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CYTOLOGY OF THE PITUITARY GONADOTROPHS, HISTOLOGICAL CHARACTERISTICS OF INTERRENAL AND CHROMAFFIN CELLS IN RELATION TO TESTICULAR ACTIVITIES IN MYSTUS VITTATUS (SILURIFORMES, BAGRIDAE) DURING GROWTH, MATURATION AND SPAWNING PHASES

M. Banerjee[1], S. Ghosh[1], P. Chakrabarti[1*]

[1]*Fisheries Laboratory, Department of Zoology, The University of Burdwan*
Burdwan-713104, West Bengal, India
*Corresponding author
E-mail: dr.pchakrabarti@yahoo.in

Interrenal and Chromaffin Cells in Relation to Testicular Activities in *Mystus vittatus* (Siluriformes, Bagridae) During Growth, Maturation and Spawning Phases. Banerjee, M., Ghosh, S., Chakrabarti, P. — The histological changes observed in the pituitary corticotrophs, gonadotrophs, adrenocortical tissues and testicular cells in *M. vittatus* (*Bloch,* 1794) have been studies during growth, maturation and spawning phases. The studies based on the changes observed in the cell types, shape and size of the cells of the adrenocortical tissues, testes and the overall percentage of gonadotroph (GTH) and thyrotroph (TSH) cells of the pituitary. However, during growth phase, in proximal pars distalis (PPD) the considerable increment of GTH and TSH have been observed having intense aniline blue stain. The corticotrophs (ACTH) also showed significant accumulation of fuchsinophilic cytoplasmic granules. The cytoplasmic features and the architecture of the interrenal cells were well coincident with the increase of different spermatogenic cells. During the maturation phase dense granulation in the GTH and TSH cells appeared to be concomitant with the spermiation. The amount of cytoplasmic granules of the interrenal cells increased than chromaffin cells and was well coincidence with the increase of spermatids and spermatozoa. The hyperactive and vacuolated features of the interrenal cells during spawning phase appeared to be concomitant with the final process of spermiation.

Key words: histology, pituitary, adrenocortical tissues, testes, growth, maturation, spawning, *Mystus vittatus.*

Introduction

The pituitary gland has a key position in the endocrine orchestra and plays a detective role in reproduction. In teleosts, the adrenal homologue is composed of intermingled groups of interrenal and chromaffin cells surrounding the main blood vessels localized in the head kidney are extremely diverse. It has been intimated that the interregnal cells are homologous to the mammalian medullary cells (Gazola et al., 1995). Secretion of adrenocortical hormone is under control of adrenocorticotropic hormone (ACTH) of hypophysis. The ACTH secreted by the rostral pars distalis (RPD) controls the interrenal synthesis and release of cortisol (Henderson and Garland, 1980). Civinini et al. (2001) hypothesized that in the stickleback the interrenal cells could also produce precursors of sexual steroids by analysis of the steroidogenic enzymes 3β-hydroxysteroid dehydrogenase (3βHSD) and 17β-hydroxysteroid dehydrogenase (17βHSD). Pituitary gonadotropic hormones and hypothalamic GnRH are important in implicating these hormones in gonadal maturation and sex steroid production which plays a very important role in gametogenesis, final maturation of oocytes and spermiation

(Parhar et al., 2003; Lethimonier et al., 2004). Gonadal steroids are generally known to participate in regulation of some aspects of reproduction in fishes. However, pituitary is required for gonadal maturation; in its absence vitellogenesis in suppressed with atresia of the large developing oocytes; spermatogenesis is blocked at the spermatogonia-sprematocyte stage, steroidogenesis does not occur in thye gonadal endocrine tissue (Krishnan and Diwan, 1990; Wen et al., 2003; Ursani et al., 2012). The structural pattern and distribution of interrenal and chromaffin cells in various teleosts have been studied by several workers (Joshi and Sathyaneson, 1980; Borella et al., 1999; Civinini et al., 2001; Sampour, 2008). However, there is dearth of works regarding the relationships of pituitary gonadotrophs, changes in the interrenal and gonadal tissues in teleosts.

The present studies were undertaken with a view to determine the correlative changes in the cytology of and chromaffin cells with testicular cells during growth, maturation and spawning phases in *Mystus vittatus* (Bloch, 1794).

Materials and methods

Adult living male specimen of *M. vittatus* of average length 11.5 ± 1.20 cm with average body weight 22.5 ± 2.10 gm were procured from particular local freshwater body during the second week of every month from December 2015 to August 2016. The fishes were housed in an aquarium and treated with methylene blue for 15 min. The fishes were acclimatized for 5 days by feeding finely chopped goat liver and oligochaetes.

As the pituitary gland of *M. vittatus* is lodged inside the sella turcica, this entire brain was exposed by dissection from the dorsal aspect and subsequently immersed in 10 % neutral formalin for hardening. After 30 minutes, the brain including the pituitary gland were carefully dissected out from the cranium and subsequently fixed in aqueous Bouin's fluid and Zenker's fluid respectively.

After proper fixation, pituitary glands were placed in 70 % alcohol for overnight and subsequently dehydrated through ascending ethanol series followed by acetone and cleared in benzene. Tissues were then embedded in paraffin wax (56–58 °C melting point). Mid-sagittal sections of pituitary gland were cut at 4 μm thickness using a Leica RM 2125 RT microtome. Deparaffinized sections of pituitary were stained by adopting various techniques which are as follows:

1. Mallory's Triple stain (MT) (Mallory, 1936)
2. Chrome Alum Haematoxylin Phloxine (CAHP) (Gomori, 1941)
3. Heidenhan's-Azan stain
4. Perodic Acid Schiff's (PAS) technique of Mc Manus (1948) using Orange G (OG) as the counter stain
5. Alcian blue-Orange G-Acid Fuchsin (AB-OFG) (Slidder's, 1961)

Gonadosomatic index (GSI)

The total body weights of male fishes were taken during the experimental periods. The testes were dissected out after giving incision along the midventral line and were weighed. The mean gonadosomatic indexes were calculated using the formula:

$$DSI = \text{Total weight of the tested / Body weight – weight of the tested} \times 100.$$

Histological methods for testis and adrenal tissues

For histological studies after decapitation of the fish, the head kidneys containing the adrenal homologue and testes were dissected out, cut into small pieces and were fixed in aqueous Bouin's fluid for 18 hrs. Subsequent to dehydration the tissues properly through graded ethanol, followed by acetone the tissues were cleared in benzene. Tissues were embedded in paraffin wax (melting point, 56–58 °C) and serial sections of tissues were cut at 4 μm thickness. The sections were stained with Delafield's Haematoxylin–Eosin (H&E) and Mallory's triple stains respectively. From the histological preparations, the measurement of interrenal and chromaffin cells, the diameters of various testicular cells were measured with the help of reticulo-micromiter and ocular-micrometer respectively.

Observation

The pituitary gland in *M. vittatus* is attached ventrally to the floor of the diencephalon of the brain. In *M. vittatus* the pituitary gland is divided into two compartments namely the adenohypophysis and neurohypophysis. Based on histological characteristics and its cell types the adenohypophysis is divisible into antero-dorsal smallest rostral pars distalis (RPD), middle massive proximal pars distalis (PPD) and the posterior pars intermedia (PI). The infundibulum branches out into the adenohypophysis to form neurohypophysis (fig. 1). The RPD zone packed closely with mostly acidopilic cells interspersed with a few basophils. The

acidophilic prolactin cells (PRL) occupy the major part of the RPD, exhibited positive reaction in the cytoplasm with azocarmine (fig. 2). These cells are usually 5.6 μm × 6.8 μm to 7.5 μm × 8.8 μm in diameter. Other type of acidophilic cells which are spherical or oval in shape and generally dispersed among the PRL cells. These corticotrophs (ACTH) cells have strong affinity to azocarmine and the average diameter of the cells ranges from 7.2 μm × 8.2 μm to 8.6 μm × 9.2 μm (fig. 2). During the end of growth phase some aniline blue positive cells have been identified as the gonadotrophs (GTH) found to dispersed among the PRL and ACTH cells (fig. 2). In the anterior part of the proximal pars distalis (PPD) the spherical or oval acid fuchsin positive somatotrophs (STH) cells are scattered in between gonadotrophs
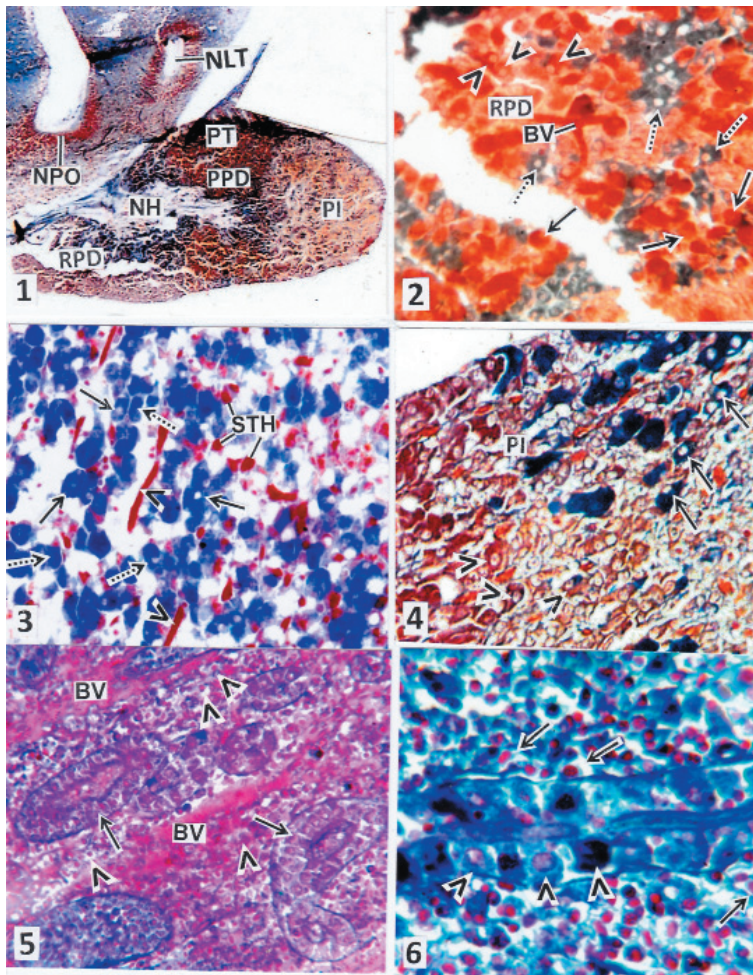

Plate I

N o t e . Chrome alum haematoxylin phloxine: CAHP; Hedenhain's Azan: HA; Mallory's triple: MT; Alcian blue-Orange G-Acid fuchsin: AB-OFG; Romies, Azan: RA; Delafield's haematoxylin-Eosin: HE; Iron alum haematoxylin: IAH; Periodic acid Schiff's — Orange G: PAS-OG.

1 — sagittal section of pituitary (PT) with short stalk. Note NPO and NLT and attachment of PT with brain during growth phase. Note the presence of RPD, PPD and PI. Note also ramification of axonal fibres of neurohypophysis (NH). (CAHP) ×100; 2 — RPD showing PRL cells (arrow heads), densely staining ACTH cells (solid arrows) and scattered GTH cells (broken arrows) during growth phase. BV indicates blood vessels. (HA) ×400; 3 — PPD showing GTH cells (solid arrows), TSH cells (broken arrows), fuchsinophil STH cells during growth phase. Arrow heads indicate blood vessels. (MT) ×400; 4 — PI showing alcian blue positive MSH cells (arrows) and fuchsinophilic MCH cells during growth phase. (AB-OFG) ×400; 5 — adrenocortical tissue showing interrenal cells (IR) arranged within lobule (arrows) and scattered chromaffin cells (CC) (arrow heads) adjacent to blood vessels (BV) during growth phase. (RA) ×400; 6 — higher magnification of IR arranged within lobule (arrow heads) having prominent nucleus and dense cytoplasm during end of growth phase. Note scattered (CC) adjacent to IR. (MT) ×1000.

(GTH) cells (fig. 3).The diameter of the STH cells measuring approximately from 8.5 μm × 9.1 μm to 9.5 μm × 9.8 μm . The GTH or cyanophil I cells occupying the major part of PPD and stained deep blue with aniline blue. Majority of the GTH cells are polygonal, oval or angular, measuring from 9.5 μm × 10.6 μm to 10.2 μm × 11.2 μm (fig. 3). Another basophilic aniline blue positive thyrotrophs (TSH) cells are usually elongated in shape and lie scattered on the middle region of the PPD (fig. 3). The diameters of TSH cells are ranging from 4.8 μm × 5.8 μm to 6.2 μm × 7.5 μm. Considerable number of blood vessels of various sizes have been observed in PPD (fig. 3). The pars intermedia (PI) contain two types of cells. The MSH cells are provided with alcian blue positive homogeneous cytoplasm and the diameter of the cells measuring from 4.8 μm × 5.2 μm to 5.8 μm × 6.2 μm. The MCH cells are provided with scanty cytoplasm stained with acid fuchsin and prominent nucleus. The diameter of the MCH cells are ranging from 4.6 μm × 5.8 μm to 6.0 μm × 6.4 μm (fig. 4).

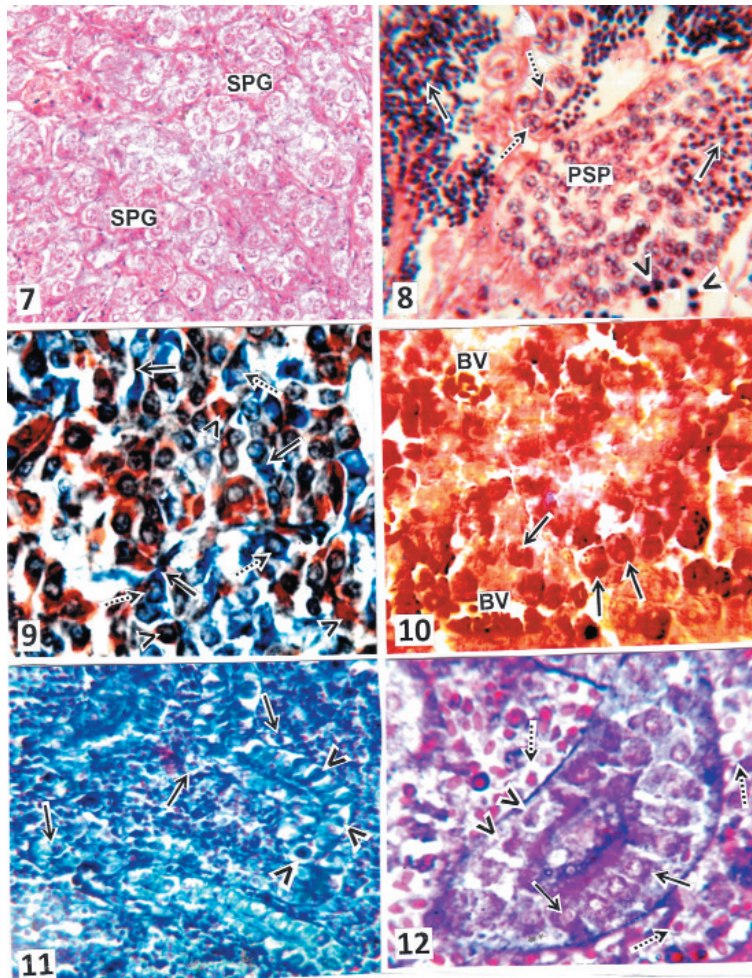**Plate II**

7 — showing spermatogonial cells (SPG) with prominent nucleus and chromophobic cytoplasm during growth phase. (HE) ×600; 8 — showing primary spermatocytes (PSP), scattered secondary spermatocytes (SSP) (arrow heads) and spermatids (STD) (solid arrows) during end of growth phase. Broken arrows indicate few SPG. (HE) ×600; 9 — terminal zone of RPD and PPD showing cytoplasmic mass in GTH cells (broken arrows) and elongated TSH cells (solid arrows) during maturation phase. Note some scattered ACTH cells (arrow heads). (MT) ×400; 10 — PPD showing dense glycoprotein materials in the GTH cells (arrows) adjacent to BV during end of maturation phase. (PAS-OG) ×400; 11 — adrenocortical tissue showing fully active phase of IR (arrow heads) and CC (arrows) during maturation phase. (MT) ×400; 12 — higher magnification of IR showing hypertrophied IR (solid arrows) and scattered CC (broken arrows) during the end of maturation phase. Note some fully active hypertrophied IR (arrow heads). (RA) ×600.

Histologically, the adrenal gland is composed of renal tubules and clusters of interrenal and chromaffin cells. The interrenal cells are oval or elongated and are arranged in the form of tubules of varying thickness adjacent to cardinal veins. The interrenal cells are provided with homogeneous cytoplasm and a distinct nucleus (fig. 5). The chromaffin cells are generally present in clusters or dispersed between hemopoietic tissues and interrenal tubules in close proximity to the blood vessels (figs 5, 6).

The value of GSI exhibits remarkable and regular changes in growth, maturation and spawning phases. The lowest value of GSI 0.54 ± 0.08 has been observed in December i. e. onset of growth phase. GSI in January rises to 0.68 ± 0.12. Improvement of GSI values continues in the end of growth phase and maturation phase. During February the GSI value increases to 0.868 ± 0.17 and from March, April and May the GSI shows the value 8.801 ± 0.14, 0.898 ± 0.13 and 1.12 ± 0.17 respectively. The increasing trend of GSI value has been observed during the spawning period due to maximum proliferation of cysts of spermatids and spermatozoa. The mean GSI recorded during June 1.20 ± 1.2 whereas in July the value increases to 1.32 ± 1.25. The highest 1.38 ± 1.19 has been recorded in the month of August.

Histologically the testis is made up of large number of lobules. The spaces in between lobules are filled with connective tissues, blood capillaries and a few interstitial leydig cells (figs 13, 18). The sequence of histological changes occurring within the testicular lobules during spermatogenesis is spermatogonia, primary and secondary spermatocytes, spermatida and spermatozoa. Spermatogonia are relatively larger and spherical in shape having centrally placed basophilic nuclei and chromophobic cytoplasm. The diameter of these cells ranges from 7.8 μm × 8.0 μm to 8.2 μm × 8.6 μm; whereas the diameter of the nucleus varies from 2.4 μm to 2.8 μm (figs 7, 8). The primary spermatocytes contain relatively lesser amount of chromophobic cytoplasm and the nuclei are deeply stained with haematoxylin. They are oval or spherical in shape and the diameter of these cells are approximately measuring from 7.4 μm × 7.8 μm to 6.5 μm × 7.0 μm (fig. 8). In the secondary spermatocytes the cytoplasm is hardly seen and the condensed nuclei darkly stained with haematoxylin. The diameter of the cells are measured approximately from 4.8 μm × 5.6 μm to 4.5 μm × 4.0 μm (fig. 8). They last for a short duration. The spermatids are further reduced in size and the chromatin matter stains more deeply with haematoxylin. They occur in cysts and the diameter of the nucleus measures approximately from 2.0 μm × 2.5 μm to 2.8 μm × 3.2 μm (figs 8, 13). The spermatozoa are the smallest cells in the testis and occurring in the central portion of the lobules. The diameter of the nuclei are approximately from 1.6 μm × 1.8 μm to 1.4 μm × 1.7 μm (figs 13, 18). The interstitial cells are oval in shape. They contain an oval or rounded nucleus and associated with the blood vessels. The diameter ranges from 6.5 μm × 8.0 μm ± 0.65 to 6.8 μm × 7.4 μm ± 0.40 (figs 13, 18).

Seasonal changes in the cells of pituitary and testis during growth, maturation and spawning phases

Growth phase (December to February)

In *M. vittatus*, the RPD consists of carminophilic PRL cells and a small number of azocarmine positive ACTH cells. During January and February the ACTH cells stain strongly with azocarmine having dense cytoplasm adjacent to blood vessels. The rim of cytoplasm of PRL cells stained with azocarmine (fig. 2). At the end of growth phase some scattered aniline blue positive GTH cells are found to be scattered in between acidophilic cells (fig. 2). The PPD zone comprises of aniline blue positive GTH, TSH and scattered acid fuchsin positive STH cells. An increase in the number and cytoplasmic content of GTH cells during end of growth phase occurs. The diameter of GTH cells are calculated to be 10.2 μm × 10.5 μm to 9.8 μm × 10.8 μm. During the end of growth phase aniline blue positive TSH cells attain their maximum size 5.1 μm × 5.8 μm to 6.2 μm × 6.6 μm (fig. 3). At the end of growth phase the PPD comprises of 50 % MSH and 40 % MCH cells (fig. 4).

Changes in the activities of adrenocortical tissues have been studied in terms of their number, cell size along with reduction or increment of cytoplasm. During the growth phase the interrenal cells are oval or elongated with prominent centrally or basally placed nuclei and the cytoplasm is acidophilic. The chromaffin cells are situated in groups or intermingled with interrenal cells (fig. 5). At the end of growth phase the condensed rim of cytoplasm is found encircling the nucleus. The cells are arranged in two layers in tubules, separated from each other and from the parenchyma of haemopoietic cells. The diameter ranges from 4.2 μm × 4.6 μm to 5.0 μm × 5.5 μm (fig. 6). The chromaffin cells (3.5 μm × 4.0 μm to 3.8 μm × 4.5 μm) are situated in groups or adjacent to interrenal cells exhibit a pale cytoplasm and spherical nucleus (fig. 6).

In December, the spermatogonia are the predominant germ cells (fig. 7) and lie arranged along the wall of the lobule. During February, spermatogonia undergo division, a gradual increase in size of the lobules take place. Scattered primary spermatocytes and nests of secondary spermatocytes and spermatids appear. The spermatogonial cells decrease numerically (fig. 8).

Maturation phase (March to May)

In the RPD zone ACTH cells increased considerably during maturation phase which are deeply stained with acid fuchsin (fig. 9). On the other hand the GTH and TSH cells are strongly aniline blue positive. The number and concentration of cytoplasmic mass in the GTH and TSH cells also increase progressively (fig. 9). During the month of May i. e. end of maturation phase the size of the GTH cells increase to 14.2 μm × 15.6 μm to 15.2 μm × 16.2 μm. The cytoplasmic mass of the GTH cells increases enormously and are densely packed with PAS positive glycoprotein materials adjacent to blood vessels (fig. 10). During maturation phase the PPD comprises of 56 % GTH cells and 38 % TSH cells.

During this phase, the clusters of interrenal and chromaffin cells are oriented encircling the blood vessels. The diameter of interrenal cells increased to 5.5 μm × 6.0 μm to 6.2 μm × 6.4 μm and undergo hypertrophy and are arranged in tubules (fig. 11). The chromaffin cells also increased in diameter (4.5 μm × 4.8 μm to 5.0 μm × 5.3 μm) and undergo hypertrophy (fig. 11). At the end of maturation phase the concentration of cytoplasmic mass in the hypertrophied interrenal cells progressively decrease encircling the condensed nuclei. The chromaffin cells are also found to be hypertrophied (fig. 12).

Histologically, it has been observed that while apermatogenesisi is in progress, a gradual increase in the semineferous lobules takes place. Spermatids are numerically abundant during April and May. Spermatozoa also appear abundantly in May (fig. 13). Interstitial leydig cells are present with densely stained nuclei. However, these cells become more active and prominent during May and diameter ranges from 8.0 μm × 8.5 μm to 8.4 μm × 8.8 μm (fig. 13).

Spawning phase (June to August)

During the spawning phase the PPD in densely populated by alcian blue positive hypertrophied GTH and TSH cells (fig. 14). In the month of July the cytoplasmic content of GTH cells considerably reduced and formed alcian blue positive cytoplasmic rim encircling hypertrophied nuclei (fig. 14). The GTH cells are 10.6 μm × 11.8 μm yo 11.5 μm × 12.0 μm in size. No remarkable changes in found in TSH cells (fig. 14).

In the spawning phase the interrenal cells undergo momentous changes. During June the hypertrophied interrenal cells are depleted of their cytoplasmic contents but the nuclei become enlarged (fig. 15). However, during July in shape encircling the central blood vessels (fig. 16). The hypertrophied chromaffin cells are provided with scanty chromophobic cytoplasm encircling the active nuclei (fig. 15).

During the spawning phase the lobules attain a maximum width and the lobule boundary wall becomes very thin and the seminiferous lobules habour an enormous number of mature spermatozoa (figs 17, 18). The maximum activity of the interstitial cells are found in association with blood cells. The nucleus of the interstitial cell is also strongly positive to haematoxylin (fig. 18).

Discussion

The teleostean pituitary gland is known for its remarkable structural diversity in topography, size, shape, mode of attachment and basic histology (Green and Maxwell, 1959). The pituitary gland in *M. vitiates* is leptobasic type having a short infundibular stalk and composed of glandular adenohypophysis and the nervous neurohypophysis. In *M. vittatus* rostral pars distalis (RPD) occupies the antero-dorsal position of the gland and mainly
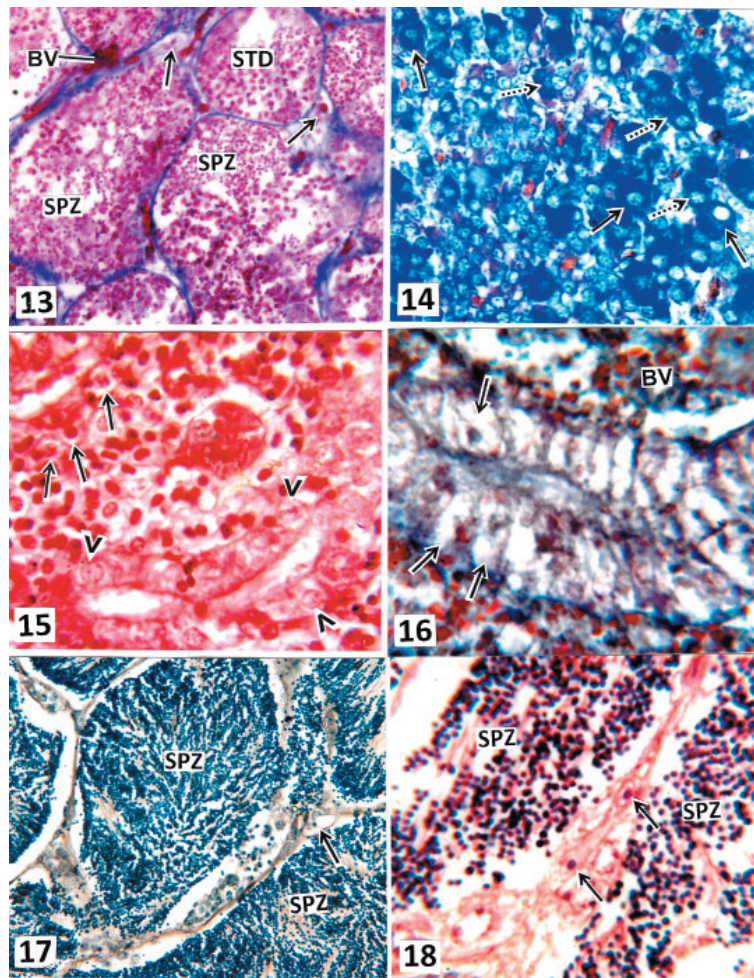

Plate III

13 — showing proliferation of spermatids (STD) and compact mass of spermatozoa (SPZ) within the testicular lobules during maturation phase. Arrows indicate fully active interstitial cells. (HE) ×400; 14 — PPD showing intense alcian blue reaction in the cytoplasm of GTH cells (solid arrows) and TSH cells (broken arrows) during spawning phase. (AB-OFG) ×400; 15 — adrenocortical tissue showing hypertrophied IR (arrow heads) and CC (arrows) during spawning phase. (HE) ×400; 16 — higher magnification showing hypertrophied and vacuolated IR (arrows) during end of spawning phase. (MT) ×1000; 17 — showing compact mass of SPZ bordered by cyst of STD during spawning phase. Note active interstitial cell (IC) (arrows) in between lobules. (IAH) ×400; 18 — higher magnification of testes showing fully packed SPZ within the lobule and hypertrophied IC (arrows) fully active hypertrophied IR (arrow heads). (RA) ×600.

consists of fuchsinophilic prolactin (PRL) cells which are probably responsible for the control of a carrier for sodium ion transport in the chloride cells of the gills, stimulation of mucus secretion both in gills and skin (Ball and Baker, 1969). Mandal and Sinha (1985) also reported about the random distribution of the PRL cells in the RPD of *Heteropneustes fossis*, *Labeo rohita* and *Catla catla*. The corticotrophic (ACTH) cells in *M. vittatus* have also strong affinity to erythrosine and acid fuchsin. The ACTH cells are interlocated between the neurohypophysis and prolactin cells. Numerically they are few and become hyperactive during the maturation phase. Assem and El-Boray (2001) also reported that ACTH cells are generally found at the interphase between PRL cells and neurohypophysis. In proximal pars distalis (PPD) of *M. vittatus* only one type of gonadotrophs (GTH) cells are occupying the major portion of PPD and stained blue with aniline blue and deep blue with alcian blue. These cells are also PAS positive. In the inactive cells the cytoplasm are densly stained and nuclei are formed to small. In the active phase, the cytoplasm of GTH cells are not stained intensly and show extensive vacuolation while the nuclei are found to be enlarged. Replacement therapy with pituitary extracts of gonadotropin is stated to restore both gonadal activity (Ahsan, 1966) and secondary sexual characteristics (Pickford and Atz, 1957). Single type of gonadotropin located in the PPD has been observed in *Oreochromis niloticus* (El-Sakhaway et al., 2011) and in *Clarias lazera* (El-Zoghby et al., 2008). In *M. vittatus* the thyrotroph (TSH) cells are normally elongated in shape and lie scattered on the anterior and middle region of the PPD. They are also aniline blue and PAS positive. The TSH cells are found to exhibit seasonal changes in their activity during non-breeding and breeding season. During the maturation and spawning season it has been noticed thai in *M. vittatus* the GTH and TSH cells outnumbered the acidophilic somatotroph (STH) cells and this result is in complete agreement with of the other workers who have noticed the changes of the pituitary during various reproductive phases (Sathyanesan, 1960; Kharat and Khillare, 2013). In *M. vittatus* the pars intermedia (PI) contains two types of cells, the larger cells provided with homogeneous cytoplasm which stained deeply with alcian blue and are identified as melanotroph stimulating hormone (MSH) cells. The smaller cells are provided with rim of cytoplasm stained with Orange G and identified as melanotroph concentrating hormone (MCH) cells. El-Sakhaway et al., 2011 reported that aniline blue positive cells and their cellular characteristics closely resemble the GTH cells of PPD during non-breeding season.

The interrenal tissue of teleost is homologous to the mammalian adrenal cortex and is the source of cortical steroids (Jones and Phillips, 1986). In *M. vittatus* the interrenal steroidogenic and chromaffin cells are mainly associated with the cardinal veins and their tributaries. Similar observations were also made by Civinini et al. (2001) in *Gasterosteus aculeatus* and Sampour (2008) in *Carassius auratus*. In *Cichilasema dimerus* the interrenal gland components were found exclusively within the posterior portion of cephalic kidney arranged in a relatively diffused manner is close association with the wall of the cardinal veins, its tributaries and sinusoids (Morandini et al., 2014).

In *M. vittatus*, the interreanl cells are comparatively larger than the cromoffin cells, and they are basophilic. The chromaffin cells contained pale cytoplasm and slightly basophilic nuclei.

The GSI has been considered to be a good marker of reproductive activity to study the gonadal development in teleosts (Delahunty and de Vlaming, 1980; Gadekar, 2014). In *M. vittatus* the active division of spermatogonial cells and eventual formation of the resultant primary spermatocytes, the testes gradually increases from growth phase onwards as indicated by the gradual increase in GSI. The highest GSI value in the spawning phase is due to the active proliferation of advanced stages of spermatogenic cells. Similar changes of the GSI value in relation to the spermatogenic activity in the testes of different teleosts have been described by various authors (Mukhopadhyay and Sinha, 1986; Ganguli and Chakrabarti, 1995; Chakrabarti, 2014). In the present investigation in *M. vittatus* spermatogonial cells are formed throughout the entire duration of different phases but they have been found to occur in great abundance during growth phase. During the process of

differentiation the spermatogonia into spermatozoa through the subsequent intermediate stages, the cytoplasm and nuclei of spermatogonia pregressively decrease in size and volume.

Changes in the pituitary histology, interrenal and chromaffin cells and testes during growth, maturation and spawning phases

In *M. vittatus*, the ACTH cells of RPD, the gonadotrophs and thyrotrophs of the PPD exhibit changes concomitant with testes. Though the physiological role of adrenocortical tissue during sexual maturation and spawning is not clearly understood but significant hyperactivity of interrenal tissues corresponds with the breeding phases of *M. vittatus*. During growth phase the GSI of testis slightly increases and the storage of glycoproteineceous material begins at this moment in GTH cells which are clearly reflected in the tinctorial reactions. The proliferation of testicular cells in *M. vittatus* is the main feature at the end of growth phase. Most of the GTH cells are in their initial phase of cytoplasmic depletion, suggesting the idea that proliferation of testicular cells is not under the influence of hormone. Rai (1966) reported the possibility of the feedback reaction of the testes to the pituitary gland. The presence of a number of highly granular and intensely staining GTH cells of the PPD of pituitary during the maturation phase can be attributed possibly to a decreased release of the pituitary gonadotrophin due to the feedback action on the pituitary by the testicular hormones. In *M. vittatus* the accumulation of cytoplasmic contents of the interrenal cells coincided with the transformation of various testicular cells and spermatogenetic activities continued until spawning Yadav et al. (1970) reported that epinephrine content is higher in *Heteroneustes fossilis* during reproduction but that the non-epinephrine content does not fluctuate. According to him, the rise of epinephrine content might be associated with the increased active phosphorylase levels required for metabolism during the breeding period. In the present study, the accumulation of the glycoprotein materials, degranulation and vacuolation of the GTH cells coincide with with the transformation of the gonocytes to spermatocytes, subsequent maturation and beginning of spermiation. In the later period of the spawning season, the rapid exhaustion of the male germ cells is accompanied by a rapid process of degranulation and vacuolation in almost all the gonadotrophs. The accumulation of cytoplasmic contents of interrenal cells in *M. vittatus* coincided with the transformation of various spermatogenic cells and the spermatogenetic activities continued until spawning. The hyperactivity of the interrenal cells could be attributed to the higher level of corticosteroid production required during maturation and spawning phases. In the present study, the chromaffin cells in *M. vittatus* were more or less uniform in appearance except during maturation and spawning phase when they were hypertrophied. Reid et al. (1998) opined that in teleost, chromaffin tissues were associated with the synthesis and secretion of the catecholamines. Sampour (2008) with the halp of electron microscopic studies, observed numerous mitochondri in different shapes in the cytoplasm of chromaffin cells probably produce energy for the activities of cells during synthesis of catecholamine hormone.

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