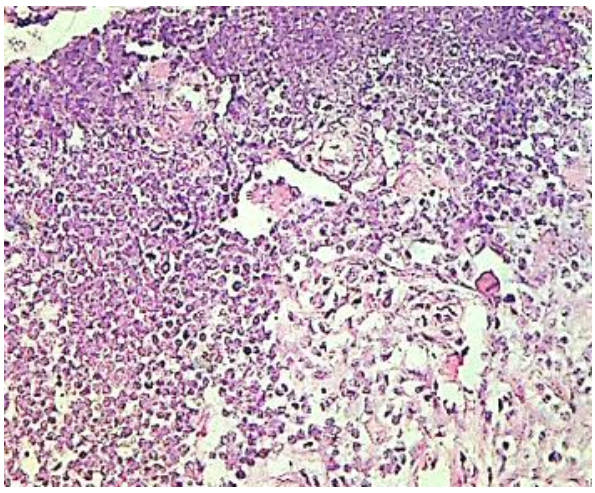
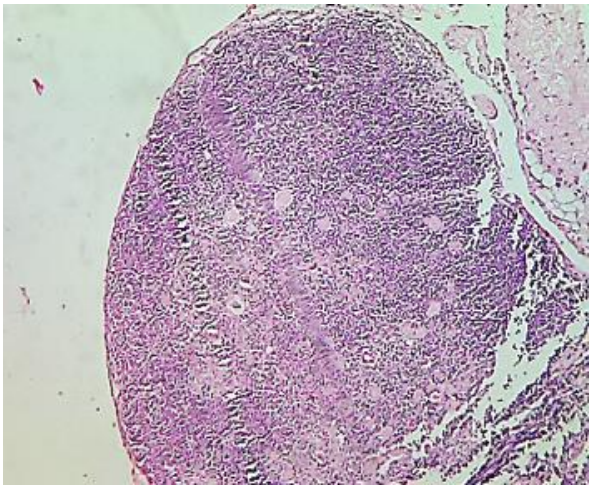
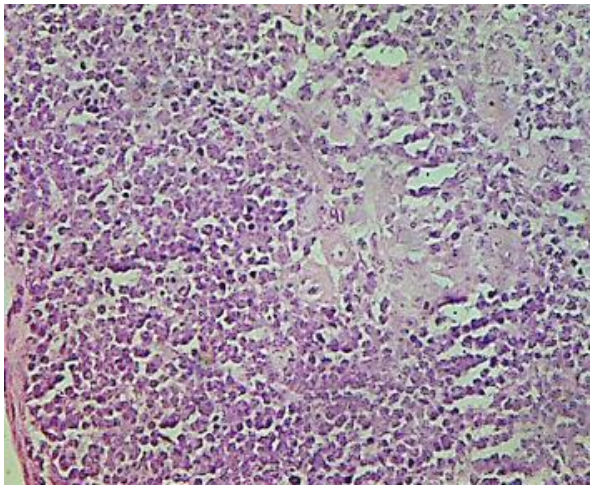
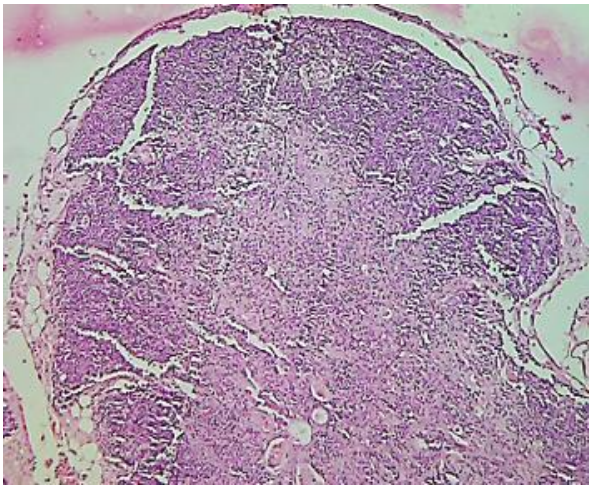


Fig.6b; Thymus, Enlarged view (400X) showing reticular cells andthymocytes.







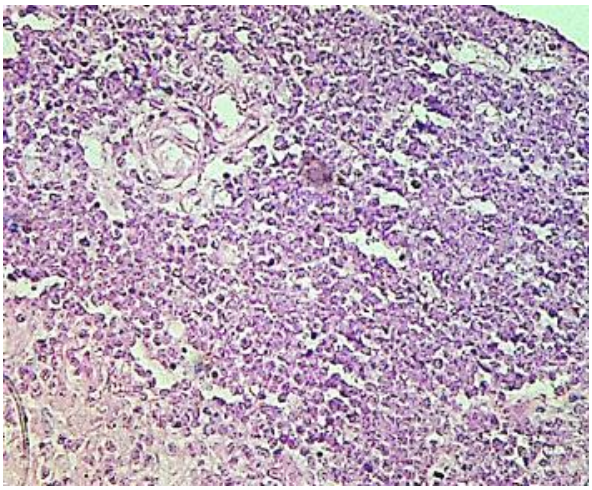


Fig.7a; Thymus (100X) showing distinct cortex and medulla .

Fig.7b; Thymus, Enlarge view (400X) showing Thymocytes, reticular cells and macrophages.

Fig.8a; Thymus (100X) showing distinct corticomedullary region.

Fig.8b; Thymus, Enlarged view (400X) showing developed thymus with different cells.

DISCUSSION

Sex hormone regulates the development and functioning of thymus through hypothalamo-pituitary and immune system. The normal histological variability of thymus also gets exposed to dietary supplements, under the influence of hormone secretion from endocrine glands.

The knowledge regarding thymic activities has been elaborated the effect of hormones which was synthesized and secreted from gonads .These hormones were influenced the activities of different organs via sex steroid and immunomediators. Sex steroids have ability to regulate the development and functioning of thymus as aging caused drastic architectural changes in thymus as reported by Heather E. Lynch et.al., 2009. The involuted condition of thymus in lizards which takes place during breeding phase has also reported by Rao M.A., 1954. Thymus involution takes place, when sex steroid comes in action during the breeding phase with development of the testes and similarly well developed during non-breeding phase as it has been established in previous chapter with effect of environment along with endocrine control of thymic serum (Fabris N.et.al., 1985).

In present study, during breeding phase testes were removed and effect of testosterone blocked, thymus morphologically enlarged and it mimicked as non-breeding phase(Lal et.al.,2009,2010). Thymus endocrinology was sponsored in development of thymus which consist thymocytes , thymic epithelial cells and thymic stromal cells in active thymus during non-breeding phase (Hadden J.W.1992 and Li Lin et.al., 1993) as in *Calotes* during non-breeding phase which has been established in previous chapter. After removal of testes, thymus undergone in developmental phase and cortex and medulla were significantly distinct. Enhanced amount of reticular cells and thymocytes, macrophases and Hassall`s corpuscles were also observed.

During non-breeding phase, thymus was well developed and showed all cellular differentiations with thymocytes, macrophages, reticular cells and Hassall`s Corpuscles. After removal of testes there was no significant variations observed in thymus morphology (Heather E. Lynch et. al., 2009). The cellular variations were also not significantly obtained as it was observed in castrated lizards (Murrey F.W. et. al., 2000).

In conclusion, the present study demonstrated the thymic development under influence of gonads as revealed by histological changes due to castration during different phases such as breeding and non-breeding in *Calotes versicolor* .The findings also suggest that celluar changes in thymus might have influence the immunomodulation and effect the reproductive performance through hypomedulary axis.

REFERENCES

1. **Al-Sadoon, M.K., El-Banna, A.A., Ibrahim, M.M.M., Abdo, N.M. and Al-Rasheid, K.A. (1990).** Effect of gonadal steroid hormones on the metabolic rate of the cold-acclimated gonadectomized male and female *Chalcidesocellatus*(forskal).Gen. and comp. endocrinology. **80:**345-348.
2. **Bram Lutton and Ian Callard (2006).** Evolution of reproductive-immune interactions. Integrative and Comparative Biology.vol.**46**.no.**6**: 1060-1071.**Dardenne, M. (1999).** Role of thymic peptides as transmitted between the neuroendocrine and immune systems. Ann. Med. Supp. **2**:34-39.
3. **Fabris, N. and Mocchegiani, E. (1985).** Endocrine control of thymic serum factor production in young-adult and old mice. Cellular immunology, vol**91**, issue **2**:325-335.
4. **Gouder, B.Y.M., and Nadkarni, V.B. (1979).** Histometric and histochemical changes in the seminiferous epithelium, Leydig cells and sertoli cells in the testes of *Calotesversicolor*. Biol. Bull. India .**1**:15-22.**Hadden J.W. (1992).** Thymic endocrinology. Int. Journal Immonopharmacol. **14(3)**:345-352.
5. **Heather, E. Lynch; Gabrielle L. Goldberg; Ann Chidgey; Marcel R.M.Van; Den Brink; Rechard Boyd and Gregory D. Sempowski (2009).** Thymic involution and immune reconstitution. Trens Immunlogy.**30** (**7**):366-373.
6. **Lal, R., Nirmal, B.K. and Saxena, A.K. (2009).**Interactive seasonal changes in testes and thymus of lizard *Calotesversicolor*Daudin. J. Endocrinol. Reprod. 13(1):13-16.
7. **Lal, R., Nirmal, B.K. and Saxena, A.K. (2009). Lal, R., Nirmal, B.K. and Saxena, A.K. (2010).** Environmental influence on development and differentiation of testes in *Calotes versicolor* .ASIAN J. EXP. BIOL. SCI. VOl 1 (3) ;706 -709.
8. **Lal, R., Nirmal, B.K. and Saxena, A.K. (2010).**Study of histological variations of thymus under gonadal influence in male *Calotes versicolor* . J. Experimental Zoology ,India Vol. 13 No. 1 pp.193-195.
9. **Lal, R., Nirmal, B.K. and Saxena, A.K. (2014).** Hormone induced histological changes in testes of Garden Lizard *Calotes versicolor*: International Research Journal of Natural and Applied Sciences.Volume-1, Issue-6 :42-51.

10. **Li, Lin; Zhou, Jin-Huang; Xing, Shan-Tian and Lau, B.H.S. (1993).** Thymus – neuroendocrine - liver pathway. Article. Medical Hypotheses. Vol.**41**, Issue **5**: 470-472.
11. **Munickasundari, M. and Pitchappam, R. (1988).** Structural, functional relationship of derived cells of the lizard *Calotes versicolor*. Developmental Comparative Immunology .**12**(**3**): 603-610.
12. **Munoz, Francisco J. and Fuente, Monica De Ia (2001).** The effect of the seasonal cycle on the splenic leukocyte functions in the Turtle *Mauremyscaspica*. Physiological and biochemical zoology .**74**(**5**):660-667.
13. **Murray, R.W., Wilson, J.G., Xiang, L. and Lagoo, A. (2000).** Progestrone inhibits glucocortcoid-induced murine thymocyte apoptosis. Int. Journal of Immonopharmacology .**22**(**11**):955-965.
14. **Nicholas, R.S., Hall, M., Grady, P.O. and Menzies, Robert A. (1992).** Thymic regulation of the hypothalamic-pituitary-gonadal axis. International Journal of immunopharmacology, vol.**14**, issue **3**:353-359.
15. **Olson, N.J. and Kovacs, W.J. (1996).** Gonadal steroids and immunity. Endocr.Rev.Vol.17.No.4:369-384.
16. **Pitchappam, R.M. and Muthukkaruppan, V.R. (1977).** Thymus dependent lymphoid regions in the spleen of the lizard, *Calotes versicolor*. Journal experimental zoology. **199:177**-188.
17. **Rao, M. Appaswmy (1954).** The involution of the thymus of the lizard,*Calotesversicolor*(DAUD).Proc. Nat. Inst. Sci. India, Vol.**21**.B.No.**1**. **Summer, Cliff H. (1995).** Regulation of ovarian recrudescence; Different effects of corticotrophin in small and large female liazrs, *Anoliscarolinensis*. The Journal of Experimental Zoology. **271**:228-234.
18. **Sutherland, J.S., Goldbeg, G.L., Hammett, M.V., Uldrich, A.P., Berzins, S.P., Heng, T.S., Blazar, B.R., Millar, J.L., Malin, M.A. Chidgey, A.P. and Boyd, R.L. (2005).** Activation of thymic regeneration in mice and humans following androgen blockade. Journal of Immunology. **175** (**4**): 2741-2753.
19. **Radder, R.S. and Saidpur, S.K. (2000a).** Maternal size determines clutch mass, whereas breeding timing influences clutch and egg sizes in the tropical lizard, *Calotesversicolor* (Agamidea). Copia (**4**) : 1062-1067.

20. **Saad, A.H. and Zapat A. (1992).** Reptilian thymus: An ultrastructure over view. Thymus, **20**:135-152.

21. **Shambhag, B.A. and Prasad B.S.K. (1993).** Follicular dynamics and germinal bed activity during the annual ovarion cycle of lizard, *Calotes vesicolor*.J.Morphol.**216**:1-7.

22. **Shambhag, B.A., Radder, R.S. and Saidpur, S.K. (2000a).** Maternal size determines clutch mass, whereas breeding timing influences clutch and egg sizes in the tropical lizard, *Calotesversicolor* (Agamidea). Copia (**4**) : 1062-1067.

23. **Zakharova, L.A. (2009).** Plasticity of neuroendocrine-immune interactions during ontogeny:role of perinatal programming in pathogenesis of inflammation and stress-relateddiseases in adults. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery.Vol.**3**. No.**1**: 11-27.

24. **Zapata, A.G., Varas, A. and Torroba, M. (1992).** Seasonal variations in the immune system of lower vertebrates. Immuno. Today, **13** (**4**): 142-147.

25. **Zimmerman, L.M., Vogel L.A. and Bowden, R.M. (2010).** Understanding the vertebrate immune system: insights from the reptilian perspective. The Journal of experimental biology.**213**: 661-671.