Role of vertebrate steroids in the regulation of testicular development in the fresh water crab, *Oziotelphusa senex senex*

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Abstract

**Background:** Crustacean reproduction is under the control of various peptide and steroid hormones including vertebrate steroids. In the present study, we examined the role of vertebrate steroids, progesterone and 17β-estradiol in the regulation of testicular development in the crab *Oziotelphusa senex senex*.

**Methods:** The crabs were divided into eight groups, first group served as initial control and second group served as concurrent control received vehicle. Third, fourth and fifth groups were injected with progesterone and six, seven and eight groups were injected with 17β-estradiol each at the doses of $10^{-6}$, $10^{-7}$ and $10^{-8}$ mole/crab respectively on 1st, 7th, 14th and 21st day of the experiment. After 28 days of the experiment the crabs were sacrificed on 30th day and testis was collected and measured testicular indices and testicular follicle diameter.

**Results:** The mean testicular indices and testicular follicle diameter were increased significantly (p<0.0001) in progesterone and 17β-estradiol injected crabs in a dose dependent manner when compared with controls. In the histological studies, the testes of controls and concurrent controls were in immature stage only. Whereas testes of vertebrate steroids injected were in mature stage.

**Conclusion:** The results of the present study provide evidence that vertebrate steroids, progesterone and 17β-estradiol are involved in the regulation of testicular development and spermatogenesis in crabs.

**Keywords:** Steroids, Progesterone, 17β-estradiol, Testicular development, *Oziotelphusa senex senex*

1. Introduction

Aquaculture industry is one of the fields of producing protein food source to meet the food demand for ever growing human population. Besides fish culture, the cultivation of crustaceans occupies an important role in the aquaculture industry. Decapod crustaceans like shrimps, prawns and lobsters are valuable sources of food. Apart from prawns and shrimps in recent years crab also becomes a most popular food item which has high economic demand in the National and International market. But in several domestication programmes poor performance of brood stock in crustaceans is the major bottleneck. The common practice of eyestalk ablation (1, 2) used to induce precocious reproduction in many commercially important crustacean species, but this technique resulted with mortality and inferior quality of seed. Hence, over the past few years crustacean aquaculture industry concentrating on different other methods to induce gonad development and maturation. In this connection, there are different compounds such as opioid peptides, biogenic amines, vertebrate steroids, prostaglandins, gonadotropins etc. were identified which able to enhance the reproduction of crustaceans without damaging animals (3-5). Most of crustacean species are dioecious and regulation of reproduction is controlled by different hormones. In females, reproduction is regulated by two neurohormones, i.e. gonad-inhibiting hormone (GIH) and gonad stimulating hormone (GSH). GIH is synthesized and secreted from the sinus gland of eyestalk which inhibits the gonad maturation (6, 7). GSH, which secreted from the brain and thoracic ganglion, stimulates gonad maturation (8-10). Methyl farnesoate (MF) a sesquiterpenoid secreted from mandibular organ also regulates the crustacean female reproduction (11-13). In males, androgenic gland a non-neural endocrine gland produces androgenic hormone (14) which regulates spermatogenesis in the testes and responsible for the development and maintenance of sexual characteristics (15). The activity of the androgenic

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gland thought to be regulated by neurohormones, the gonad-inhibiting hormone inhibits the activity of androgenic glands and gonad-stimulating hormone triggers the androgenic gland to induce spermatogenesis (16-19). Methylfarnesoate is also known to stimulate male reproduction in few crustaceans (20-21).

Vertebrate steroids regulate a wide variety of physiological functions in vertebrates. Different aspects of reproduction are controlled under the sex steroid hormones and are involved in the regulation of reproduction. Similar to vertebrates, vertebrate steroid hormones, such as progesterone, estradiol and testosterone are also regulates the crustacean female reproduction (3-5) and several studies reported the presence of vertebrate-type steroids in the hepatopancreas, ovary and hemolymph of crustaceans (22-24). The regulatory role of vertebrate-like steroids on reproduction in crustaceans is performed in the presence of specific receptor within the cell (25) and presence of vertebrate steroid receptors were reported in few crustaceans. Presence of progesterone receptor was identified in the ovary and both progesterone and 17 β-estradiol receptors in the hepatopancreas of the crayfish, Austropotamobius pallipes (26). The presence of vertebrate like hormones is well documented in tissues of female crustaceans and few studies reported the role of these steroid hormones on the regulation of reproduction in females. But very few studies focused on the occurrence and role of vertebrate steroids on male reproduction. The positive effect of progesterone administration on spermatogenesis was demonstrated in freshwater prawn, Macrobrachium kistnensis (27) and in tiger shrimp Penaeus monodon (28). Recently the occurrence of vertebrate-like steroids, were reported in the male crayfish Astacus leptodactylus (29). Further, no similar studies were reported the effect of administration of vertebrate steroids on testicular development and spermatogenesis. In view of this the present work was undertaken to examine the role of vertebrate steroids, progesterone and 17β-estradiol on male reproduction in Indian rice field crab, Oziotelphusa senex senex.

2. Materials and Methods
Oziotelphusa senex senex were collected from paddy fields in and around Tirupati (India) and maintained at room temperature at 28 ± 1°C in plastic tanks, half submerged in filtered tap water. They were fed daily with sheep meat ad libitum and the medium was changed 4hrs after feeding. Only adult intermolt stage C4, intact male crabs with a body weight of 28-32g were used. The crabs were divided into eight groups of 20 animals each.

The first group served as an initial control and these animals were sacrificed on the first day of the experiment. Crabs in group 2 received 10µl of crustacean saline through the arthroidal membrane of the coxa of the third pair of walking legs and served as concurrent controls. Crabs in group 3, 4 and 5 received a dose of 10⁻⁶ mole/crab, 10⁻⁷ mole/crab, and 10⁻⁸ mole/crab of progesterone respectively. Crabs in groups 6, 7 and 8 received a dose of 10⁻⁶ mole/crab, 10⁻⁷ mole/crab, and 10⁻⁸ mole/crab 17β-estradiol respectively by injection in 10µl per volume. The injections were given on the 1st, 7th, 14th, 21st day and the crabs were sacrificed on 30th day. The crabs were weighed and the reproductive organs were isolated, immediately placed in crustacean saline. The organs were removed from saline and blotted with paper towels and weighed wet. The crabs from control and experimental groups the gonad indices were determined using the following formula:

\[
\text{Wet weight of the gonad} = \frac{\text{Wet weight of the gonad}}{\text{Wet weight of the crab}} \times 100
\]

The diameters of 25 randomly chosen testicular follicles were measured using an ocular micrometer on a microscope. The data were analysed using one-way analysis of variance.

After 24 h of fixation the testis were dehydrated through an alcohol series and then embedded in paraffin wax (58–60 °C). Sections were cut at 7 µm thickness and affixed to gelatinized slides. After being deparaffinised in xylol the testicular sections were stained with haematoxylin and counter stained with eosin (30). The diameters of randomly chosen testicular follicles were measured using an ocular micrometer under compound microscope.

3. Results
Administration of progesterone and 17β-estradiol significantly (p<0.0001) increased the mean testicular index in a dose dependent manner (Progesterone: 10⁻⁶, 10⁻⁷ and 10⁻⁸ mole/crab - 0.842 ± 0.05, 0.793 ± 0.07 and 0.766 ± 0.07 respectively; 17β-estradiol: 10⁻⁶, 10⁻⁷ and 10⁻⁸ mole/crab - 0.812 ± 0.08, 0.738 ± 0.03 and 0.711 ± 0.07 respectively) when compared with controls (Table 1). The mean testicular follicle diameter was also significantly (p<0.0001) increased in a dose dependent manner in progesterone (10⁻⁶, 10⁻⁷ and 10⁻⁸ mole/crab 47.57 ± 3.75, 45.28 ± 3.82 and 42.45 ± 3.85 respectively) and 17β-estradiol (10⁻⁶, 10⁻⁷ and 10⁻⁸ mole/crab 45.22 ± 2.25, 43.87 ± 2.16 and 41.45 ± 3.85 respectively) injected crabs from the control crabs. No significant change was observed in the testicular index and testicular follicle diameter of
the concurrent control crabs from initial controls (Table 1). The process of development of sperm cells is called spermatogenesis and it is divided into different stages, i.e., immature, maturing and mature, with the indication of spermatogonia, spermatocytes, spermatids, spermatozooids and spermaphores. In Oziotelphusa senex senex, the testis contains 10-15 lobes, each built up by many seminiferous tubules, whose shape changes according to the stage of spermatogenesis. During spermatogenesis, a process whereby the spermatids are transformed to spermatozoa, a lumen is formed in the central tubuli. The spermatozoa are stored in the lumini until they are extruded into spermiducts where they are maintained during the period before mating. The process of spermatogenesis takes place in the seminiferous tubuli. The spermatogenesis develop into spermatocytes, and then to spermatide which undergo a functional maturation in the testis.

### Table 1. Effect of injection of progesterone and 17β-estradiol on testicular index and testicular follicle diameter in the fresh water crab, Oziotelphusa senex senex

<table>
<thead>
<tr>
<th>Group</th>
<th>Testicular index</th>
<th>Testicular follicle diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.582± 0.05</td>
<td>35.82 ± 3.65</td>
</tr>
<tr>
<td>Concurrent control</td>
<td>0.585 ± 0.24 (0.51)</td>
<td>36.24 ± 2.64 (1.17)</td>
</tr>
<tr>
<td>Progesterone 10⁻⁶ mole/crab</td>
<td>0.842* ± 0.05 (44.67)</td>
<td>47.57* ± 3.75 (32.80)</td>
</tr>
<tr>
<td>10⁻⁷ mole/crab</td>
<td>0.793 *± 0.07 (36.25)</td>
<td>45.28* ± 3.82 (26.40)</td>
</tr>
<tr>
<td>10⁻⁸ mole/crab</td>
<td>0.766*± 0.07 (31.61)</td>
<td>42.45* ± 3.85 (18.50)</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>10⁻⁶ mole/crab</td>
<td>0.812*±0.08 (39.51)</td>
</tr>
<tr>
<td>10⁻⁷ mole/crab</td>
<td>0.738*±0.03 (26.80)</td>
<td>43.87*±2.16 (22.47)</td>
</tr>
<tr>
<td>10⁻⁸ mole/crab</td>
<td>0.711*±0.07 (22.16)</td>
<td>41.45* ± 3.85 (15.71)</td>
</tr>
</tbody>
</table>

p value: <0.0001 <0.0001

Values are mean± S.D (n= 20 for testicular index; n=25 for testicular follicle diameter).

Values are percent change from control; Values with * show significantly changed from controls.

Histological observations of testes observed in both initial control crabs concurrent control are in immature stage at the end of the experiment. Whereas the testes of crabs received vertebrate steroids such as progesterone and 17β-estradiol were in mature stage (Figure 1). Formation of spermatogonia starts in the procini, which are undifferentiated seminiferous tubules at the top of each lobe. In this stage the lobules contain mostly primary spermatocytes (Figure 1, A). In the next stage the differentiation of primary spermatocytes to secondary spermatocytes were shown (Figure 1, B). Differentiation of secondary spermatocytes and spermatides takes place in the seminiferous tubules, which lay in the center of each lobe (Figure 1, C). All those seminiferous tubules are filled with spermatocytes and spermatides surrounded by a layer of the oocytes from mesodermal origin (Figure 1, D).

4. Discussion

In crustaceans different types of methods were employed to study the reproduction, gonad index has direct bearing on the gonad development and maturity (25-31) and histological observation of the gonads was used to determine the phases of gonad maturation in reproduction (32-33). In crustaceans, androgenic gland plays a key role in the regulation of male reproductive activity and male maturation by releasing male hormone androgenic hormone (34-35). However, the hormonal control of the spermatogenesis in crustaceans is not completely understood. In the present study injection of progesterone and 17β-estradiol significantly increased testicular index and testicular follicle.
diameter including histological alterations in the testis of crab, *Oziotelphusa senex senex*. There are no studies reported on the effect of vertebrate steroids in the testicular development in *Oziotelphusa senex senex*. But the administration of progesterone stimulated the spermatogenesis in freshwater prawn *Macrobrachium kistnensis* (27) and injections of progesterone and estradiol increased gonadosomatic index in *Penaeus monodon* (28), were support the present results. However, the role of progesterone and 17β-estradiol in female reproduction has been reported in different crustaceans. Administration of progesterone induced ovarian maturation in the marine penaeid shrimp *Parapenaeopsis hardwickii* (36) and in *Macrobrachium kistnensis* (27), 17α-hydroxyprogesterone and 17β-estradiol stimulated ovarian growth of the red swamp crayfish *Procambarus clarkii* (37). Administration of 17α-hydroxyprogesterone stimulated the ovarian growth, ovarian maturation and vitellogenesis in the kuruma prawn *Penaeus japonicas* (38), and in the freshwater crab, *Oziotelphusa senex senex* (39). Injections of estradiol and progesterone stimulated the vitellogenin in *Chemys albidus* (40). Fluctuating levels of progesterone and 17β-estradiol in the ovary and hemolymph of the crab *Scylla serrata* at different reproductive stages were reported (23). Recently, the role of sex steroids on reproduction in male crayfish *Astacus leptodactylus* and in tiger shrimp *Penaeus monodon* were studied (28-29). The level of vertebrate steroid, testosterone was reported in *Astacus leptodactylus* in reproductive and non-reproductive periods. From the results, high levels of testosterone were reported in hemolymph of male, *Astacus leptodactylus* in the months of November and January, whereas high levels of estradiol reported in the month of January. But levels of progesterone not related to the reproductive cycle (28). This study also reported that the increased gonad somatic index of testis by the injection of testosterone, estradiol and progesterone in male *Astacus leptodactylus*. In another study similarly injection (5 μg/g, b.w) of testosterone hormone induced sperm development and subsequent mating success in tiger shrimp *Penaeus monodon* (29). This study also reported that testosterone induces androgenic gland to stimulate spermatogenesis during 6 hrs, 12 hrs and 24 hrs and its effect reduced at 48 hrs by histological observations and concluded that testosterone hormone is more effective on androgenic gland initially for 60 days and then enhancing the growth/molting by stimulating Y-organ in shrimp *Penaeus monodon* (29). From above studies the result of the present study is very clear that the injection of vertebrate steroids (progesterone and 17β-estradiol) increases the testicular indices by inducing spermatogenesis as evidenced by histological studies in male crabs, *Oziotelphusa senex senex* are in agreement with the existing reports.

**Conclusion**

In the present study for the first time we demonstrated vertebrate type steroids, in the male crab *Oziotelphusa senex senex*. Progesterone and 17β-estradiol in the testicular development provides evidence that vertebrate steroids also involved in the regulation of male reproduction in crustaceans. Furthermore the studies may focus on inducing male reproduction in commercially important crustaceans and studies are needed to establish in the future.

**Conflict of interest**

The authors declare no conflict of interest.

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**References**


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