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CELLULAR PARAMETERS OF THE JUVENILE TESTES IN RELATION TO THE SECONDARY SEXUAL CHARACTERS (SSC) OF *MYSTUS (M) VITTATUS* (BLOCH) (SILURIFORMES: BAGRIDAE)

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Markandey Misra and Kamleshwar Pandey (1983) Cellular parameters of the juvenile testes in relation to the secondary characters (SSC) of *Mystus (M) vittatus* (Bloch). *Bull. Inst. Zool., Academia Sinica* **22**(1): 37-41. Histocytological testicular structures of juvenile *Mystus (M) vittatus* have been described to study the possible role of interstitial Leydig's cells in the differentiation of secondary sexual characters in this fish. Despite numerical increase and cytological prominence these cells fail to initiate the development of secondary sexual characters. This functional failure of the Leydig's cells in controlling the development of secondary sexual characters in juvenile fishes has been discussed.

Literatures on testicular histology and cytology are abundant (Turner, 1919; Van Oordt, 1925; Craig-Bennett, 1931; Matthews, 1938; Jones, 1940; Weisel, 1943; James, 1946; Cooper, 1952; Ghosh and Kar, 1952; Gokhale, 1957; Sathyanesan, 1959; Honma and Tamura, 1962, 1963; Rai, 1965; Khanna and Pant, 1966; Swarup and Srivastava, 1978, 1979; Pandey and Misra, 1981 and also reviews by Hoar (1957, 1969); Lofts (1968) and De Vlaming (1974) are now available. However, the information available on juvenile testicular histocytology and its relation to SSC remains all the more scanty. Present study thus aims a comprehensive account of the testicular parameters in the juvenile *Mystus (M) vittatus* particularly to assess cellular changes in the interstitial Leydig's cells related to the differentiating SSC.

MATERIALS AND METHODS

Samples of juvenile *Mystus (M) vittatus* collected from local Ramgarh lake in the month of September, were utilized for the present work. The weight (g) of fish and testes were recorded, small pieces of the testes were fixed in Bouin's (both aqueous and alcoholic) solution, Baker's Formol-Calcium and Picro-mercuro formol (Pandey, 1979) fixatives. Sections cut at 5-6 μm were stained with Heidenhain's haematoxylin, Heidenhain's Azan, Herlant tetrachrome and Sudan Black B for histological studies. The data for size of interstitial Leydig's cells in juvenile and at different phases of sexual cycle in adult fish was analysed statistically using analysis of variance and $P<0.001$ was taken as the limit of statistical significance (Table 2).

RESULTS

Adult *Mystus* (*M*) *vittatus* shows sexual dimorphism (Swarup and Swaroop, 1975) and males differ from females in having

1. well developed genital papilla and
2. a spear shaped thickening at the base of the caudal fin at the level of lateral line.

Morphology

The testes of juvenile *Mystus* (*M*) *vittatus* are small (1.5 ± 0.04 cm average in length), paired, whitish structure of 0.04 ± 0.003 g average in weight. Anteriorly they are free but united posteriorly and open to the exterior through urinogenital pore.

Histocytology

The juvenile testis consists of numerous seminiferous tubules bounded together by a thin covering of connective tissue. These tubules are separated by interlobular septa of connective tissues containing blood capillaries and interstitial Leydig's cells. The lobular wall is lined by a single layer of germinal cells. The lobules are 55.63 ± 3.31 μm average in diameter and display sperm mother cells, primary spermatocytes and secondary spermatocytes as chief germ cell types. Thus, except quantitative and certain cellular differences the juvenile testis ressembles in all respect with adult males.

Sperm mother cell: Sperm mother cells are of large (9.86 ± 0.33 μm average in diameter) size with indistinct cell boundary and a conspicuous nucleus and nucleolus. Their number recorded is $27.20 \pm 0.4\%$ which is higher than the adult specimens (Table 1).

Primary spermatocyte: Repeated mitotic division of sperm mother cells gives rise to these cells of 5.00 ± 0.44 μm average in diameter. Their cellular features indicate active division and are $31.80 \pm 0.3\%$, again a higher percentage in juvenile testis than the adult (Table 1).

Secondary spermatocyte: They are the smallest germinal cells (4.11 ± 0.06 μm average in diameter) and their number recorded is $34.60 \pm 0.39\%$ higher than those of adult counterpart (Table 1).

Interstitial Leydig's cell: They are (2.20 ± 0.04 μm average in diameter) spherical cells with a large nucleus located in the middle of thin non-granular cytoplasm. The cytoplasm without marked granulation appears to be smooth and hyaline showing frequent deep invaginations. These are either uniformly distributed or present in clusters in the interlobular septa and show positive reaction with Black dye. The interstitial Leydig's cells are $6.4 \pm 0.13\%$ in juvenile *Mystus* (*M*) *vittatus* while

TABLE 1

Showing a comparative testicular data in the juvenile and adult male *Mystus* (*M*) *vittatus*

Fish testis	Diameter of IC (μm)	Number of IC (%)	Number of SMC (%)	Number of PS (%)	Number of SS (%)	Number of Sptd/Sptz (%)	SSC
Juvenile	2.20±0.04	6.40±0.13	27.20±0.40	31.80±0.30	34.60±0.39	Absent	Not apparent
Adult							
Preparatory phase	2.25±0.05	4.80±0.26	26.85±0.03	27.65±0.41	16.80±0.37	23.90±0.39	+
Maturation phase	2.81±0.02	10.63±0.36	8.43±0.21	12.73±0.33	18.66±0.35	49.53±0.55	++++
Spawning phase	2.83±0.01	14.34±0.31	4.96±0.21	2.96±0.10	2.54±0.16	75.20±0.91	++++
Post-spawning phase	2.11±0.02	8.25±0.23	29.25±0.43	18.10±0.39	10.05±0.25	34.35±0.66	+++

IC, Interstitial cell; SMC, Sperm mother cell; PS, Primary spermatocyte; SS, Secondary spermatocyte; Sptd, Spermatid; Sptz, Spermatozoa; SSC, Secondary sexual characters. Each value represents the Mean±SE of 40 measurements.

TABLE 2
Analysis of variance for size of interstitial cell of juvenile and at different sexual phases of adult *Mystus (M) vittatus*

Sources of variance	Sum of squares	Degree of freedom	Mean square	Variance ratio	Level of significance
Between levels	4.9	4	1.22	122	$p<0.001$
Residual	0.5	45	0.01		
Total	5.4	49			

their per cent number differs in different phases of testicular cycle of adult fish. They are $4.80\pm0.26\%$ in preparatory phase (January-February), $10.63\pm0.36\%$ in maturing phase (March-May), $14.34\pm0.31\%$ in spawning phase (June-October) and $8.25\pm0.23\%$ during post-spawning phase (November-December) in the adult *Mystus (M) vittatus*. To a greater extent their cytological features, per cent number and diameter correspond to the preparatory phase of adult fish.

DISCUSSION

Cellular features of steroidogenic structures related to the SSC in teleosts have unfortunately could not draw much attention either of the endocrinologist or of cell biologists at least in the past. The reasons for this may be chiefly two folds, firstly, majority of teleosts do not depict sexual dimorphic features and secondly due to the lack of precise techniques, the steroidogenic tissue in fish testis remained a controversial structure for fairly long time and was almost in a dormant state. However, Marshall and Lofts (1956) and Lofts and Marshall (1957) have histochemically demonstrated that the endocrine elements in the teleost testes show either a typical vertebrate pattern or a pattern of lobule boundary cells. The interstitial Leydig's cells in the testes of juvenile *Mystus (M) vittatus* are found arranged in the typical vertebrate pattern corresponding to the former type. Lobule boundary cells according to Marshall and Lofts (1956), Lofts and Marshall (1957) and Robertson (1958) have been regarded as modified gland cells in the later period of gonadal maturation. However, in agreement to Oota and Yamamoto (1966), present investigation demonstrates distinctly differentiated interstitial cells in the stroma of juvenile testis of *Mystus (M) vittatus* and that they are to a great extent identical in form and distribution to the immature interstitial Leydig's cells of mammalian testis.

The nuptial colouration (SSC) in minnow *Phoxinus laevis* was for the first time found dependent to testicular hormone by Kopec (1918, 1928). This was further confirmed in stickle back *Gasterosteus pungitis* and *Gasterosteus aculeatus* by Van Oordt (1923, 1924), Van Oordt and Vandermass (1927), Bock (1928), Craig-Bennett (1931) and Ikeda (1933). Almost identical results were recorded by Niwa (1965a, 1965b) in medaka. Tozawa (1923) proved that the development of pearl organs of goldfish *Carassius auratus* are greatly controlled by testicular hormones. However, in the testes of juvenile *Mystus (M) vittatus* the interstitial cells responsible for the development of SSC are though more or less numerically equivalent and cytologically identical to its adult counterpart yet SSC remain undifferentiated. Such a functional diversity of these cells may thus be bimodal, firstly the secretion of interstitial cells might not achieve the threshold concentration to initiate the differentiation of SSC and secondly the testicular hormone may simultaneously be involved in overall development of the testis and consequent gonadal maturity. Gradually as the fish testis approaches maturation the secretion of interstitial cells achieve the threshold concentration initiating the differentiation of SSC which is being prominently marked at the onset of the breeding season.

During the differentiation of SSC in *Colisa fasciatus* only caudal fin appears to be sensitive to androgens while other fins remain unaffected (our unpublished data). Thus, the development of genital papilla and a spear shaped thickening at the base of the caudal fin during the breeding season, seemingly is due to the fact that when germinal elements acquire final development the secretion of interstitial cells is diverted to be utilized for the manifestation of SSC at this time. It has been observed during the course of this investigation that a decline in the number and cell size (diameter) of Leydig's cells (in the succeeding phases) dwindles the SSC. Up to a greater extent these cells in juvenile fish though numerically correspond to the similar cells in the preparatory phase of adults yet the SSC does not even appear. Our present investigation statistically compared with adult fishes during different phases of sexual cycle thus points that along with the per cent number of interstitial cells their size plays a decisive role in the development of SSC (Table 1). Broven from the above data it may, therefore, be inferred that a combined impact of the number and more so the diameter of these cells is significant and responsible for this functional aspect. Such cytometric variations perhaps make room for relevant subcellular changes which increase the secretory function of these cells and thus both the cytometric variations and cytoarchitecture may be studied together and not separately.

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條紋黃顙魚幼魚睾丸組織構造與萊氏細胞控制第二性徵之關係

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本研究報告 *Mystus vittatus* 幼魚睾丸之組織構造與其精間萊氏細胞控制第二性徵之相互關係。雖然在幼魚期間，萊氏細胞數目增生以及細胞形態構造有顯著變化，但並不引起第二性徵出現。關於萊氏細胞在此段期間，其功能未有顯現之原因，詳加討論。