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Ovaprim induced effect on testis of *Channa gachua*

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Abstract

The testis of Channa gachua, after administration of Ovaprim, showed changes in the morphology of testis and in the serum androgen level within period of 72 hrs. Morphological changes included spermatogonial proliferation, activation of leydig's and sertoli cells, organization of seminiferous lobules and formation of lobular lumen in testis. Leydig cells were enlarged, exhibited characteristics of steroid producing cells. Sertoli cells became elongated, shown signs of high cellular activities and remained in close contact with spermatogonia. The lobular organization was achieved much earlier than the progression of spermatogenesis.

1. Introduction

In vertebrates various components of testis forms well defined cellular organisation. The association of the cells and sequences of their appearance in the seminiferous tubules are highly organised. However, in the fishes, each spermatogenic cycle, followed by a resting stage for the release of spermatozoa during the process of late spermatogenesis by the rearrangement of germ cell cysts and somatic cells especially Sertolli cells.

Induction of spermatogenesis and maturation has been observed by the administration of Ovaprim doses for different duration. In present investigation, an attempt has been made to study the organization of testis of germ cell and observations after induction of spermatogenesis in fresh water fish, *Channa gachua* under the influence of Ovaprim.

2. Material and Methods

Live species of *Channa gachua* were collected from Kham river, near Aurangabad (M.S) India. Fish were collected during period of March 2007 to September 2008. They were brought to Laboratory, weighted (35.6 to 298 gm and length 8.5 to 20.5 cm) and kept in freshwater aquarim. Ovaprim was administered intraperitoneally to fish at a dose 0.25ml/kg (Syndel laboratory, Canada). A single dose is normally sufficient to induce maturation (0.25ml/kg).

3. Results

The testis of *Channa gachua* consists of germinal tissue and intermingles with connective tissue (Fig. 1A). The germinal tissue was disposed into chord–like testicular lobules containing spermotogonia and few sertoli cells The interlobular connective tissue also contains interstitial cells and blood capillaries. The spermotogonia were rounded in shape with rounded nuclei and prominent nucleoli. The cell boundaries, nuclear boundaries, and darkly stained granules were distinctly visible (Fig. 1B). The sertoli cells were found amongst spermotogonia were irregular shaped with well defined nuclei.

In present study, activation of sertoli cells and Leydig cells after 12 hrs, spermatogonial division after 24 hrs and lobular organization after 72 hrs were achieved by injecting a single dose of Ovaprim (Fig. 1D). After 72 hrs when the primary spermotogonia and secondary spermotogonia along with sertoli cell organize themselves in such a manner that a quite distinct lobular structure with the lumen was formed as compared to controlled (Fig. 1C). The interlobular connective tissue was reduced.

Spermatogonia have a sheet of cytoplasm around large rounded (Fig 2B), homogeneneously dense nuclei compared to control (Fig 2A). Single sometimes double nucleoli with dense granules were observed. Sertoli cells were found surrounded the spermotogonia, whether the cysts were in cluster of the seminiferous lobules. They posses irregular nuclei and contain round lipid globules. Rounded but sometimes elongated Leydig cells were disposed

singly or in groups at the periphery of the testicular cysts separated by basal lamina along with fibroblast cells and other connective tissues. The testicular organization remains the same but the spermotogonia were increased (Fig. 2D). Compare to control (Fig. 2C). Sertoli cells were elongated with an irregular nucleus containing more electron dense material towards its periphery. Sertoli cells were received invaginations of spermatogonial cytoplasm indicating very close physiological association between them Leydig cells were further activated.

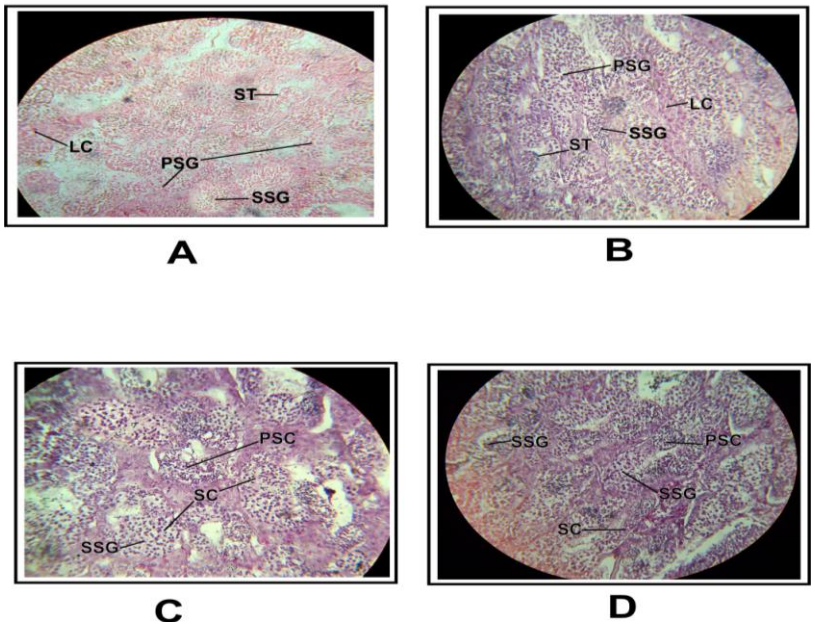

Plate No. 1 T.S.of Testis. A- Controlled (Preparatory Phase), Note the connective Tissue (CT). Note the less No. of primary spermatogonia (PSG), B- Injected (Preparatory Phase) Note the primary and secondary spermatogonia (PSG and SSG) abundant in No. and large in size, Dark stained indicated the large secretion of gonadotropin, C- Controlled (Pre-spawning Phase) Less No. of Secondary spermatogonia (SSG), D- Injected (Pre-spawning Phase) Secondary spermatogonia (SSG) and primary spermatocyte (PSC) more in No. Sertoli cell (SC) and leydig cell (LC) becoming active with better cytoplasm.

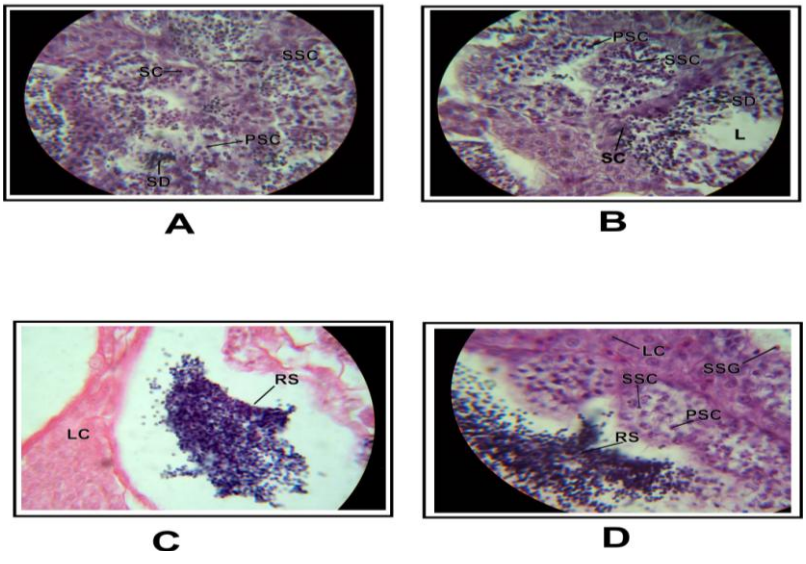

Plate No. 2 T.S. of Testis. A- Controlled (Spawning Phase) Less No. of primary and secondary spermatocyte (PSC and SSC). Note signs of secretary activity, B- Injected (Spawning Phase) Elongated sertoli cell (SC). Note the association between secondary spermatocyte and sertoli cell. Leydig cell with better cytoplasm. C-Controlled (Post-spawning Phase) Note the No. of residual sperm. Leydig cell with less cytoplasm. D- Injected (Post-spawning Phase) Note the No. primary secondary spermatocyte and residual sperm.

Seventy two hours after injection, spermatogonia were produced. These cells have dense nuclei with heterogenous distribution of electron dense granules. The cellular bridges between these cells were also observed. Sertoli cells were much elongated. The lipid globules were usually present in the sertoli cells. Leydig cells at this stage were large and found in the groups and assume characteristics of steroids synthesizing cells.

The testicular lobules contain spermatogonia and sertoli cells. Early spermatogonia took part in the cysts formation. The sertoli cells were found surrounded the germ cells and located in clusters filled up the spaces in chord like lobules. With increased spermatogonial division, and number and size of testicular cysts were increased and groups of sertoli cells becomes localized in the centre. Further, increased in the size of cysts took places due to division of spermatogonia. As the result, central sertoli cells were pushed apart leaving a gap in between, in the shape of tubular lumen. The testicular lobules thus formed consist of cysts containing spermatogonia surrounded by sertoli cells with a lumen in the centre and Leydig cells were lying just outside the cysts.

4. Discussion

The testis in most teleosts consists of compact paired structures lying in the abdominal cavity and composed of mass elongated, branched tubular structure with thin fibrous walls which lack a permanent lining, seminiferous epithelium and because of this reason , they are generally referred to as lobules, crystal or canals (Lofts,1968). On the basis of distribution of spermatogonia and spermatogenetic pattern, two kinds of testicular structures namely, tubular and lobular types have been identified (Hoar 1969; Billiard *et al.,* 1982; Nagahama 1983; 1986; Redding and Patino, 1993).

The pituitary gonadotropic function seems to be responsible for suspended maturity in male *Channa gachua* in river. Successful attempts have been made to induced sexual maturity in *Channa gachua* by means of hormonal injections. Colombo *et al.,* (1987) and Khan *et al.,* (1987) induced spermatogenesis and production of spermatozoa in European eels by administering a single dose of hCG after 1month and 3 months, respectively. Sugimoto and Takahashi (1979) have shown that interstitial (leydig) cells were activated in the testis of Japanese eel during hCG induced maturation. Recently, Miura *et al*., (1991a) had induced sexual maturation in Japanese male eel with in 18 days by administering a single dose of hCG. The histoarchitecture of testes after 6, 12, and 18 hours of treatment remained unchanged. After 24 hrs of hormonal injection, the dramatic changes took place and lobular organization after the 72 hrs. Thus the pituitary-gonadotropic function was restored by exogenous administration of Ovaprim resulting in the induction of spermatogenesis.

The structure and deposition of spermotogonia were similar to the description of earlier workers (Gresik *et al.,* 1973; Sugimoto 1979; Grier 1975; Billard 1984; Colombo *et al.,* 1987; Miura *et al.,* 1991a). In *Channa gachua* primary spermatogonia were always surrounded by sertoli cells, while secondary spermatogonia were restricted to testicular cysts and continue to divide until spermatozoa formation. Each cysts was enclosed in a covering of sertoli cells and the cells extended in between and remains in close contact with spermatogonia. Clusters of sertoli cells were also occured inside the testicular lobules. The leydigs cells occurring singly or in groups always lies the outside the interstitial cells separated by basement membrane.

The sertoli cells in the teleost testis perform several functions including support and structural organization of the cysts, lobules and tubules help in the formation of spermatogonia and eventual conversion of metabolites and hormones towards the germ cells or central cavity phagocytosis of germ cells and in isolation of cysts compartments beyond the spermatocytic stage (Billard, 1986). These cells have also been implicated with steroid production in certain species (Weib 1969; Bara 1969; Van den Hurk *et al.,* 1980). The sertoli cells remains in very close and direct association with germ cells which they support physically and nurture by modifying the chemical environment (Redding and Patino, 1934). The sertoli cells becames active within 12 hrs of Ovaprim treatments as evidenced by increasing cell and nuclear size, organization and distribution of dense globule material. The peak activity was achieved after 72 hrs.

The Leydig cells were typically interspersed in the connective tissue surrounding germ cells–sertoli cells unit and their primary function is to produce steroids needs for gametogenesis. These are characteristically steroids–producing cells in *Channa gachua* testis which becomes elongated after Ovaprim treatments. The Leydig cells in the present study also became activated within 72 hrs synthetic hormones treatments. Same results were found by Sugimoto and Takahashi, 1979; Colombo *et al.,* 1987; Miura *et al.,* 1991b; Follinius and Porte, 1960.

It might be concluded that, Ovaprim first stimulated androgen; testosterone and mainly 11-kitotestosterone in turn induce spermatogonial proliferation and organization of testicular lobules with a wide lumen within a 72 hrs. The sertoli cells remain in close contact with the spermatogonia, and maintained supply of metabolites and other substances later. They also took part in the lobular organization and formation of its lumen. This further strengthened our earlier conflict regarding the stimulatory effect of Ovaprim on the induction of spermatogenesis and sexual maturation.

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