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Research Article

Tentative Differentiation of Interstitial Cells during Preparatory Phase of *Heteropneustes fossilis* (Bl.) Under Long Photoperiod

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Abstract: Present paper deals with the impact of a long photoperiod (18 h) per day for 45 days on steroid secreting interstitial cells during preparatory phase of a freshwater cat fish, *Heteropnuestes fossilis* (Bl.). Depending upon the histocytological structure and secretory status, interstitial cells have been grouped into four categories revealing their corresponding functional status.

Keywords: *Heteropneustes fossilis,* photoperiods, preparatory phase of the reproductive cycle

INTRODUCTION

It has been conclusively reported that interstitial cells are present in all the teleost species, as well as cyclostomes, elasmobranchs, dipnoans and latimeria (Marshall and Lofts, 1960). An endocrine function of these cells was suspected much earlier in fishes. The role of photoperiod as an environmental factor controlling reproduction in fishes has been advocated by various workers (Sathyanesan, 1959; Crim, 1982; Borg *et al*., 1986; Duston and Bromage, 1986, 1987 and1991; Bromage *et al*.,1993; Bromage *et al*., 2001; Amano *et al*., 1994,1995; Bjornsson *et al*., 1998; Bon *et al*. 1999 and Campus *et al*., 2003), though direct evidence that they are the main source of testicular androgen has emerged comparatively recently (Campus *et al*., 2003; Folenius, 1968; Hyder, 1970; Nicholis and Graham,1972; Gresik *et al*., 1973; and Poncin, 2006). These cells secrete androgen under the influence of pituitary gonadotropins. Testosterone synthesized by these cells under the influence of LH, acts as the principal stimulus for the germ cells differentiation in the tubules. The secretary behavior of these cells shows functional parallelism with the entire process of spermatogenesis and spermiation. This aspect of reproductive physiology was reviewed by Marshall and Lofts (1960); Sathyanesan (1959); Rai (1965); Hyder (1969); Belsare (1973); De Valming (1974); Guraya (1976); Pandey and Mishra (1981); Mishra and Pnandey (1984 a, b) and Pandey and Agarwal (1988).

However, the earlier workers did not furnish definite proof neither regarding the occurrence of these cells nor have paid attention to study their cellular architecture and secretary status (Hoar, 1955 and Dodd, 1960). Present study, therefore, has been undertaken to accentuate the process of spermatogenesis by subjecting the fish *H. fossilis* to a long photoperiod (18 h). Our study is seemingly of great value in recording the testicular events corresponding such changes that are shown by interstitial cells. Such a tentative differentiation of these cells may thus provide a remarkable scalar index for judging the testicular activity in fishes.

MATERIALS AND METHODS

Freshwater cat fish *H. fossilis* were collected from Ramgarh lake and acclimatized for a week in the laboratory conditions. Adult and healthy male fishes were divided into two groups (control and experimental group), each containing 30 individuals. Each group of fishes were transferred into glass aquaria filled with equal amount of water. Temperature in all the aquaria was almost same and did not vary more than ±1°C. Fishes were given shrimp powder mixed with flour in a ratio of 1:2 as food every day. Feeding and renewal of water was done every day during lighted period.

The exposure of light to both control and experimental group was done in a wooden dark chamber covered with a black cloth to make it light proof. The chamber was filled with a 4' fluorescent tube of 40 watts. The control fish were exposed to light for 10 hours (from 6.00 A.M. to 4.00 P.M.) whereas the experiment fish were exposed for 18 hrs (from 4.00 A.M. to 10.00 P.M.). The timings were maintained through automated timer fitter in the circuit.

The preparatory phase, a period when the fish shows over all growth and cellular multiplication in their gonads is thus the most suitable period for this study.

After exposing the fishes (both control and experimental) for 45 days they were weighed and

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immediately dissected. Testes were taken out, weighed and fixed in Bouin's fluid and Picro-Mercuro Formol (Pandey, 1979). After routine paraffin embedding sections, were cut at 6 µm and stained with heidenhains iron haematoxylin.

RESULTS

The interstitial cells are present in the inter lobular spaces of the testes and are structurally different from that of the germ cells. The degree of activity of these cells is manifested through their hypertrophy or hyperplasia during the reproductive cycle. However, cell division has not been observed in these cells.

Depending on the secretary status and functional involvement in the spermatogenesis, these cells can be tentatively studied under four categories as follows:

Category I: The cells belonging to this category are characterized by their round, well defined boundaries with more or less centrally placed nucleus having few nucleoli. The cytoplasm shows extremely fine granulation and is rather hyaline. These characteristics indicate that these cells are in a state of active hormone synthesis and yet to start their secretion.

Category II: Cells belonging to this category are more or less identical to those of the cells belonging to category I. However, their nucleus appears to be shifted towards periphery and the cytoplasm incorporates darkly stained heavy granulation in which even a few vacuoles may also be marked. Presence of such vacuoles and certain degree of degranulation indicates that they are now involved in active secretion. They appear to show a trend of a hyperplasia.

Category III: Cells of category III show radical differences from the earlier two categories. Their shape shows a change from circular to oval outlines. Nucleus becomes more chromophillic with the cytoplasm showing heavy granulation. Some of these cells show cytoplasmic concentration being pushed to one side in a crescent form. Increased degranulation and vacuolization point towards an enhanced secretary state.

Category IV: The cells of category IV are characterized by a more a less circular to oval form. Some of them even look fusiform or shrunken. Their nuclei show pycnosis and the secretary material is almost exhausted, showing no sign of either synthesis or secretion.

The testes of *H. fossilis* shows an uniform tempo of spermatogenesis throughout the year. However, an increased rate of spermatogenesis has been recorded in those months which correspond with the active phase of female fish. These observations in the present study have been confirmed from the quantitative studies of these interstitial cells.

Fig. 1: Showing interstitial cells with the dominance of category I (→), Category II (→) and category III (- - -►). (Control×40)

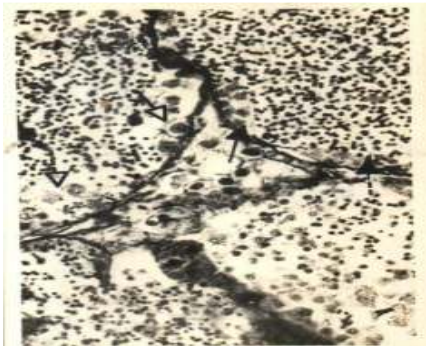

Fig. 2: Showing a mixed population of different categories of interstitial cells (→)**, (**Control×40**)**

A correlative study of the aforesaid types of interstitial cells with that of spermatogenesis under the long photoperiod along with the respective control is described as under:

Control group: Due to active growth and division of germ cells in the preparatory phase, the lobules are inflated, due to which the interlobular space is compressed and consequently become thin containing a low percentage 2±0.03% of interstitial cells. In this group they show a mixed population of category I, II and III (Fig. 1and 2). More or less 50% of these cells belong to category I, showing hyaline cytoplasm with small centrally placed nucleus and circular outline. Almost structurally similar to them, category II calls are less about 25-30% show massive granulation with large size. Some of them incorporate cytoplasmic vacuolization showing a change in their shape and belong to category III.

The overall picture, therefore, reveals that the steroid synthesis is appreciably to augment the steady spermatogenesis. Experimental group (18L:6D) under this long photoperiod of 18L:6D, the interstitial cells are 3±0.08%. There is a dominance of category II over category I. However, countable number category III and IV may also be seen at the outskirts of the stroma.

Fig. 3: Showing lobules having all the categories of interstitial cells with the dominance of category II (→), category I (→), category III (- - -►) are also present which show hypotrophy and hypolasia. (18L: 6D×40)

Fig. 4: Showing degranulated and vacuolated pycnotic interstitial cells (→). (18L: 6D×40)

Later categories of these cells have undergone pycnosis whose size has been appreciably reduced and consequently become fusiform. The percentage of degranulated and vacuolised category III cells along with prominent vascularity around cortical lobules indicate an increased steroid demand possibly to accomplish early spermatogenesis (Fig. 3 and 4).

DISCUSSION

The relative percentage of interstitial cells and lobular picture reveals that a long photoperiod is stimulatory for both the early spermatogenesis as well as steridogenesis.

A perusal of earlier literature (Hoar, 1955; Dodd, 1960 and Craig, 1931) reveals that workers have not arrived at any unanimous inference regarding the occurrence of interstitial cells and their role in teleost testes. Possibly, therefore, little attention has been paid to study the cellular features pointing the secretary status of these cells in relation to the spermatogenesis in teleost testes. Interstitial cells, as in other vertebrates are known to play significant role in controlling the reproductive physiology in fishes as well.

Few workers (Craig, 1931) suggest lymphatic origin of these cells while nutritive or trophic role has been assigned to them by several others (Gokhale, 1957 and Polder, 1971) respectively. Sathyanesan (1959); Crim (1982); Borg *et al*. (1986); Duston and Bromage (1986, 1987, 1991); Bromage *et al*.(1993); Bromage *et al*. (2001); Amano *et al*. (1994, 1995); Bjornsson *et al*. (1998); Bon *et al*. (1999) and Campus *et al*. (2003), though direct evidence that they are the main source of testicular androgen has emerged comparatively recently (Campus *et al*., 2003; Folenius, 1968; Hyder, 1970; Nicholis and Graham,1972; Gresik *et al*., 1973; and Poncin, 2006 ; Sunundaraj, 1960; Rai, 1965 and Lehri, 1967). Though others have reported vacuolization in these cells but without attributing any precise function to them while some others (Rasquine and Hafter, 1951; Swarup, 1959; Barr, 1963; Srivastava and Rathi, 1959; Nayar and Sundarraj, 1970 and Neaves, 1973) could not record any evidence of glandular activity in these cells. One of the reason for such divided option may be attributed to the poorly persuaded testicular cytology in the earlier experiments. Nevertheless, in the present study, the stimulatory role of photoperiod particularly of a long photoperiod during the preparatory phase has been found to produce interesting histocytological change leading to conclusive result, regarding their tentative differentiation and consequent secretary status.

Long photoperiod has been found to be more stimulatory for the testicular activity which is regulated and governed by the interstitial cells which show pronounced changes in their cellular architecture and contour depending upon the tempo of their secretary involvement.

In the beginning these cells show their well defined outline and cytoplasm with very fine granulation indicating the commencement of the steroid synthesis. Soon after, their cytoplasm becomes impregnated with darkly stained heavy granulation with few vacuoles pointing their secretary involvement. Though at a low ebb. Later on, the contour of these cells gradually changes from circular to oval with increased vacuolization and degranulation indicating an enhanced tempo of secretary activity. Finally their shape change to fusiform with pycnotic nulei and almost complete degranulation of the cytoplasm and consequent decline in their size.

The changing histocytology, cellular features and corresponding specific activity of these cells can be thus adopted as a beneficial index in the assessment of the degree of spermatogenesis.

It has correlated the presence of steroid synthesizing interstitial cells at the site where secondary spermatogonia are formed, suggesting a functional relationship (Hurk *et al*., 1978).

A more detailed observation has been recorded where interstitial cells seem to be associated with the developing germ cell cycle and lobular cycle of *Colisa fasciatus* (Pandey and Mishra, 1981).

In the present study, a long photoperiod has been found to be highly stimulatory for manifesting histocytological changes and the steroidogenic activity in the interstitial cells. Though a comparative study on fish testes under different photoperiod is highly scanty (Rowah, 1925) yet in quails and in Bufo marius has observed that increased activity of these cells corresponds to the faster testicular maturation under a long photoperiod and vice versa (Saxena and Lal, 1981). It has also been recorded a significant change in the secretary activity of seminal vesicle in *H. fossilis* under a long photoperiod (Garg and Sundarraj, 1985).

Similarly reports reveal the stimulatory impact of long photoperiod on the testes of *Gasterosteus aculeatus* (Borg *et al*., 1986)**.** Further has observed increased blood GTH level in the gold fish, rainbow trouts and carps under the influence of photoperiod along with other environmental factors (Peter, 1981). Such endocrine fluctuation has also been recorded by few (Crim, 1982).

However, a similar study dealing with the hisocytological changes in the leydig cells under a sensitive photo regime has hardly been pursued. Nevertheless, the present work clearly shows that interstitial cells undergo well marked histocytological changes during the period of active spermatogenesis in fishes and owing that they have been classified under four categories. Further, the present authors are inclined to emphasise that since the preparatory phase is a period of active cellular growth and development, therefore, more receptive for a long photoperiod incorporating pronounced change in these cells.

It may, therefore, be inferred that interstitial cells undergo such well marked cytometric and histocytological changes that show positive relationship with the degree of their functional involvement and consequent testicular activity.

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