HORMONE INDUCED HISTOLOGICAL CHANGES IN TESTES OF GARDEN LIZARD CALOTES VERSICOLOR

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ABSTRACT

Testosterone plays important role in development and proliferation of testicular cells along with environment in animals. Calotesversicolor is seasonal breeder influenced by environment along with temperature and day length. In present investigation, different doses of testosterone were given in different group of lizard to observe the histological variations in testicular cells during breeding and nonbreeding phases. Different doses of testosterone have brought the variations in testicular cell development in reproductive phases. During nonbreeding phase high dose of testosterone influenced the highly intesticular cell development along with other essential factors. High doseof testosterone had great response in proliferation of seminiferous tubules and formation of central lumen. During breeding phase testosterone showed more enhanced activities of seminiferous tubule as compare to the control.

KEY WORDS: Testes, Testosterone, *Calotesversicolor*

INTRODUCTION

The vertebrate reproduction involves successful interaction between external (physical, climatic and social environments) and internal (neuroendocrine) factors. However, little is known about the role of social clues governing the reproduction.Shambhag B.A.et.al., 2002, explained the role of socio-sexual factors in gonadal recrudescence with various social situations under semi-natural conditions in *Calotesversicolor*.

The environmental regulation in vertebrate reproduction had been subject of intensive investigation by different researchers and yet to need more attention over the study of endocrine glands. Seasonal change brings variations in reproductive events and plasma steroid levels are well documented in several species of Lizard (Arslan M. et.al., 1978, Bona-Gallo A. et.al., 1980, Ando S. et.al., 1991, Amey Andrew P. et.al., 2000 and Radder R.S.

et.al., 2001). The change in size and histology of the testis along with accessory sexual organs and thymus are well known in many reptiles (Callard I.P. et.al., 1976, andSingh R.et.al., 1988). The reproductive biology of certain reptiles has relationship with endocrine, where seasonal influence plays major role. The monthly changes in androgen concentration in peripheral plasma and in the testes of *U. hardwicki* are described and observed by Arsalan M. et.al; 1978 where annual androgen rhythms are correlated with changes in testicular weight and the spermatogenic cycle in the species. Shambhag and Prashad,1993 has reported *Calotesversicolor* widely distributed in India is a seasonal breeder and Gouder and Nadkarni; 1979 suggested that males are spermatically active during April to September(Lal R and Nirmal B.K. 2010).Gravid lizards are encountered during May-October (Shambhag and Prashad,1993;Shambhag et.al.,2000 a). Furthermore Lofts B. et.al., 1973 has documented that sexually regressed house sparrow shows reduction in testicular weight which can be activated by testosterone treatment. According to Al-Sadoon M.K. and El-Banna A.A. et.al., 1990, a cold acclimated gonadectomized male and female *Calcidesocellatus* shows significant increase in rate of oxygen consumptions, which results the development of other organs due to increase in testosterone and estradiol activity. Increased activity of testes leads the regression of thymus in *Calotesversicolor* due to the environmentalinfluences are also documented by Lal R. et.al., 2009.

MATERIAL AND METHODS

Indian garden lizard *Calotesversicolor* has procured from locally by supplier to study the hormone administered histology of testis and thymus. Adult male Garden lizard (Average wt. $23.56^{\pm}7.2$ gm; SVL $8.67^{\pm}$ 0.48 cm) were purchased during breeding and non-breeding phase and maintained in wire netted wooden cages and acclimated to natural day length, temperature and other environmental factors. Lizards were provided a mixed diet of live cockroaches, grasshoppers, crickets and maggots every day and water was provided ad libitum.

The lizards were grouped into four groups for testosterone treatment. First grouptreated with 25 µg/ lizard of testosterone, second group treated with 50 µg/ lizard of testosterone,third group treated with 100 µg/ lizard of testosterone and 4^{th} group (Control) of animals received only i.e. 7% saline water in which testosterone diluted for different doses.After 5 days of acclimatization lizards were given subcutaneous injection of

testosterone (Testosterone entherate, 250mg/100ml) .The different doses of injections were given to the different group of lizards three times after 3 days interval during breeding and non–breeding phase. After 15 days of injection (testosterone) lizards were dissected to remove the testes for observations, the month of February and month of August considered as peak of non-breeding and breeding phases respectively. Testes were fixed in Bouin's solution, tissues were further processed for double staining technique, which includes washing, dehydration, embedding in paraffin wax and sectioned serially at 5µm thickness. Sections were stained with haematoxylin and eosin and observed the variations in development and differentiation in histology of testis.

RESULTS

Testes of*Calotesversicolor* showed dose dependent response with exogenous administration of testosterone which included significantly increase of testicular volume as well as testicular weight. During **non-breeding** phase seminiferous tubules were undeveloped and with involuted epithelial lining as shown in **control group** of animals (Fig.1a). Inactive spermatogonial cells closely associated with epithelial tissues could be seen in Fig.1b in non-breeding testes. It was observed that the seminiferous tubules found to be developed in different experimental doses of testosterone. First dose of 25 µg testosterone, seminiferous tubules were slightly enlarged, spermatogonial cells also enlarged and showed initial stage of spermatogenesis as compared to the control. Seminiferous tubules found to be filled with gonial cells (Fig.2a &2b). When50 µg testosterone administered further development in seminiferous tubules and spermatogonial cells were observed in which Leydig cells along with lumen formations in seminiferous tubule were found (Fig.3a &3b). Much higher dose of testosterone (100 µg), the histology resembled with breeding season testes except the formation of spermatozoa at this dose, testes were enlarged, seminiferous tubules round shaped, active spermatogonial cells were homogeneously around the completely formed central lumen. Hence the appearance of central lumen within seminiferous tubule and proliferation of spermatogonia were the major events that could be observed under influence of testosterone (Fig.4a &4b).

In **breeding phase** seminiferous tubules were well developed showing all cellular differentiation of mature testis. Different developmental stages were observed where the maturation of spermatozoa with active interstitial cells in between the connective tissue

during different experimental groups. Spermatozoa were visible in central lumen in mass along with primary spermatocytes, secondary spermatocytes, spermatid and Seminiferous tubules were well differentiated and filled with spermatozoa in **control** (Fig.5a &5b) although testes were fully functional in breeding phase however few noticeable changes could be seen with exogenous administration of testosterone in breeding phase. When 25µg testosterone administered it showed enhanced germinal activities and in seminiferous tubule, proliferation and spermatogenesis were in advance phase in comparison of control. Embedded spermatozoa with spermiogenesis and gonial cells were arranged in cord like structures (Fig. 6a &6b).Dose of 50µg testosterone, showed histological development in seminiferous tubules with the availability of released and embedded spermatozoa in lumen. All phases of spermatogenesis could be visualized along with spermiogenesis in Fig.7a &7b. With 100 µg testosterone**,** advancement in spermatogenesis was observed with large number of spermatozoa and lumen development of testis could also been seen with large number of spermatozoa (Fig. 8a & 8b).

DISCUSSION

The testicular activities were studied in present investigation which was also observed in other species of lizard (Susan et.al., 1997andCaroline et.al.,2011). Some reports are also available which discuss the regulation of testicular development. Different dose specific variations were investigated in this work through histology as it is treated with testosterone and it has been supported by Arselan M.et.al., 1978. Bourne A.R.et.al; 1985, who has investigated the annual cycle of plasma and testicular androgens level in lizard *Tiliquarogosa.*According to Lofts B., Phillips J.G., and Tom W.H. (1966),the **s**easonal changes in testis of cobra, *Najanaja*(Linn.) were also supported the activities of gonadal development due to steroidal effect. The endocrine control of the dogfish shows histological and ultra-structural changes in testis after hypophysectomy (Dobson S.et.at., 1977).

During non-breeding phase due to exogenous testosterone administration, significant increase of testicular size and development of the seminiferous epithelium was presumed that sharp advancement in plasma as well as testicular androgen level. During non-breeding phase different doses of testosterone influenced the seminiferous tubule activities. Spermatogonia were homogeneously arranged in higher dose and lumen formation had been taken place which was filled with gonial cells. This finding was supported by Callard I.P.et.al; 1976, who

has detected peripheral testosterone level which influences the development of the interstitial cells and showed the active seminiferous epithelium. Histological observations in present investigation provided evidences towards enhanced testicular activities as Vijay Kumar B., Ramjaneyulu T. et. al., 2002 has reported the FSH and LH activities on testis during non-breeding season in *Calotesversicolor*.

During breeding phase testicular activities were well enhanced due to different testosterone doses. The rise in plasma testosterone concentration coincides with enhanced testicular weight, active spermatogenesis and appearance of mature spermatozoa in the testis. Present work gets supported from the study of Arslan M. et. al; 1978, who observed that the annual androgen rhythm affects the testicular weight and spermatogenic cycle.

Hence the study brought the information regarding hormone administered histological changes in testis along with environmental influence. It also elaborates environmental influence on endocrine glands, which leads towards higher vertebrates as well as in human reproduction and health.

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HORMONE (TESTOSTERONE)ADMINISTERED HISTOLOGY OF TESTES DURING NON- BREEDING PHASE

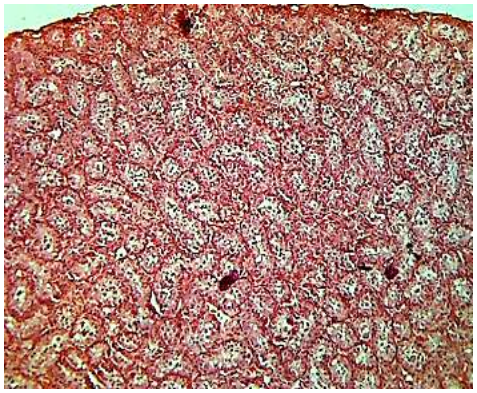
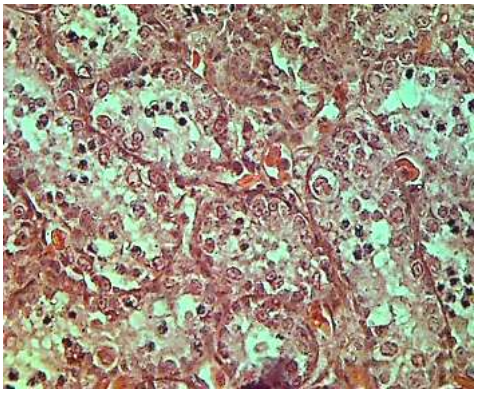


Fig 1a; Testis (100X) Control(C)



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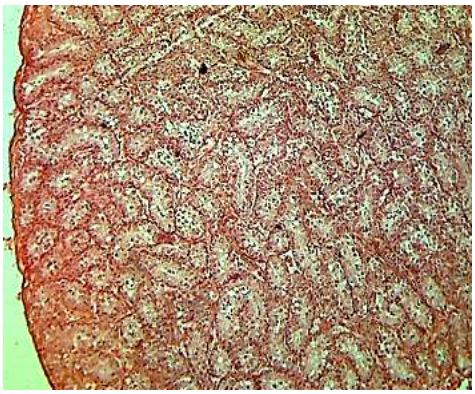


Fig.2a; Testis (100X) Testosterone 25µg dose

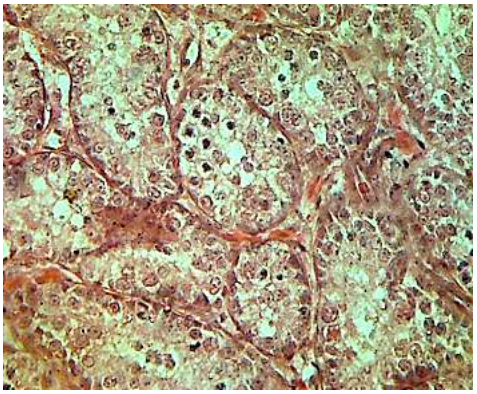


Fig.2b; Testis, (400X) Testosterone 25µg dose

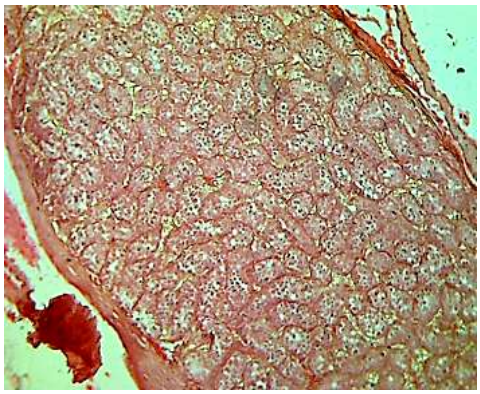


Fig.3a;Testis (100X) Testosterone 50µg dose

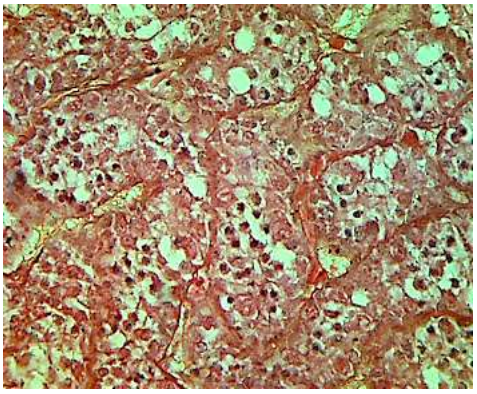
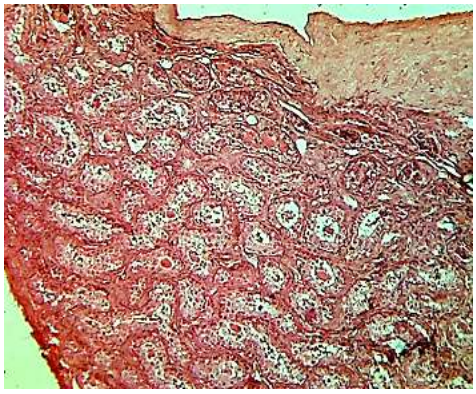


Fig. 3b; Testis (400X) Testosterone 50µg dose



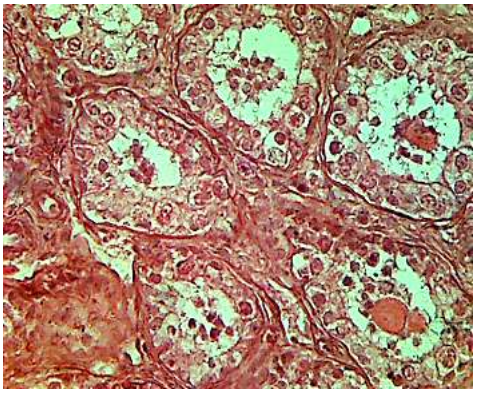
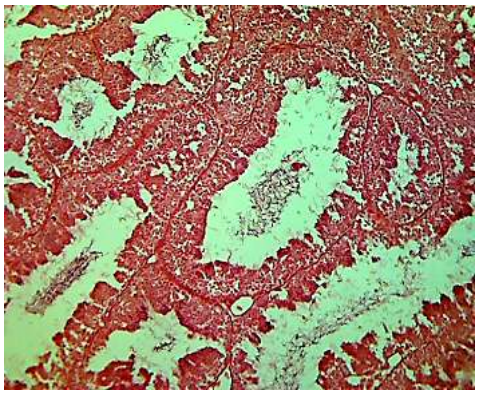


Fig.4a; Testis (100X) Testosterone 100µg dose.Fig.4b; Testis (400X) Testosterone 100µg dose.

HORMONE (TESTOSTERONE)ADMINISTERED HISTOLOGY OF TESTES DURING BREEDING PHASE



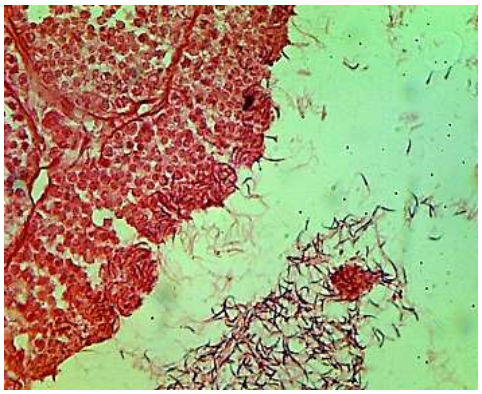
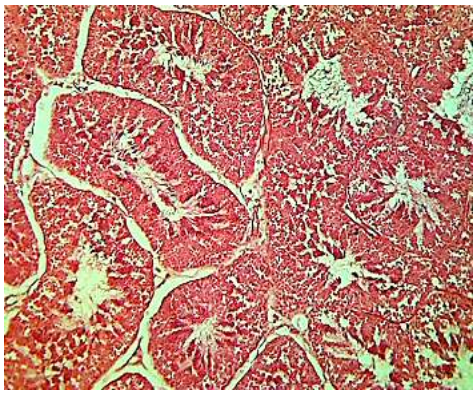


Fig.5a; Testis (100X) control (C) Fig.5b; Testis (400X) Control(C)



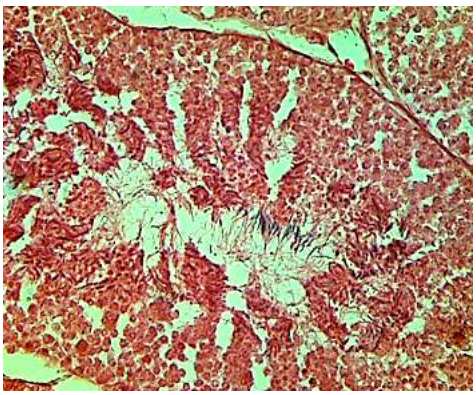
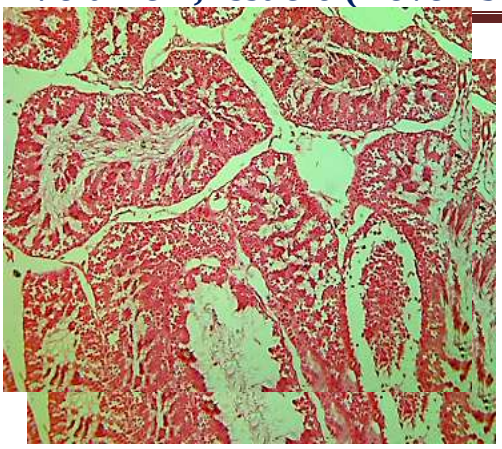


Fig.6a; Testis (100X) Testosterone 25µg dose Fig 6b; Testis (400X) Testosterone 25µg dose



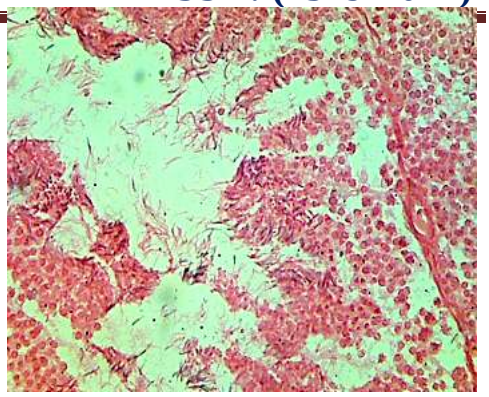
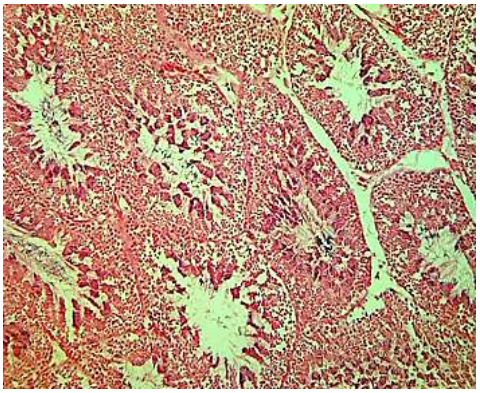


Fig7a; Testis (100X) Testosterone 50µg dose Fig.7b; Testis (400X) Testosterone 50µg dose



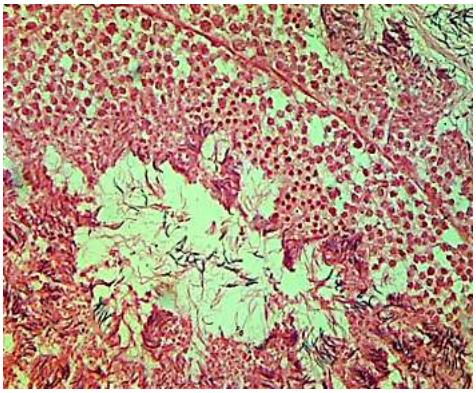


Fig.8a; Testis (100X)Testosterone 100µg dose Fig.3.8b;Testis (400X) Testosterone 100µg dose