Effect of cortisol on testis of freshwater fish Notopterus notopterus (Pallas)

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Received 26 April 1999; revised 10 June 2000

Cortisol (20, 40 and 60 μg / fish for 10 days) treatment caused an increase in testicular-somatic index (TSI) in immature N. notopterus whereas in mature fish no change from that of controls was observed. Histology of testis indicated that spermatogenic activity was activated in immature fish while it was inhibited in mature fish. Testicular cholesterol exhibited a similar response. The results indicate that cortisol inhibits spermatogenesis during mature phase while it stimulates spermatogenesis during immature phase of the reproductive cycle in N. notopterus.

Cortisol is quantitatively the most important steroid found in teleost plasma in different species. Cortisol is generally considered a metabolic hormone in fish and has an important role in the regulation of intermediary metabolism. The adrenocortical hormone cortisol and the adrenohypophysal prolactin are well known as important triggers for electrolyte and osmotic adjustment. Although its role in female for the regulation of ovocyte maturation, ovulation, spawning behaviour and mobilization of energy stores required for reproduction is known, its effect on male fish reproduction is not clear. Deleterious effects of cortisol on reproductive function are reported. In Pacific salmon hypercortisolism may be a cause of "programmed death" after spawning. Hence, in the present study an attempt has been made to understand the comparative effects of cortisol on the testis of mature and immature fish Notopterus notopterus (Pallas).

Materials and Methods

Notopterus (100) were collected from Bheema river which is around 40 Km away from Gulbarga city. The weight of the mature fish ranged 80 - 120 and length 20 - 25 cm. The live fish were brought to the laboratory and kept in large plastic pool tanks (diameter 90 cm; height 60 cm). Each tank accommodated 8-10 fish. About 8-10 days were needed for the fish to acclimatise. Fish of both control and experimental groups were fed with live earth worms and small fishes (Gambusia).

Cortisol (11β : 17α : 21 trihydroxy-4-pregnenone – 3:20 – dione) was obtained from S.D. Fine Chemicals Ltd. BIOSAR. It was dissolved in olive oil and administered in two different stages of the reproductive cycle i.e., during August (mature stage) and December (immature stage). The fish were divided into 3 groups of 10 each. The hormone was injected interperitonally near the caudal region below the lateral line once in a day for 10 days. Group – I served as control and received only olive oil. Group – II received 20 μg of cortisol / 100 g body weight (0.2 μg/g body weight) / day. Group III and IV received 40 μg/100g body weight (0.4 μg/g body weight) and 60 μg / 100g body weight (0.6 μg/g body weight), respectively.

After 10 days of treatment, all the experimental and control fish were sacrificed by decapitation, the testes were dissected out carefully weighed and processed for histological and biochemical studies. Before fixing, the testis weights were measured for the determination of testicular - somatic index (TSI) by using the formula:

$$ TSI = \frac{\text{Weight of the testis}}{\text{Weight of the body}} \times 100 $$

Data were expressed as mean ± SD and Student’s, t test was used as described. After the termination of the experiment, the dissected out testis was fixed in aqueous Bouin’s fluid for histology (haematoxyline and eosine staining). The testicular cholesterol content was determined by following the method of Liberman Burchard reaction as described by Netelson.

Results

The reproductive cycle of N. notopterus can be divided into 4 stages during one year period. The
Fig. 1—Testis of control *N. notopterus* during mature stage showing different stages of spermatogenic elements H & E x 1200. Fig 2—Testis of *N. notopterus* after 20 μg cortisol treatment during mature stage Interstitial Leydig cells (LC) and spermatogenic cells (SC) are seen H & E x 1200. Fig 3—Testis of *N. notopterus* after 40 μg cortisol treatment during mature stage showing less number of spermatogenic cells (SC) and interstitial Leydig cells (arrow) H & E x 1200. Fig 4—Testis of *N. notopterus* after 60μg cortisol treatment during mature stage. Less number of spermatogenic cells (SC) and empty lobules (EL) H & E x 1200. Fig. 5—Testis of a control *N. notopterus* during immature stage showing spermatogenic (SC) cells undergoing divisions H & E x 1200. Fig 6—Testis of *N. notopterus* after 60 μg cortisol treatment during immature stage showing some dividing spermatogenic cells (SC) H & E x 1200.
January (spent). This study was based on the stages are prespawning, spawning, postspawning and spent. Mature gonad was found during July – August (prespawning) and immature during December – January (spent). This study was based on the histological observation made during different months of one year period. TSI of the cortisol treated fish did not exhibit any significant change in mature fish while in immature fish it was increased under the doses selected. In 20 µg treated mature fish, the testicular histology was similar to that of control (Fig.1) having all the stages of spermatogenesis including spermatocytes in the lobular lumen (Fig.2). In 40 and 60 µg treated groups, the testicular histology exhibited inhibitory changes, the lobules containing spermatogenic cysts are thought organised, spermatogenic divisions are less, the interstitium between the lobules increased and their lumen become empty (Figs.2 and 4). Thus these observations indicate that the normal spermatogenic activity is affected by cortisol administration in the mature fish.

The testis of immature control fish has dividing spermatogonial cells (Fig.5). In the cortisol treated fish, the testis has different stages of spermatogenic elements undergoing active divisions (Fig.6) indicating that the cortisol treatment caused activation of spermatogenesis.

The cholesterol content was determined in both mature and immature fish after cortisol treatment. Cholesterol was reduced in mature fish testis but increased in immature fish testis (Table 1) indicating its availability for steroid hormone synthesis during spermatogenesis.

**Discussion**

The adrenocortical cells in teleosts are steroidogenic and have the capacity to synthesize cortisol under different experimental conditions. Cortisol treatment in the mature *N. notopterus* caused a decrease in TSI, inhibition of spermatogenesis and reduction in cholesterol whereas increased TSI, active spermatogenesis and elevated cholesterol in immature fish indicates cortisol has both negative and positive action on the reproductive activity in males. In this context there are some studies showing cortisol having deleterious effects on reproductive function such as reducing normal sex steroid production, pituitary gonadotrophin content, gamete quality and subsequent larval quality. Reduction in testicular activity in mature *N. notopterus* in response to cortisol treatment may be either reduction in the normal sex steroid production through decreased pituitary gonadotrophin release or may be also due to excess. Cortisol may have probably induced gonadotropin receptor loss resulting in the down regulation. The cortisol treatment in the immature fish induces spermatogenic activity with the increases of TSI and cholesterol. This effect of cortisol indicates its involvement in spermatogenesis through probably inducing energy release for reproductive activity at a required level. It is known that seasonal increase in steroid hormones in fish is for gearing up of metabolism to provide energy for protein and lipid synthesis. Hence, in the present study cortisol induced increased testicular activity may be due to an increase in the metabolic activity specially during nonbreeding period providing expenditure of energy required for reproduction.

**References**


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**Table 1** — Effect of different doses of cortisol on testicular-somatic index (TSI) and cholesterol content in the freshwater fish *N. notopterus*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mature TSI</th>
<th>Immature TSI</th>
<th>Mature Cholesterol</th>
<th>Immature Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>0.23 ± 0.0458</td>
<td>0.065 ± 0.032</td>
<td>104.33 ± 108.82</td>
<td>145.00 ± 23.23</td>
</tr>
<tr>
<td>II. 40 µg cortisol</td>
<td>0.28 ± 0.0736</td>
<td>0.254 ± 0.124</td>
<td>105.51 ± 23.23</td>
<td>279.16 ± 36.11c</td>
</tr>
<tr>
<td>III. 60 µg Cortisol</td>
<td>0.228 ± 0.0459</td>
<td>0.219 ± 0.060a</td>
<td>142.35 ± 106.41a</td>
<td>271.16 ± 65.77b</td>
</tr>
</tbody>
</table>

*P values:* a < 0.05, b < 0.01, c < 0.001; Student's *t* test
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