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Annual Cyclical Changes in the Testicular Activity of an Indian Freshwater Major Carp, *Labeo Rohita* (Hamilton)

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Abstract: *Testicular development of Labeo rohita was associated with increasing day-length and temperature, whereas testicular maturity and spermiogenesis during spawning phase seem to be correlated with the lowering of water temperature, attributable to rainfall. Different male germ cells identified on the basis of histological and cytological characteristics in the testicular lobules of Labeo rohita have been grouped into primary spermatogonia, secondary spermatogonia, spermatocytes, spermatids and spermatozoa. The seasonal changes of the testis in Labeo rohita have been described according to its morphological peculiarities as well as to its variations in gonadal volumes, GSI values and percentages of the different male germ cells occurring in the testicular lobules. Consequently, the entire testicular cycle in Labeo rohita may be categorised into 5 distinct phases viz., resting, preparatory, prespawning, spawning and postspawning.*

Keywords: *Labeo rohita*, reproductive phases, testes, GSI and Spermatogenic stages

1. INTRODUCTION

Photoperiod and temperature are important environmental factors regulating gonadal development and maturation and other reproductive events in most of seasonally breeding teleosts (Lam, 1983) including cyprinids (Hontela and Stacey, 1990). Long photoperiod and increasing temperature were found to be favourable for gonadal development in, *Heteropneustes fossilis* (Sundararaj and Vasal, 1976), *Clarias batrachus* (Singh and Singh, 1983) and *Cirrhinus mrigala* (Singh and Singh, 1984). The spawning of Indian major carps was correlated with the rainfall and lowering of temperature (Sinha *et al.*, 1974). Normally five spermatogenic stages, *i.e* primary spermatogonia, secondary spermatogonia, spermatocytes, spermatids and spermatozoa (sperm) are described in the testes of fish (Agarwal, 1996). The morphocytochemical changes in interstitial Leydig cells have been correlated with steroidogenesis in fishes (Guraya, 1976; Kanwar and Sheikhar, 1978). In view of lack of similar correlative information in male of *Labeo rohita*, the present study was undertaken.

2. MATERIAL AND METHOD

Monthly collections of the fishes were made for one complete year. Length and weight of each individual and testes were recorded and gonadosomatic index was calculated by formula: weight of testes ×100/ weight of body. Fixation of testes was done in Bouin's fluid for 24 hours. Sections were cut ranging from 6-10µ and stained by Delafield's haematoxylin, counterstained by eosin. The diameters of the testicular lobules were measured by the oculometer standardized against a stage micrometer on random sampling basis.

3. RESULT

3.1. Histological Changes in the Testes

The testes of Labeo rohita are paired structures lying in the abdominal cavity. Each testis was attached to the abdominal cavity by a thin layer of membrane called as mesoarchium. Each testis

was made up of a number of lobules. The interlobular space contains connective tissues, blood capillaries and interstitial cells (Fig 1and 2). The lobules in the peripheral area of the testes show lobule boundary cell-like structures, but those in the central area did not possess such cells. The lobular wall shows variation in the thickness during different phases of reproductive cycle. During testicular development a variable number of spermatogenic cysts at different stages are found in each seminiferous lobule.

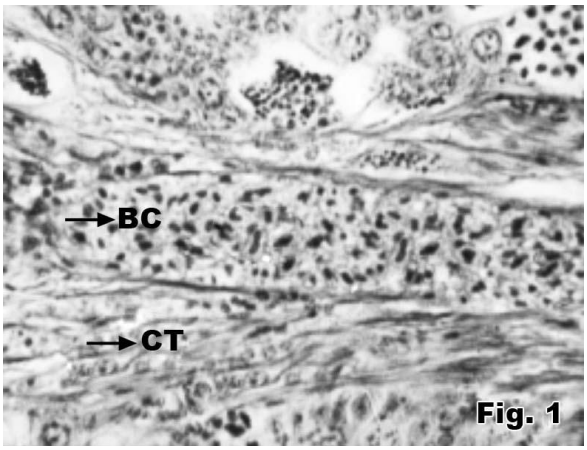

Fig 1. *Photomicrograph of section of testis showing connective tissue (CT) and blood capillaries (BC) X400.*

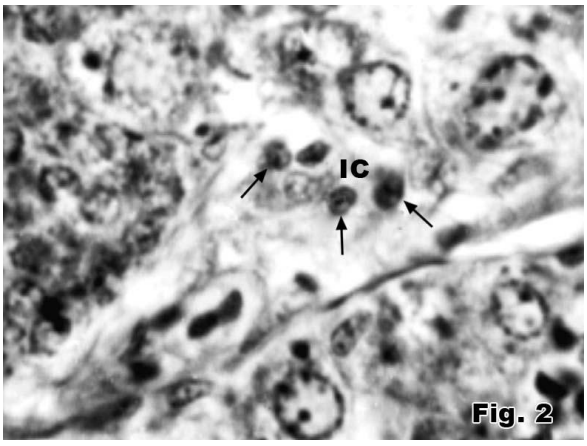

Fig 2. *Photomicrograph of section of testis showing Interstitial cells (IC) in the interlobular space X400.*

The different stages found during development and growths of testis are:-

1. Primary Spermatogonia (PSG)

These are larger in size (diameter 6.48 to 7.80 µm), located along the periphery of the lobule. The primary spermatogonia are prominent with cell boundaries and have a large spherical nucleus with prominent and darkly stained nucleolus (Fig 3).

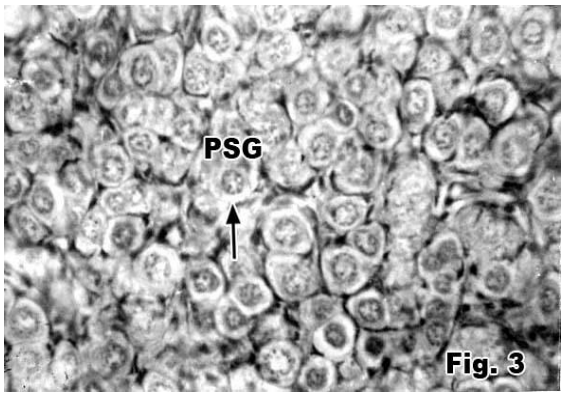

Fig 3. *Photomicrograph of section of testis showing prominent Primary spermatogonia (PSG) (arrow) with cell boundaries and large nuclei X 400.*

2. Secondary Spermatogonia (SSG)

These cells are slightly smaller (diameter 4.40 to 5.90 µm) than primary spermatogonia. The nuclei are darkly stained with prominent nucleolus and cytoplasm was lightly stained (Fig 4).

3. Spermatocytes (SC)

SC was still smaller in size than secondary spermatogonia (diameter 2.50 to 4.25 µm) and contains thick chromatin matter and deeply stained nuclei (Fig 4)

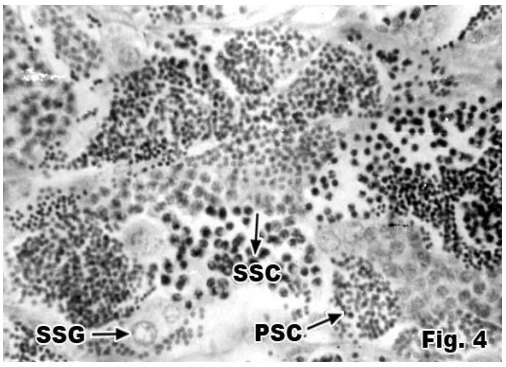

Fig4. *Photomicrograph of section of testis showing Secondary spermatogonia (SSG), Primary spermatocytes (PSC) and Secondary spermatocytes (SSC) X 400.*

4. Spermatids (ST)

The spermatids are smaller (diameter 1.92 to 2.18µm) and spherical. They have dense chromatin in their nuclei. They look like dark spots and stain brightly (Fig 5).

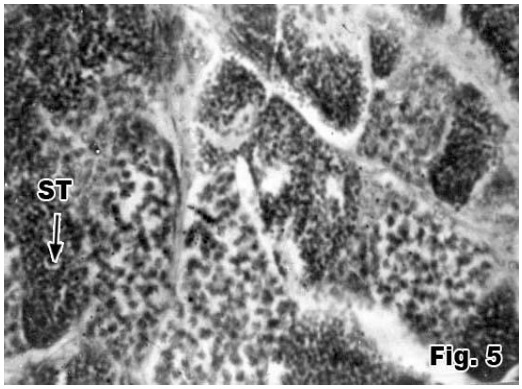

Fig 5. *Photomicrograph of section of testis showing Spermatids (ST) X400.*

5. Spermatozoa (SZ)

Spermatids are converted into motile spermatozoa. In spawning period the testes are full of spermatozoa. They migrate to the centre of the lobules after their formation. The lobule boundaries are not prominent and the average diameter of spermatozoa is about 0.68 to 1.28 µm (Fig 6).

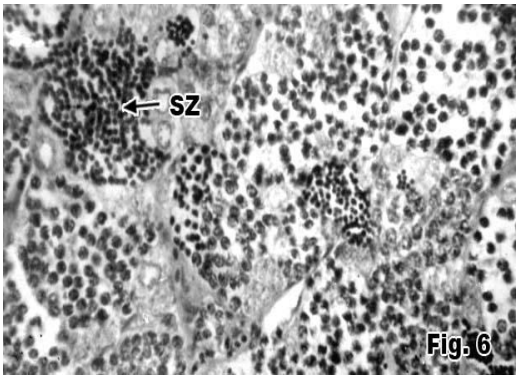

Fig 6. *Photomicrograph of section of testis showing Spermatozoa (SZ) X400.*

3.2. Seasonal Changes in the Testis

On the basis of the seasonal changes in the testes, variation in the GSI (Table 1), lobule diameter of the testes (Table 2) and percentage of spermatogenic stages present in the testis (Table 3), the annual cycle in the male has been divided in to the following five phases:-

1. Resting (November- January)

In resting phase, the testes are very small and thread like. GSI value is 0.087±0.004. This phase was dominated by primary spermatogonia and few secondary spermatogonia which constitute about 97.66±0.16% and 2.33±0.05% respectively. The average diameter of seminiferous lobules was 51.39±2.00 µm. Primary spermatogonia are larger in size in comparison to secondary spermatogonia and are located along the periphery of the lobule. In primary spermatogonia nucleus was spherical with prominent nucleolus (Fig.7).

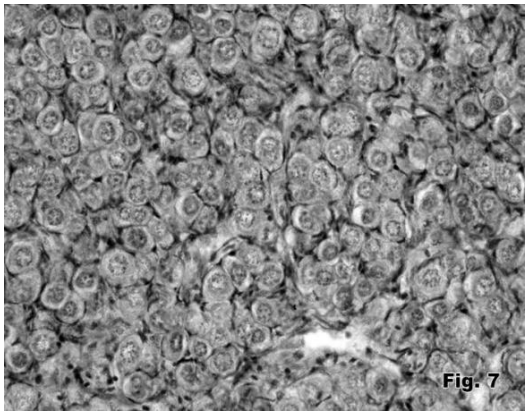

Fig 7. *Photomicrograph of section of testis in the resting phase showing Primary spermatogonia (PSG) X400.*

2. Preparatory (February- March)

In this phase testes increases in size and vascular supply also increases. GSI value was 0.285±0.058. In preparatory phase, PSG, SSG and SC are visible which constitute about 41.25±0.81%, 39.50±0.28% and 19.25±0.54% respectively. The average diameter of lobules increases to about 90.82 ± 6.98 µm. A prominent interlobular demarcation is observed in this phase (Fig.8).

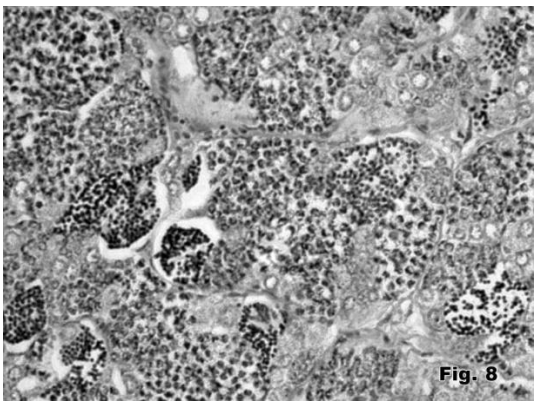

Fig 8. *Photomicrograph of section of testis in the preparatory phase showing various stages such as PSG,PSC and SSG X400.*

3. Prespawning (April- June)

In this phase there is rapid increase in GSI in this phase. The GSI was 1.71±0.08. Diameter of lobule increases to about 199.40±15.53 µm. This phase was predominated by spermatocytes, spermatids and spermatozoa which constitute about 36.41±0.71%, 19.83±0.21% and 37.0±0.64% respectively. This phase also has PSG and SSG which are about 1.58±0.02% and 5.08±0.09% respectively. In this phase spermatocytes and spermatids are found in clusters (Fig.9).

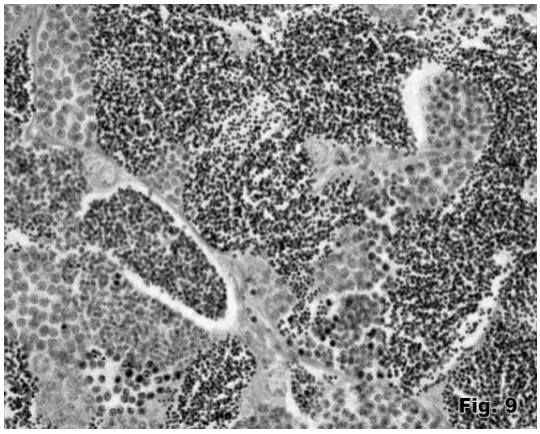

Fig 9. *Photomicrograph of section of testis in the prespawning phase showing SSC, ST and SZ X400.*

4. Spawning (July- August)

In this phase testes bulge out in abdominal cavity occupying one third of the cavity. Vascular supply increases and testis become red in color. GSI value in this phase was about 2.02±0.18. Wall of seminiferous lobules was not very clear due to rupture of seminiferous lobules which are filled with spermatozoa. In spawning phase, spermatids and spermatozoa are prominently noticed which constitute about 6.25±0.89% and 93.75±1.09% respectively. The average lobule was about 221.50±25.28 µm in diameter (Fig.10).

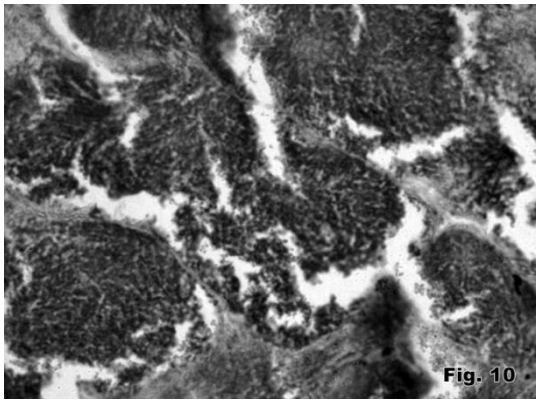

Fig 10. *Photomicrograph of section of testis in the spawning phase showing seminiferous lobules filled with SZ X 400.*

5. Postspawning (September- October)

In this phase, size of the testis decreases, GSI value also falls down and was about 0.53±0.045. Testis become mostly empty and shows a few stages of spermatogenesis near the lobule wall i.e. primary spermatogonia and in the centre of lobule leftover spermatozoa are seen. The percentage of primary spermatogonia and spermatozoa was 83.50±0.87% and 16.50±0.57% respectively. The average lobule diameter in this phase was much reduced to about 77.94 ± 4.08 µm (Fig. 11).

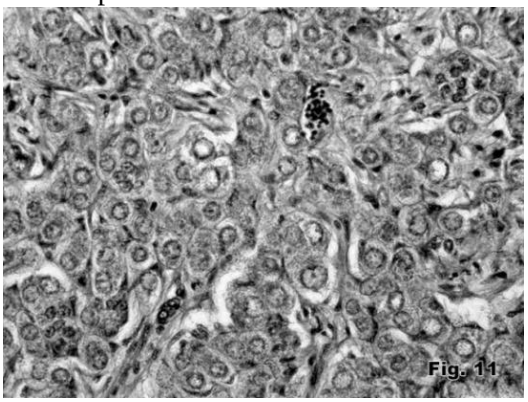

Fig 11. *Photomicrograph of section of testis in the postspawning phase with pre-dominance of PSG with left over spermatozoa in the lobule X400.*

Table 1. *Seasonal gonadosomatic indices of Male Labeo rohita.*

Phases	Months	GSI	Mean
Resting (Control)	November	0.081±0.008	0.087± 0.004
	December	0.051± 0.012	
	January	0.120± 0.003	
Preparatory	February	0.130± 0.007	0.285±0.058 P < 0.01
	March	0.440± 0.109	
Prespawning	April	0.506± 0.046	1.71± 0.08 P < 0.01
	May	1.505±0.09	
	June	3.145 ±0.117	
Spawning	July	3.00± 0.425	2.02± 0.181 P < 0.01
	August	1.04 ±0.137	
Postspawning	September	0.59 ± 0.075	0.536±0.045 P < 0.01
	October	0.089±0.015	

Values represent mean ± SE of observation based on 140 fishes.

Table 2. *Average lobules diameter of the testis during different phases of the reproductive cycle in Labeo rohita.*

Phases	Months	Testicular lobule diameter in µm	Mean
Resting (Control)	November	54.71± 3.36	51.39 ± 2.00µm
	December	49.06 ± 4.05	
	January	49.91 ± 1.58	
Preparatory	February	68.84 ± 1.20	90.82± 6.98 µm
	March	92.80 ± 2.01	
Prespawning	April	137.20 ± 1.50	199.40 ± 15.53 µm
	May	186.10 ± 3.44	
	June	274.83 ± 4.55	
Spawning	July	252.60± 2.28	221.50 ± 25.28 µm
	August	130.40 ± 2.05	
Postspawning	September	86.44 ± 1.99	77.94 ± 4.08µm
	October	69.44 2.65	

Values represent mean ± SE of observation based on 140 fishes

Table 3. *Percentage of primary spermatogonia, secondary spermatogonia, spermatocytes, spermatids and spermatozoa during different phases of the reproductive cycle in Labeo rohita*

Phase	PSG	SSG	SC	ST	SZ
Resting (Control)	97.66 ± 0.16	2.33 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Preparatory	41.25 ± 0.81 NS	39.50 ± 0.28 P<0.01	19.25 ± 0.54 P<0.01	0.00 ± 0.00	0.00 ± 0.00
Prespawning	1.58 ± 0.02 NS	5.08 ± 0.09 P<0.01	36.41 ± 0.71 P<0.01	19.83 ± 0.21 P<0.01	37.0 ± 0.64 P<0.01
Spawning	0.00±0.00	0.00±0.00	0.00±0.00	6.25 ± 0.89 P<0.01	93.75 ± 1.09 P<0.01
Post spawning	83.50 ± 0.87 NS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16.50 ± 0.57 P<0.01

Values represent mean ± SE of observation based on data on 140 fish.

NS- Non Significant

4. DISCUSSION

The annual cyclic changes occur mainly due to maturation of testis. The shape, size, colour and length of the testes of *Labeo rohita* undergo variations during different reproductive phases of life cycle. Similar changes have been reported in other teleosts by other workers (Umeda and Hesengawa, 1984).

The testes attain maximum weight between July to August in spawning season and they show maximum GSI (2.02 ± 0.181) during this phase. It is then followed by a rapid decline. The GSI values during resting phase is very low (0.087±0.004), the seminiferous lobules are small and they are mostly predominated by primary spermatogonia (97.66%). There is a gradual increase in the GSI during preparatory phase (0.285±0.058). However, during this period different stages of spermatogenesis i.e. primary spermatogonia (PSG), secondary spermatogonia (SSG) and spermatocytes (SC) are present. Increase in the GSI is very rapid in prespawning phase (1.71±0.08). In this phase, the testes are predominated by spermatocytes (36.41%), spermatids (19.83%) and spermatozoa (37%). GSI attains its maximum peak during the spawning phase in the month of July (2.02±0.181). From the postspawning phase onwards, the males show a sudden fall in the GSI which becomes 0.53 ± 0.045. This low GSI in the postspawning phase is due to the discharge of milt. In this phase, the wall of seminiferous lobules ruptures and spermatozoa are released out.

In *Labeo rohita* spermatogenic activity starts from preparatory and continues upto spawning. In the spawning phase, testes are full of sperms. In late spawning, the seminiferous lobules are filled with resting germ cells. The testes are lobulated structures in *Barbus tor* (Tor) (Rai, 1965) and *Clarias batrachus* (Lehri, 1967). In Gadus (Gokhale, 1957), the testes may show coiling and convolutions due to which the surface shows protrusion. The paired glandular sac like structure sometime present in teleosts as outgrowth at the posterior end of vasa differentia have been variously referred to as seminal vesicles, accessory sex organs, accessory male organ or testicular lobes. Such so called seminal vesicles are well developed in *Heteropneustes fossilis* (Sundararaj, 1958) and *Clarias batrachus* (Lehri, 1967) which undergo seasonal changes related to the size of the testes (Nawar, 1959) and are responsible for storage (Nair, 1960). Such seminal vesicles are however not found in *Labeo rohita* and testis open directly into the sperm duct. Seminiferous lobules are separated from one another by partitions in the testes of *Labeo rohita.* These partitions become prominent in preparatory phase.

Spermatogenesis in the testis of fish comprises mitotic proliferation of spermatogonia, meiotic cell divisions, spermiogenesis (Billard, 1984) and formation of spermatozoa (Winkoop and Timmermans, 1990). In *Labeo rohita* sperm mother cells line the wall of the lobules and subsequent stages descend down towards the centre of the lobules. Spermatogenesis commences in the month of June and testes are full of spermatozoa in July and August month.

Interstitial cells undergo seasonal changes according to the reproductive cycle (Follenius, 1953) while some teleosts do not show secretary activity as well as cyclic changes in the interstitial cells (Sundararaj, 1960). Interstitial cells in between the seminiferous lobules are evident in *Labeo rohita* from prespawning phase.

Swarup (1958) has reported that the testes of *Gasterosteus aculeatus* remain mature at any time of the year but their functional maturity is attained only in the breeding season (April-May). In *Heteropneustes fossilis* (Ghosh and Kar, 1952) there is no clear seasonal testicular cycle, but in the same species distinct seasonal periodicity in testis is reported by Hunge and Baile (2003) and maximum GSI is reported during spawning phase for this fish. In *Labeo rohita*, the testes are fully mature only during spawning phase (July-August) of reproductive cycle. The variation in view may be because of different environmental factors which play an important role in gonadal maturation and development. The maturation of testes in *Gasterosteus aculeatus* (Swarup, 1958) may be because of high temperature. From April to July maximum gonadal activity is reported for *H. fossilis* (Ghosh and Kar, 1952; Hunge and Baile, 2003). Temperature and photoperiod are involved in the regulation of recrudescence of testis and relative importance of these two factors varies with annual cycle (Garg, 1987).

Seasonal testicular changes are reported in largemouth bass, *Huro salomoides* (James, 1946) and in Cyprinid fish, *Notropis bifrenatus* (Harrington, 1957). Annual cyclicity in the weight and spermatogenic activity of testes and in the secretary activity of the seminal vesicle in *H. fossilis* is reported by Garg (1987). With changes in reproductive cycle, the testicular tissue shows distinct changes which are characteristic for each species (Nath *et al*., 1996). In African catfish *Clarias gariepinus,* spermatogonial multiplication and spermatocyte formation takes place when testicular steroidogenic system is highly active and responsive to GTH, whereas differentiation of haploid germ cells is accompanied by a reduced responsiveness to GTH and by secretion of several folds lowers androgen amounts per mg of tissue (Schulz *et al.,* 1996). In postspawning phase GSI of males of *Labeo rohita* falls and testes are regressed. Basic hormonal changes underpinning sexual maturation are reported in brown trout, *Salmo trutta* (Breton *et al.,* 1983), Chum salmon, *Onchorhynchus keta* (Ueda *et al.,* 1984) and in Coho salmon, *O. kisutch* (Leather-land *et al.,* 1982).

A period of testicular quiescence is a general feature of seasonally breeding teleosts (Rosenblum *et al.,* 1987). This quiescent period can be the resting period in *Labeo rohita* during November to January months when testes show, primary spermatogonia and secondary spermatogonia. Temperature is lowest during these months.

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