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Original Article

Histological characteristic of interrenal and chromaffin cells in relation to ovarian activities in *Mystus vittatus* (Bloch) during growth, maturation, spawning and post-spawning phases

Padmanabha Chakrabarti*, Mainak Banerjee

Fisheries Laboratory, Department of Zoology, The University of Burdwan, Burdwan-713104, West Bengal, India.

Abstract: The histological status of adrenocortical tissues and the correlated seasonal changes in ovarian activities in *Mystus vittatus* was performed. The tubules and nests of interrenal and chromaffin cells were located in cephalic kidney around the main branches of posterior cardinal vein. Various female germ line cells were identified in the ovary based on size, distinctive features and histoarchitechture of the cells. However, on the basis of relative abundance and size of the different oocytes, the event of oogenesis has been found to occur in four distinct phases, including growth, maturation, spawning and post-spawning. The cytoplasmic features and the architecture of the interrenal and chromaffin cells varied during different phases of the reproductive cycle. During growth and maturation phases, the amount of cytoplasmic granules of interrenal cells increased than chromaffin cells that was in coincidence with the increase of early and late perinucleolar oocytes followed by highest frequency percentage of oocyte at stages IV and V. The cytoplasmic mass of interrenal cells was gradually elevated along with hypertrophied nuclei from the end of maturation and spawning phases also correlated with the increased frequency of mature oocytes. Therefore, gradual accumulation of cytoplasmic granules in the interrenal cells was noticed during post-spawning phase. The cytological variations in the interrenal and chromaffin cells harmonized with constitution of different ovarian cells during different reproductive phase in *M. vittatus*.

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Introduction

The morphology and distributional pattern of teleostean interrenal or adrenocortical and chromaffin cells surrounding the main blood vessels localized in the head kidneys are extremely diverse. It has been intimated that the interrenal cells are homologous to the mammalian adrenal cortex, whereas the chromaffin cells are homologous to the mammalian medullary cells (Gazola et al., 1995). In teleosts, cortisol is synthesized and secreted by steroidogenic cells which in addition to chromaffin cells constitute the adrenal gland (Grassi Milano et al., 1997). Civinini et al. (1997) noticed that interrenal steroidogenic cells present a considerable variety of cytological aspects that characterize them in relation to a metabolic cell cycle and periods of the annual cycle. Aminergic chromaffin and interrenal steroidogenic cells are found to be mixed, adjacent or completely separated and also can line the endothelium of the venous blood vessels or may be located in close proximity (Gallo and Civinini, 2003). The structural pattern and distribution of interrenal and chromaffin cells in various teleosts have been studied by several workers (Joshi and Satyanesan, 1980; Verma and Misra, 1992; Borella et al., 1999; Civinini et al., 2001; Sampour, 2008). However, few authors have been studied the relationships between changes in the interrenal and gonadal tissue in different teleosts (Singh et al., 1974; Chakrabarti, 2014; Chakrabarti and Ghosh, 2014).

As the interrenal cells and gonads are the essential elements involve in steroidogenic along with other physiological processes the aim of the present work

* Corresponding author: Padmanabha Chakrabarti
E-mail address: dr.pchakrabarti@yahoo.in

was to examine more precisely the correlative changes of the cytology of interrenal and chromaffin cells with ovarian cells during different reproductive phases in *Mystus vittatus*.

Materials and Methods

Adult living female specimens of *M. vittatus* (with length of 12-14 cm and weight of 40-55 gm) were procured from particular local freshwater body during the second week of every month from February 2014 to January 2015. They were housed in an aquarium and treated with 1% methylene blue for 15 mins. The fishes were acclimatized for 5 days by feeding finely chopped goat liver and Tubifex. Data on total body weight and after decapitation the total ovarian weight of ten fishes were taken to calculate the mean gonadosomatic index (GSI) using the following formula:

$$\text{GSI} = \frac{\text{Total ovary weight}}{\text{Body weight} - \text{weight of the ovary}} \times 100$$

Histological methods: For histological studies after decapitation of the fish, the head kidneys containing the adrenal homologue and ovaries were dissected out, cut into small pieces and were fixed in aqueous Bouin's fluid for 18 hrs. Subsequent to dehydration properly through graded ethanol, the tissues were dehydrated in acetone and were cleared in benzene. Tissues were embedded in paraffin wax (melting point, 56°C-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 (MT) (Mallory, 1936) stains, respectively. From the histological preparations the measurement of interrenal and chromaffin cells and the nuclei were measured with the help of reticulo-micrometer and ocular micrometer, respectively.

Results

Histologically, the adrenal gland is composed of tubules and clusters of interrenal and chromaffin cells surrounding the posterior cardinal veins and their branches in the anterior-most part of the pronephric kidney (Fig. 1). The interrenal cells are usually round in shape and arranged in the form of

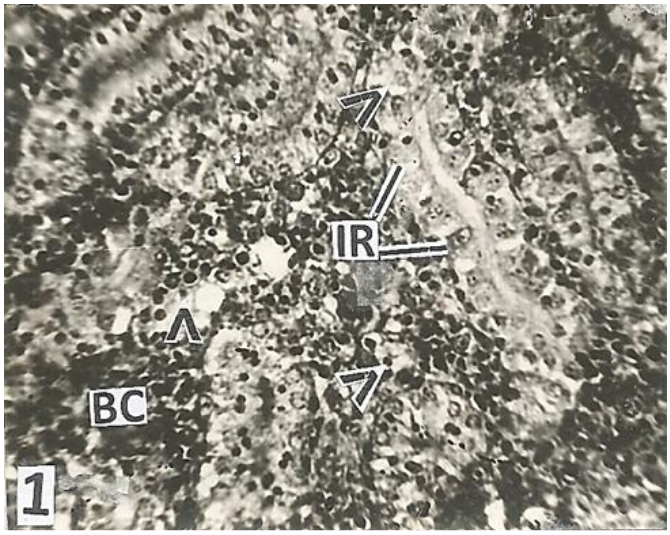

Figure 1. The section of interrenal and chromaffin cells in *Mystus vittatus*. Interrenal cells (IR) in form of tubules having prominent nucleus and cytoplasm during growth phase. Note chromaffin cells (arrow heads) adjacent to blood capilleries (BC) (H&E, 400X).

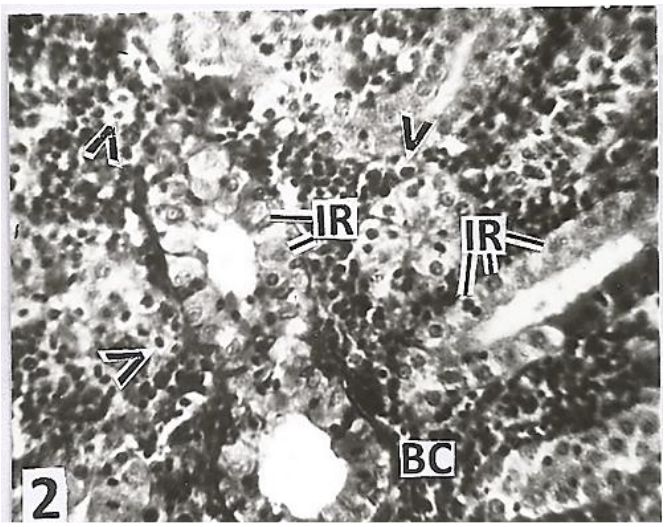

Figure 2. The section of interrenal and chromaffin cells in *Mystus vittatus*. Enlargement of interrenal cells (IR) adjacent to blood capilleries (BC) at the end of growth phase. Note prominent nucleus and scanty cytoplasm in chromaffin cells (arrow heads) (MT, 400X).

tubules varying in thickness. The tubules are closely arranged interspersed with blood vessels and haemopoetic tissues (Figs. 1, 2). The round interrenal cells provided with granular homogeneous cytoplasm and a distinct uninucleated nucleus with prominent nucleolus (Fig. 1). In the Mallory's triple stain, the cytoplasm of interrenal cells become light pink and the nuclei are stained light orange brown. The chromaffin cells are generally present in clusters in close proximity to the blood vessels and also

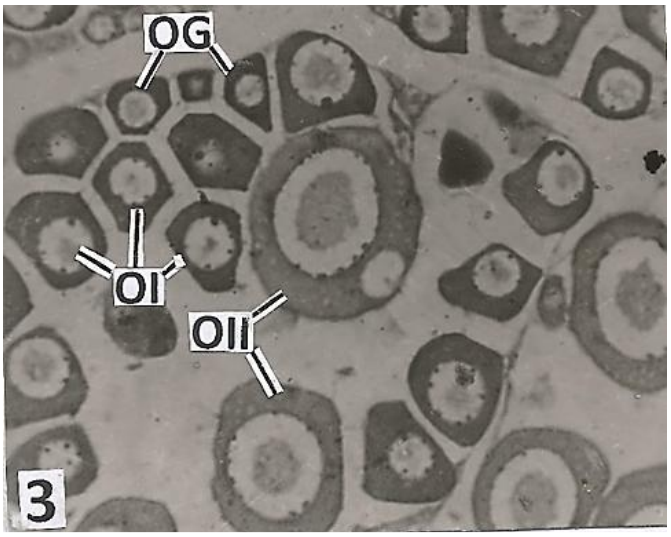

Figure 3. The section of ovary in *Mystus vittatus*. Oogonial cells (OG), oocyte I (OI) and oocyte II (OII) within the ovigerous lamella during growth phase (H&E, 150X).

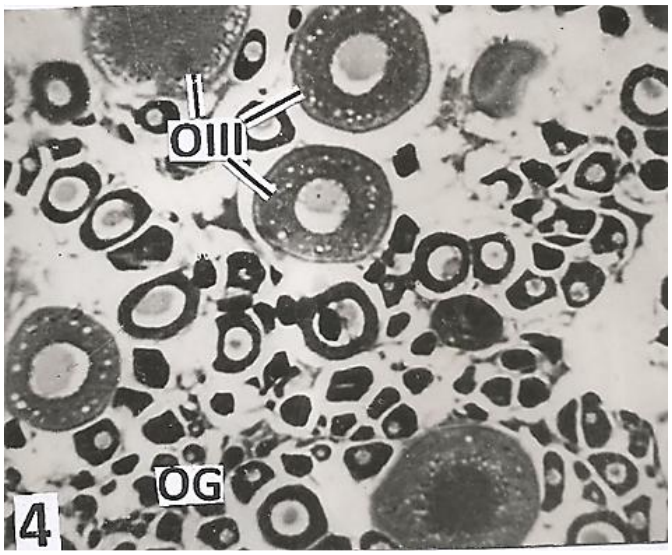

Figure 4. The section of ovary in *Mystus vittatus*. Increasing number of oocyte III (OIII) at the end of growth phase along with oogonia (OG) and primary oocytes (MT, 100X).

dispersed between haemopoietic tissues and interrenal cells (Figs. 1, 2). The nucleus of chromaffin cells appear oval or round and cytoplasmic granules are sparse than interrenal cells (Figs. 1, 2).

The inner wall of the germinal epithelium of ovary projected into the ovarian cavity is termed as ovigerous lamellae where development of new crops of oogonia take place. The sequence of oocyte maturation in *M. vittatus* has been divided into six developmental stages viz., oogonia (stage I), early and late perinucleolar stage (stage II and stage III),

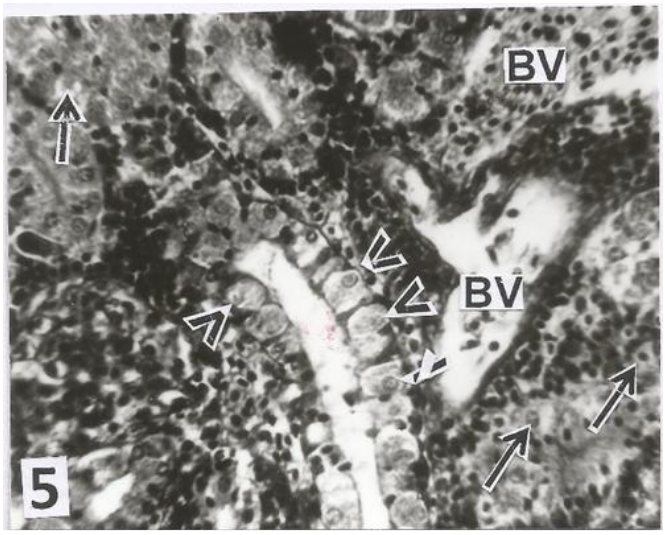

Figure 5. The section of interrenal and chromaffin cells in *Mystus vittatus*. Hypertrophied interrenal cells within the tubules with prominent nucleus and cytoplasm encircling the blood vessels (BV) during maturation phase. Arrows indicate enlarged chromaffin cells adjacent to BV (H&E, 400X).

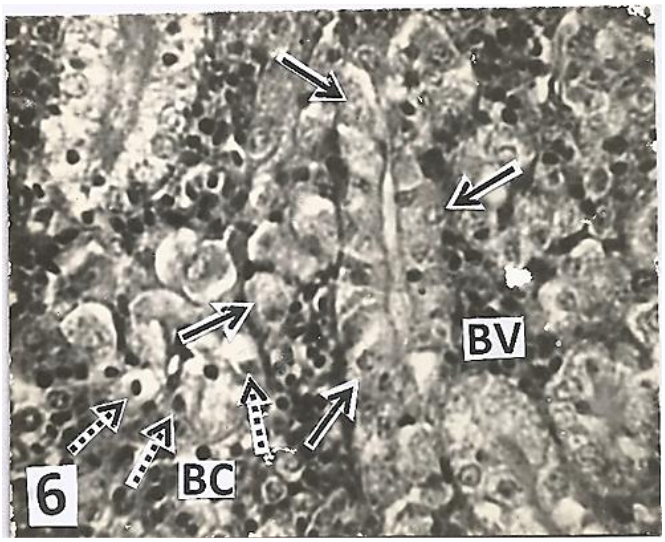

Figure 6. The section of interrenal and chromaffin cells in *Mystus vittatus*. Acidophilic homogeneous cytoplasm of enlarged interrenal cells (solid arrows) and hypertrophied chromaffin cells (CC) (broken arrows) adjacent to blood vessels (BV) and blood capillaries (BC) during maturation phase (MT, 600X).

yolk vesicle stage (stage IV), yolk granule stage (stage V) and mature follicle (stage VI).

Oogonia (stage I) (8×11 µm to 14×12 µm): Oogonia are present either singly or in small nests within the lamellar epithelium. An oogonium is made up of a large nucleus (4-5 µm) with chromatin threads (Fig. 3).

Early perinuclolus oocyte (stage II) (23×28 µm to 36×40 µm): This stage is larger than the oogonium.

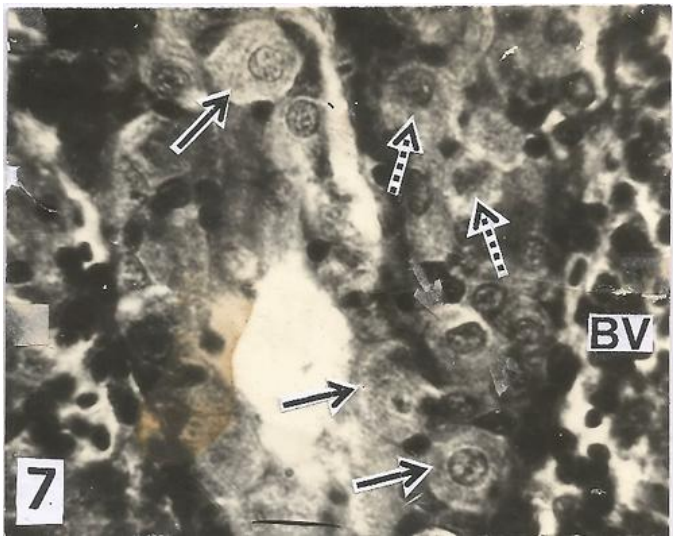

Figure 7. The section of interrenal and chromaffin cells in *Mystus vittatus*. Higher magnification of interrenal cells (solid arrows) and chromaffin cells (broken arrows) adjacent to the blood vessels (BV) at the end of maturation phase (MT, 1000X).

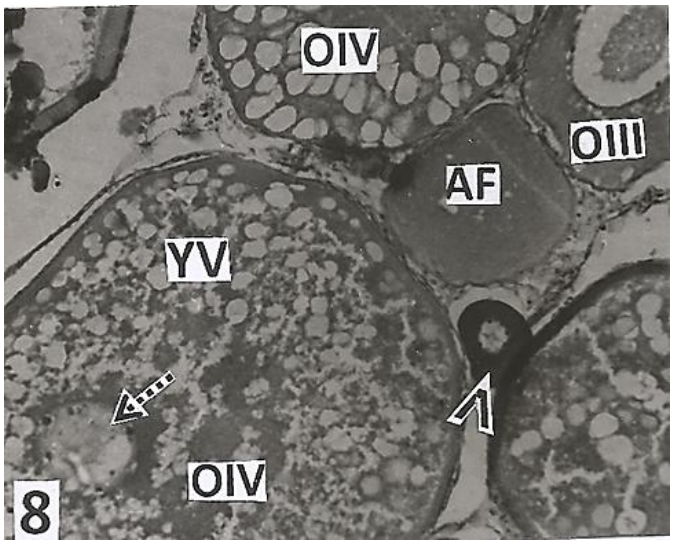

Figure 8. The section of ovary in *Mystus vittatus*. Oocyte IV (OIV) stage with prominent yolk vesicles within the ooplasm and germinal vesicle (broken arrow) at the centre. Note oogonia (arrow head), oocyte III (OIII) and atretic follicle (AF) in between during maturation phase (MT, 400X).

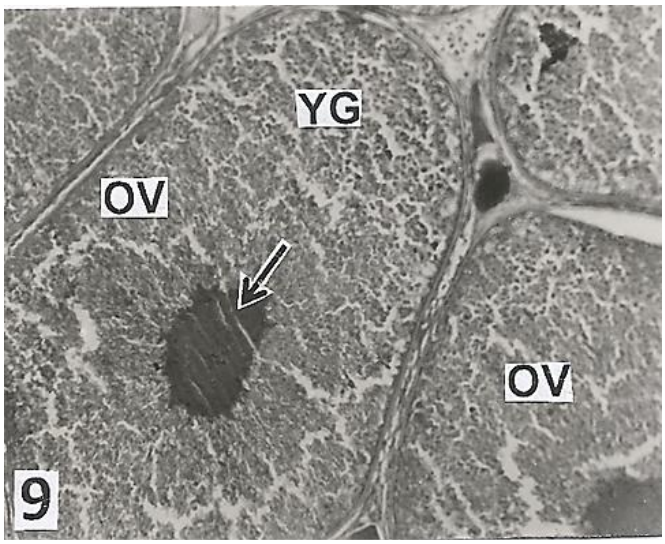

Figure 9. The section of ovary in *Mystus vittatus*. Increased number of oocyte V (OV) having condensed yolk granules (YG) and ecentric germinal vesicle (solid arrow) at the end of maturation phase (H&E, 400X).

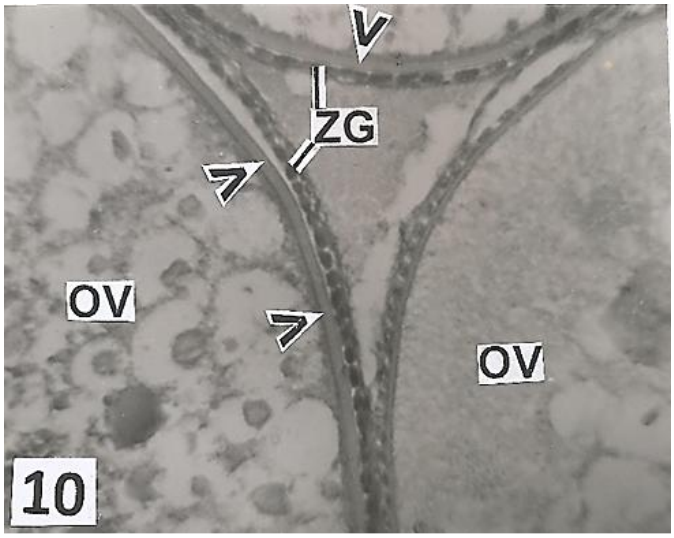

Figure 10. The section of ovary in *Mystus vittatus*. Oocyte V (OV) having prominent granulosa cells in zona granulosa and thick zona radiata (arrow heads) (H&E, 1000X).

The central nucleus (10-14 µm) is provided with a few nucleoli near the periphery of nucleus together with central chromatin mass (Fig. 3). Some oocytes at this stage increased in size and possess a yolk nucleus which lies close to the nuclear membrane in the ooplasm (Fig. 3).

Late perinucleolus oocyte (stage III) (68×72 µm to 80×88 µm): The stage is characterized by the appearance of cortical alveoli along the periphery of the ooplasm. It consists of centrally placed nucleus with an average diameter of 18-20 µm together with condensed chromatin materials (Fig. 4). A thin layer enclosing the zona radiata is also noticed in this stage.

Yolk vesicles oocyte (stage IV) (110×120 µm to 145×154 µm): The yolk vesicle finally cover the entire ooplasm of stage IV oocyte. Most of the vesicles are empty but some of them are filled with homogeneous materials (Fig. 8). The nucleus is large spherical and centrally placed having condensed materials. The oocyte is enveloped with a zona

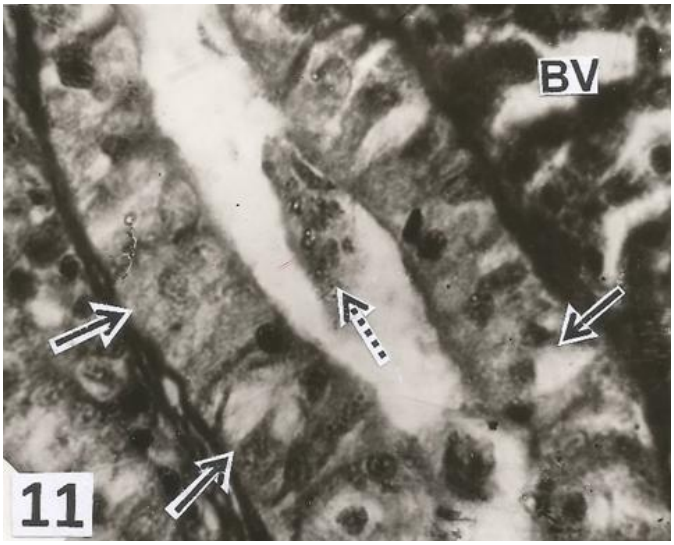

Figure 11. The section of interrenal cells in *Mystus vittatus*. Columnar or lobular interrenal cells (solid arrows) in the form of tubules encircling central blood vessels (broken arrow) during spawning phase. BV indicates blood vessels (MT, 1000X).

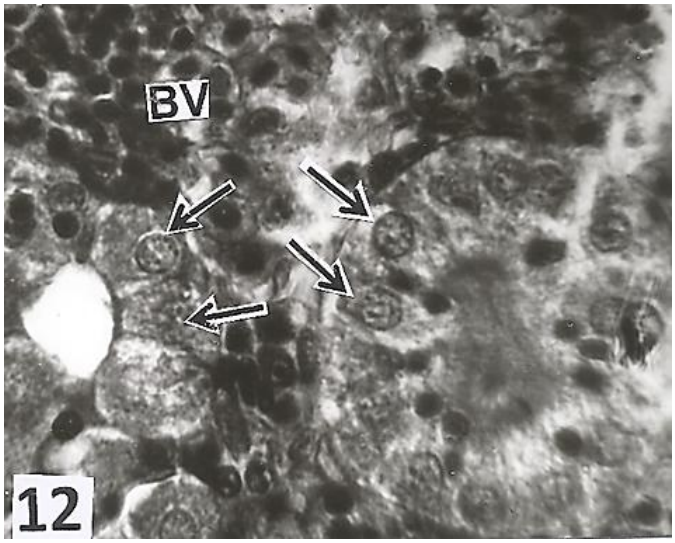

Figure 12. The section of chromaffin cells in *Mystus vittatus*. Enlarged chromaffin cells (solid arrows) close to the blood vessels (BV) (H&E, 1000X).

radiata, middle zona granulosa and outermost theca (Fig. 8).

Yolk granules stage (stage V) (230×250 µm to 280×320 µm): In this vitellogenic oocyte stage, migration of germinal vesicle from the centre of egg towards the periphery is started (Fig. 9). The yolk granules are condensed and as a result the cell volume and diameter increased considerably. The theca is thin but the granulose cells are prominent with distinct nucleus (Fig. 10).

Mature follicle (stage VI) (380×400 µm to 420×480 µm): The yolk granules coalesced and remain packed to form homogeneous yolk mass. The germinal vesicle move towards zona radiata. The thickness of theca and zona granulosa reduced considerably (Figs. 13, 14).

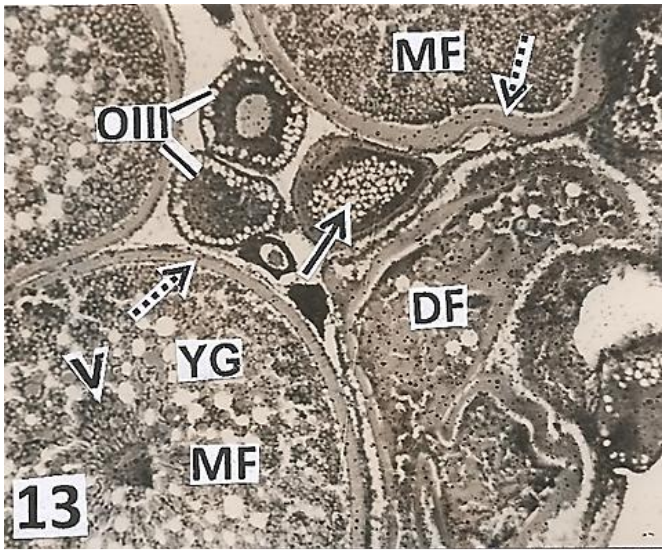

Figure 13. The section of ovary in *Mystus vittatus*. Mature follicles (MF) with full of yolk granules (YG), thick zona radiata (broken arrows) and ecentric germinal vesicle (arrow head) during spawning phase. Note the presence of discharged follicle (DF), atretic follicle (solid arrow) and few oocyte III (OIII) (MT, 400X).

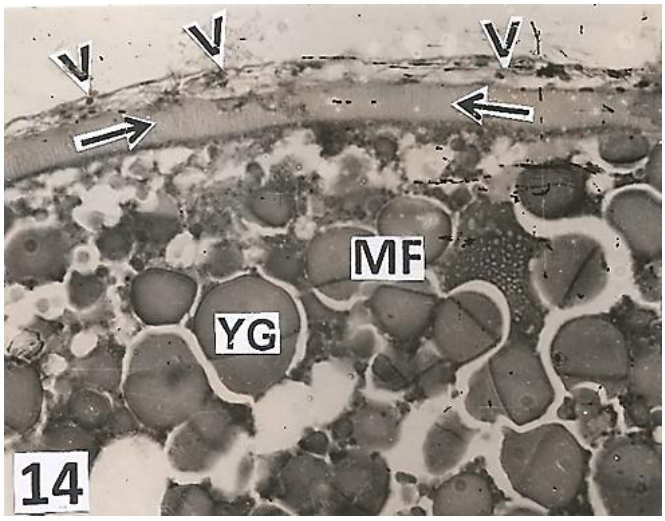

Figure 14. The section of ovary in *Mystus vittatus*. Higher magnification of mature follicles showing distorted zona granulosa cells (arrow heads) thick zona radiata (arrows) and condensed yolk granules (YG) (H&E, 1000X).

Discharged follicle: After the discharge of mature ovum, the theca and follicular cells are left behimd. The granulosa layer shows definite changes in structure (Figs. 13, 17).

Atretic oocyte: Sometimes the developing oocytes

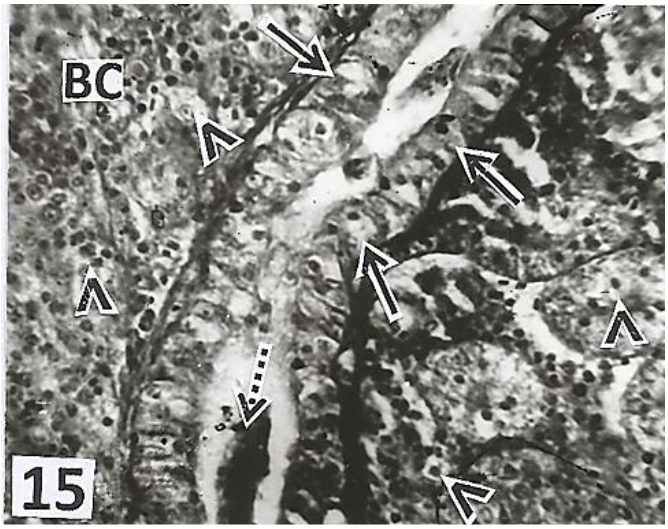

Figure 15. The section of interrenal and chromaffin cells in *Mystus vittatus*. Attenuated interrenal cells with the tubules (solid arrows) encircling central blood vessel (broken arrow) during post-spawning phase. Note the presence of chromaffin cells (arrow heads) associated with blood capilleries (BC) (H&E, 400X).

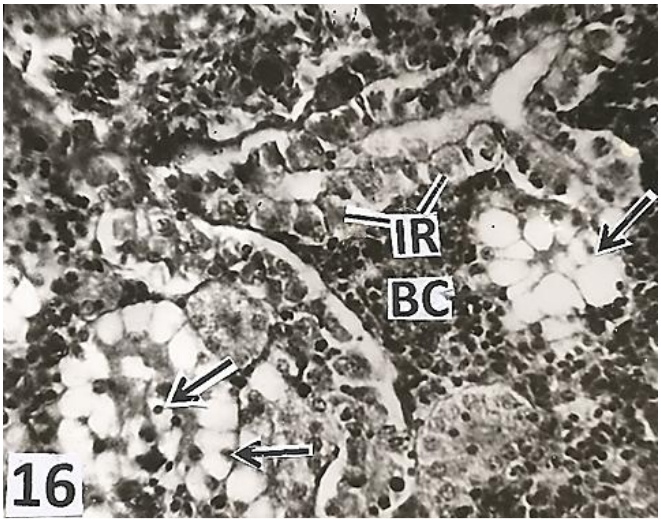

Figure 16. The section of interrenal and chromaffin cells in *Mystus vittatus*. Interrenal cells (IR) with cytoplasmic granules and prominent nuclei arranged in tubules during post spawning phase. Note vacuolated chromaffin cells (solid arrows) adjacent to blood capilleries (BC) (MT, 400X).

failed to attain maturity and they are characterize by irreregular shaped, disintegrated nuclei and liquefied yolk granules. They are found to be more apparent during maturation and spawning phases (Figs. 8, 13).

Sequential changes of interrenal and chromaffin cells in relation to ovarian cells during different reproductive phases: The activities of the interrenal and chromaffin cells undergo correlative changes during the different reproductive phases. Changes in the activities of adrenocortical tissues have been studied in terms of their number, distributional patterns and cell size along with decreases or increases of cytoplasm. Based on the histological characteristics of ovary and the gonadosomatic index (GSI) values and the frequency of occurrence of various oogenetic cells, the reproductive phases of *M. vittatus* is conveniently divided into four phases, ıncludıng growth (December to February), maturation (March to May), spawning (June to August) and the post-spawning (September to November) phases.

Growth phase (December to February): During the growth phase the interrenal cells are round or oval (4±0.12 to 5±0.06 µm) with prominent, centrally located nuclei and the cytoplasm is acidophilic. The cells are arranged two layers in tubules, separated from each other and from the parenchyma of haematopoietic cells, chromaffin cells (2±0.05 to 3±0.02 µm) are situated in groups or intermingled with interrenal cells and exhibit a pale cytoplasm and spherical nucleus (Figs. 1, 2).

The gonadosomatic index (GSI) is recorded from 0.98±0.02 in December, 1.10±0.87 in January and 1.56±0.53 in February, respectively. The oogonia are few in number and the dominant cell types in this period are early perinucleolar oocytes (Fig. 3). However, the percentage of late perinucleolar oocytes are gradually increasing during the end of growth phase (Fig. 4).

Maturation phase (March to May): During this phase, the clusters of interrenal and chromaffin cells are oriented encircling the sinusoids. The diameter of interrenal cells increased (6.0 to 6.50±0.18 µm) and undergo hypertrophy and are arranged in tubules (Figs. 5, 6). When stained with Mallory's triple stain, the homogeneous cytoplasm is lightly acidophilic while the nucleus is basophilic and mostly rounded (Fig. 6). The chromaffin cells also increased in diameter (4.0 to 4.5±0.11 µm) and undergo hypertrophy. In some areas, the interrenal and chromaffin cells are present in clusters along the wall of the blood vessels (Fig. 5).

During the onset of maturation phase in March

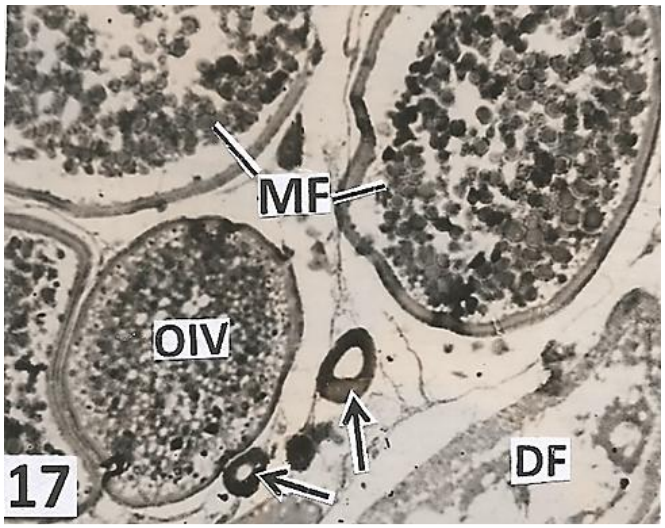

Figure 17. The section of ovary in *Mystus vittatus*. Distorted mature follicles along with oocyte IV (OIV), discharged follicle (DF) and primary oocytes (arrows) during post-spawning phase (H&E, 400X).

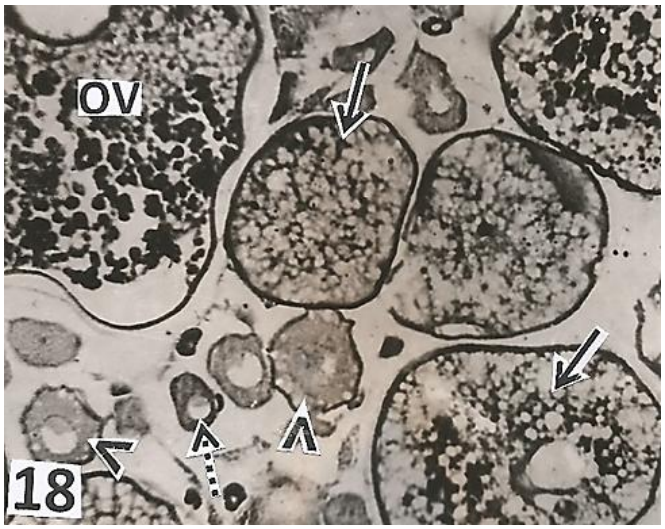

Figure 18. The section of ovary in *Mystus vittatus*. Considerable number of primary oocytes (arrow heads), oogonia (broken arrow), distorted mature follicles (solid arrows) and oocyte V (OV) during post-spawning phase (H&E, 400X).

onwards when the ovary enter into the maturation, GSI gradually increase to 2.20±0.11 in March, 5.56±0.16 in April and 9.40±0.22 in May. The highest oogenetic activity is found to occur in this phase. Majority of the developing oocytes are of stage IV and V, respectively. The immature oocytes are decrease in number (Figs. 8, 9). From the month of April to May yolk filled oocytes V are appeared and are gradually increased in number very sharply (Fig. 9). A few atretic follicles are also found in this phase (Fig. 8). The zona granulosa and zona radiata have an average thickness of 2.25±0.07 µm and 3.75±0.15 µm, respectively (Fig. 10).

Spawning phase (June to August): In the spawning phase, the interrenal and chromaffin cells undergo momentous changes. The interrenal cells are lobular or columnar cells with more acidophilic cytoplasm and hypertrophic nuclei encircling the central blood vessels (Fig. 11). The diameter of interrenal cells varies from 7.25±0.10 to 7.75±0.16 µm. However, in the late spawning phase, the interrenal cells are depleted of their cytoplasmic contents. The chromaffin cells are almost round with prominent nuclei and the cytoplasm stains brightly with eosin in haematoxylin-eosin stain (Fig. 12). The diameter of chromaffin cells ranges from 6.15±0.09 to 6.25±0.18 µm. Both the interrenal and chromaffin cells are usually arranged surrounding the blood vessels.

In June the ovary is full of mature follicles and the GSI is recorded to be 10.25±0.04. However, the GSI rises up to a peak value (12.20±0.17) in July but in August it shows a declining trend (9.52±0.08). The ovaries of this stage are full of ripe ova. The mature follicles become larger and irregular in shape with ecentric germinal vesicle and the yolk granules condensed (Fig. 13). A few discharge follicles and atretic follicles are also found (Fig. 13). At the end of this phase the zona granulosa of the mature follicles are thin and distorted in several places (Fig. 14).

Post-spawning phase (September to November): During the post-spawning phase, the interrenal cells and chromaffin cells are attenuated in size with vacuolated cytoplasm and prominent nucleus. These cells are located around the blood vessels (Figs. 15, 16), although some interrenal cells has a considerable amount of cytoplasmic mass (Fig. 15). In post-spawning phase, diameter of interrenal cells reduced considerably (3.5±0.02 to 3.8±0.05 µm) and provided with cytoplasmic granules and prominent nuclei (Fig. 16). The cytoplasm of chromaffin cells become vacuolated because of their degranulation (Figs. 15, 16) and the diameter varies from 3.0±0.17

to 3.20±0.14 µm, respectively.

In post-spawning period, the ovaries show a regression state. The GSI is recorded as 3.38±0.34 in September followed by 0.38±0.24 in October and 0.80±0.88 in November, respectively. During this phase the mature follicles are collapse and irregular in shape with distorted condition of yolk granules. Oogonia appear in large numbers along with primary oocytes in between the distorted follicles (Figs. 17, 18).

Discussion

In fish, two tissues were found in the head kidney, i.e. the interrenal tissue (cortical portion) and chromaffin tissue (medullar portion). The interrenal tissue of teleost is homologous to the mammalian adrenal cortex and is the source of cortical steroids (Jones and Phillips, 1986). The amount of interrenal tissue observed in the head kidney of fishes vary among species. In the present investigation in *M. vittatus*, the cephalic portion of the kidney is bilateral where the interrenal gland is composed of two main types of cells chromaffin and steroidogenic and are mainly associated with the posterior cardinal veins and their tributaries. Similar observations were also made by Civinini et al. (2001) in *Gasterosteus aculeatus* and Sampour (2008) in *Carassius auratus*.

Abdel-Aziz et al. (2010) also considered that interrenal and chromaffin cells were associated with the cardinal veins to be typically teleostean. In *Cichlasoma dimerus*, the interrenal gland components were found exclusively within the posterior portion of cephalic kidney arranged in a relatively diffuse manner. The steroidogenic and chromaffin cells were in close association with the wall of the posterior cardinal vein, its tributaries and sinusoids (Morandini et al., 2014). In *M. vittatus*, the interrenal cells were comparatively larger than the chromaffin cells, and they were basophilic. The chromaffin cells contained pale cytoplasm and slightly basophilic nuclei. Though the physiological role of adrenocortical tissue during sexual maturation and spawning was not clearly understood but significant hyper activity of interrenal tissues corresponds with the breeding phases of *M. vittatus*. Ball (1960) observed interrenal cells to be active during the teleost reproductive phase. Yadav et al. (1970) reported that epinephrine content is higher in *Heteropneustes fossilis* during reproduction, but that the nor-epinephrine content does not fluctuate. According to them, the rise of epinephrine content might be associated with the increased active phosphorylase levels required for metabolism during the breeding period. Nussdorfer (1986) opined that different cytological aspects of interrenal cells could be linked to steroidogenic cells undergoing different degrees of hormonal activity. In the present investigation in *M. vittatus*, the accumulation of cytoplasm contents of interrenal and chromaffin cells coincided with the transformation of various oogenetic cells and the oogenetic activities continued until spawning. There were also a few degranulated and vacuolated interrenal cells and also some chromaffin cells which could have possibly released their content for oocyte maturation. The hyperactivity of the interrenal cells could be attributed to the higher level of corticosteroid production required during maturation and spawning phases.

In *M. vittatus*, the maximum and minimum ovarian weights were recorded in July and October respectively, which coincided with the maximum and minimum nuclear diameter of interrenal cells. Therefore, the activity of these cells appeared to be closely associated with ovarian activity. The gonadosomatic index (GSI) in *M. vittatus* increased slightly during the growth period. The storage of cytoplasmic granules in the interrenal cells began at the end of this period, which was clearly reflected in tinctorial reactions. In *Channa punctatus*, the interrenal cells exhibit clear seasonal changes becoming either hyperactive or inactive during the breeding and non-breeding periods of this fish species (Verma and Mishra, 1992). During the post-spawning phase, however, only a few mature follicles and primary oocytes were noted, and the gonadosomatic index (GSI) gradually decreased. Subsequent to the release of cytoplasmic contents,

the cortical cells became less efficient to gonadal stimulation and were soon transformed into quiescent phase.

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. The occurrence of chromaffin cells close to the blood vessels indicated releasing their contents into the blood circulation. Reid et al. (1998) opined that in teleosts, chromaffin tissues were associated with the synthesis and secretion of the catecholamines. Sampour (2008) with the help of electron microscopic studies, observed numerous mitochondria in different shapes in cytoplasm of chromaffin cells probably produce energy for the activities of the cells during the synthesis of catecholamine hormones. Further studies of electron microscopy, immunocyto-chemical examinations and quantitative estimations of corticosteroids and catecholamine levels would be useful in corroborating the present findings.

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