GnRH and/or testosterone induced changes in reproductive activities during nonbreeding season in *Calotes versicolor* (Daud.)

Bhaktaraj B, Somanathreddy Patil & Saraswati B Patil*
Department of Studies in Zoology, Gulbarga University, Gulbarga 585 106, India

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Administration of Gonadotrophin releasing hormone (GnRH) to male *C. versicolor* during nonbreeding season increases the weight of testis, diameter of testis, seminiferous tubule, Sertoli and Leydig cell nuclei. It also activates the spermatogenic process. Increase in the weight of epididymis and lowered cholesterol level of testis indicate androgen production. Treatment of testosterone along with GnRH further enhances the activities of testis as a few spermatozoa appeared in the lumen of seminiferous tubule along with increase in other spermatogenic elements. It may be concluded that the exogenous GnRH can induce reproductive activities during nonbreeding season when the environmental conditions are unfavourable. Testosterone administration has the additive effect on these activities.

In reptiles the cyclic changes in the gonadal activities are influenced by endocrine and environmental factors such as temperature, photoperiod, relative humidity, rainfall, etc. which trigger the hypothalamo-hypophyseal axis. Gonadotrophin releasing hormone (GnRH) is known to stimulate the pituitary gonadotrophin release in vertebrates. Seasonal studies on the hypothalamo-hypophyseal complex in teleost fishes have clearly indicated the possible role of hypothalamic neurosecretion in regulating the function of gonads. Pituitary transplants have induced the regression of gonadotrophs, thereby resulting in decrease in gonadotrophin secretion suggesting the importance of direct hypothalamic control of the hypophysis. Testosterone administration during early regression phase inhibited the rate of regression of the testis, proliferated the activity of testis and caused significant increase in the growth of epididymis. In the present study an attempt has been made to induce the reproductive activities through GnRH during nonbreeding season and the effects of testosterone on reproductive activities of GnRH treated *Calotes versicolor*.

Materials and Methods
Adult male specimens of *C. versicolor* were collected locally during regressive phase (September and October) of nonbreeding season. The lizards weighing 30-35 g and snout vent length of 12-13 cm were selected and acclimatized to the laboratory conditions for one week prior to the experimentation. All the lizards were fed regularly with live cockroaches and water ad libitum. The lizards were divided into 4 groups of 6 animals each. The first group served as control and received 0.1 ml saline. The second group received 0.5 lgl/100 g body weight GnRH (Buserelin-Acetate, Hoechst Veterinar GmbH, W. Germany) in 0.1 ml saline. The third group received 25 lgl/100 g body weight testosterone in 0.1 ml saline. The fourth group received both 0.5 lgl GnRH and 25 lgl/100 g body weight each in 0.1 ml saline. All the injections were given intraperitoneally on alternate day for 15 days in between 1000 and 1100 hrs. Lizards were autopsied on day 16. At autopsy the weight of the body was recorded. Testes and epididymis were dissected out, freed from adherent tissue and weighed on an electronic balance. The organs from one side were fixed in Bouin’s fluid and processed for histological observations. The number of spermatogenic elements like spermatogonia, spermatocytes and speramidids was counted from randomly selected 20 sections of seminiferous tubules appeared round in cross sections from each group. For histometry, the micrometric measurements such as diameter of testis, seminiferous tubule, epididymis, Sertoli and Leydig cell nucleus and height of the epithelial cell of epididymis were made from randomly selected 20 sections appeared round in cross sections from each group by using.
ocular and stage micrometers. Biochemical estimations of cholesterol by Liebermann and Burchard’s method described by Peters and Van Slyke11; Protein12 and glycogen13, were made from the other side testis. Similarly the epididymal protein content12 was estimated. Statistical analysis was carried out by using Student’s ‘t’ test. The results were judged significant if $P<0.05$.

**Results and Discussion**

The results are presented in Tables 1-3.

The seasonal changes in the testis of *C. versicolor* have already been reported14. In reptiles the neuroaxons from supraoptic nuclei (SON) and paraventricular nuclei (PVN) of hypothalamus help in the transfer of message from hypothalamus to pituitary through portal system. In the lizard *C. versicolor* a few fibres of the peptidergic tract become associated with the portal capillaries and release the neurosecretory product which reaches pars dorsalis (PD) through portal vein15.

GnRH provides a humoral link between the neural and endocrine systems. This decapeptide is synthesized and then stored in the medial basal hypothalamus. In response to neural signals, GnRH is released in pulses into the hypophysial portal system and then conducted to the anterior pituitary, where it stimulates the release of the gonadotrophins (FSH and LH). These gonadotrophins in turn regulate the gonadal steroidogenesis and gamete maturation16. The androgens are essential for maintaining the activities of male accessory organs and testosterone seems to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter of testis (µm)</th>
<th>Diameter of seminiferous tubule (µm)</th>
<th>Diameter of Sertoli cell nucleus (µm)</th>
<th>Diameter of Leydig cell nucleus (µm)</th>
<th>Spermatogonia</th>
<th>Spermatocytes</th>
<th>Spermatids</th>
<th>Spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>561.23 ± 6.49</td>
<td>48.45 ± 1.94</td>
<td>1.58 ± 0.13</td>
<td>1.75 ± 0.13</td>
<td>16.75 ± 0.81</td>
<td>9.12 ± 0.29</td>
<td>7.37 Nil</td>
<td></td>
</tr>
<tr>
<td>GnRH</td>
<td>1020.69 ± 6.69**</td>
<td>77.57 ± 1.79**</td>
<td>3.29 ± 0.31**</td>
<td>3.03 ± 0.09**</td>
<td>20.50 ± 0.75*</td>
<td>10.87 ± 0.44</td>
<td>8.25 Nil</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>1304.34 ± 5.55**</td>
<td>83.06 ± 1.86**</td>
<td>2.83 ± 0.12*</td>
<td>2.59 ± 0.08*</td>
<td>21.37 ± 0.39</td>
<td>9.12 ± 0.07</td>
<td>6.25 Nil</td>
<td></td>
</tr>
<tr>
<td>GnRH + Testosterone</td>
<td>1163.37 ± 3.44**</td>
<td>85.86 ± 1.44**</td>
<td>3.47 ± 0.07**</td>
<td>3.21 ± 0.08**</td>
<td>31.37 ± 0.41*</td>
<td>23.12 ± 0.78*</td>
<td>10.00 A few</td>
<td></td>
</tr>
</tbody>
</table>

*P* values: *<0.01; **<0.001

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wt. of testis (mg/100g body wt.)</th>
<th>Cholesterol (µg/100mg)</th>
<th>Protein (mg/100mg)</th>
<th>Glycogen (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.37 ± 4.71</td>
<td>320.14</td>
<td>1.67</td>
<td>3.61</td>
</tr>
<tr>
<td>GnRH</td>
<td>157.95 ± 10.31**</td>
<td>33.42</td>
<td>4.54</td>
<td>1.63</td>
</tr>
<tr>
<td>Testosterone</td>
<td>95.73 ± 3.51</td>
<td>146.28</td>
<td>2.90</td>
<td>3.17</td>
</tr>
<tr>
<td>GnRH + Testosterone</td>
<td>93.17 ± 2.01</td>
<td>51.63</td>
<td>6.52</td>
<td>2.27</td>
</tr>
</tbody>
</table>

*P* values: *<0.01; **<0.001
have dual role in male reproduction. Administration of testosterone stimulates spermatogenesis in fishes and in mammals but inhibits spermatogenesis in amphibians. In *Hemidactylus flaviviridis* testosterone has no stimulatory effects on the spermatogenic epithelium.

In the present study the administration of GnRH during nonbreeding season increased the weight of testis in *C. versicolor* indicating GnRH stimulation of gonadotrophin release during nonbreeding season. It also stimulated the spermatogenesis; however the spermatozoa have not made their appearance. Though testosterone alone has not much effect on the weight of testis, it has initiated the spermatogenesis as spermatogonial number increases. As the cells show hypersecretion the diameter of testis and seminiferous tubules increased alongwith the lumen. It also stimulated the accessory sex organs growth, indicating the dependency of secondary sex organs on testosterone. The combination of GnRH and testosterone initiated the complete spermatogenesis as a result a few spermatozoa appeared in the lumen of seminiferous tubule.

In reptiles Leydig cells form the principal site for steroid biosynthesis; the seminiferous epithelium and the capsule of testis form the additional sites. Besides Leydig cells, the Sertoli cells also have the ability to synthesize androgens needed for spermatogenesis.

Increase in the nuclear size of any cell indicates its hyperactivity. Administration of GnRH or testosterone or combination of these two hormones has increased the nuclear size of Sertoli cell and Leydig cell. But there is no significant change in the number of Leydig as well as Sertoli cells. The increase in the protein content and decrease in cholesterol content indicate respectively the enhanced growth rate and utilization of precursor for steroidogenesis. The glycogen is an energy content, present in Sertoli cells and spermatogonia as stored substrate and provides source of carbohydrates for seminiferous tubular cells. In the present investigation the glycogen content of testis is reduced after GnRH and/or testosterone treatment, indicating its utilization for the reproductive activities.

The seasonal epididymal cycle closely correlates with the Leydig cell steroidogenic activity. The epididymis is androgen dependent. Administration of testosterone during the quiescent phase stimulates the epididymis. In the present study administration of GnRH alone is relatively not much effective. Treatment of testosterone alone or in combination with GnRH is highly effective. The action of GnRH is mediated through pituitary gonadotrophins which act on testicular interstitial cells that secrete androgens. Testosterone is a potential androgen having direct action on epididymis. Therefore the effect of testosterone and GnRH+testosterone is more effective in rising the growth rate and activities of epididymis.

References


