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Distribution of different cells and seasonal variations of gonadotropin cells in the pituitary gland of *Liza parsia* (Hamilton, 1822) in relation to maturation of ovary

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Abstract
The pituitary gland of *Liza parsia* (Hamilton, 1822) was of the cranio-leptobasic type and subdivided into three distinct zones innervated by the narrow and short strip of neurohypophysis. Different cell types were identified in the pituitary gland of *L. parsia* on the basis of their distribution and staining properties. The acidophilic lactotrophs or prolactin (PRL) cells and basophilic thyrotropic (TSH) cells stained with Mallory's triple and Periodic Acid Schiff's-Orange G (PAS-OG) and Aldehyde fuchsin (AF) were found in the rostral pars distalis (RPD) region. The PRL cells showed strong affinity to aniline blue. Between PRL cells and neurohypophysis (NH), adrenocorticotropic hormone secreting cells (ACTH) were found which gave faint purple colour (OG) with PAS-OG stain. The basophilic gonadotropic cells (GTH) and acidophilic somatotropic cells (STH) were distributed in the middle proximal pars distalis (PPD) region. The GTH cells were PAS, AF positive while STH cells were PAS and Acid Fuchsin positive. The melanocyte stimulating hormone secreting cells (MSH) were observed in the pars intermedia (PI) which were positively stained with PAS. No significant changes of the activities in all of the acidophilic cell types were observed throughout the year. However, The GTH and TSH cells reacted positively with PAS and exhibited both quantitative and qualitative variations during the ovarian cycle. During growth and pre-spawning period the GTH and TSH cells were characterized by homogeneous or granulated cytoplasm and recorded with maximum cellular diameters. The activity of GTH and TSH cells was reflected by low staining intensity as well as vacuolization of the cells during spawning period. During the post-spawning phase both the GTH and TSH cells stained weakly having atretic features. The substantial changes in the ovarian activity were correlated with the changes in the activity of the GTH and TSH cells of the pituitary gland in *L. parsia*.

Keywords: Cell type variation, Gonadotropin cells, Pituitary gland, Female *Liza parsia*

1. Introduction

The pituitary gland is a neuro-epithelial complex structure which is known to mediate between the external environments and the reproductive organs [1]. The identification and distribution of the cell types in the pituitary gland of the different teleosts have attracted some investigators from the histochemical, ultrastructural and immune-cytochemical techniques [2, 3, 4, 5, 6]. Most of the authors have pointed out that the secretory cells of the pituitary gland show different pattern of distribution in the three well defined zones of adenohypophysis. The acidophilic cells are located in the rostral pars distalis (RPD) region while the basophilic cells and some acidophilic cells are found in the proximal pars distalis (PPD) and pars intermedia (PI) zones although, some differences have been noticed from species to species. Nelly and Abraham [7] studied the influences of environmental salinity on the prolactin and gonadotropin secreting regions in the pituitary of *Mugil* sp. The ultrastructure of prolactin cells in the pituitary gland of *Sarotherodon* (*Oreochromis*) *mossambica* and their response to environmental salinity were studied by Dharmamba and Nizhioka [8].

One type of gonadotropin secreting cells (GTH) has been observed in some species [9, 10, 5] recorded two types of aforesaid cells in *Mugil cephalus*.
Studies on the cyclic changes in the pituitary gland and gonads of estuarine fishes are limited [3, 11]. Hence, the aim of the present work was to identify and localize the different cell types with special emphasis to GTH cells in the pituitary gland of the brackish water teleost *Liza parsia* at all stages of ovarian development. This brackish water teleost is very much popular as food fish due to its high nutritive and palatable qualities and breed once a year which seems to be suitable for successful propagation in brackish water aquaculture.

2. Materials and Methods

Forty numbers of mature specimens of *L. parsia* (15 to 20 cm in total length and 50-75 g in total body weight) were procured from Junput brackish water fish farm and estuarine region of Digha, West Bengal. Fishes were sacrificed and total body weight of the fish and of the ovary were taken to calculate the gonadosomatic index (GSI) from the following formula:

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

2.1. Histological and histochemical methods

To study the seasonal changes of the pituitary cell types and different ovarian cells, the fishes were sacrificed and pituitary glands along with the entire brain were obtained and were fixed in Bouin's fluid for 18 h. Pieces of ovarian tissues were also fixed in Bouin's fixative for the same period of time. After fixation, pituitary and pieces of ovary were then placed in 70% ethanol and subsequently dehydrated with ascending ethanol series followed by acetone and cleared in benzene. Tissues were then embedded in paraffin wax (56-58 °C melting points). Paraffin sections of ovary and mid-sagittal sections of pituitary gland were cut at 4µm thickness using a Leica RM 2125 RT microtome. Deparaffinized sections were stained by adopting various techniques which are as follows:

1. Delafield's Haematoxylin and Eosin (HE)
2. Periodic Acid Schiff's (PAS) technique of Mc Manus [12] using Orange G (OG) as the counter stain: (PAS-OG) for demonstration of thyrotrophs, gonadotrophs and somatotrophs.
3. Mallory's Triple stain (MT) [13] for demonstration of gonadotrophs.
4. Chrome Alum Haematoxylin Phloxine (CAHP) after Gomori [14] for demonstration of corticotrophs, somatotrophs and thyrotrophs.
5. Alcian Blue-Orange G-Acid Fuchsin (AB-OFG) after Slidders [15] for demonstration of somatotrophs, gonadotrophs and thyrotrophs.
6. Aldehyde fuchsin (AF) technique of Gabe [16] using OG as counter stain for gonadothrophs.
7. Heidenhain's-Azan stain for acidophil and basophil cells.

Sections of ovarian tissues were also stained with Delafield's Haematoxylin and Eosin (HE) and Iron Alum Haematoxylin (IAH) stain for identification of different oogenetic cells.

After staining the sections of pituitary and ovary were dehydrated through ascending series of ethanol, cleared in xylene, mounted permanently with DPX and then analyzed under a binocular microscope. From the histological preparation, diameters of different pituitary cells and various oogenetic cells were measured with the help of reticulo-micrometer and ocular micrometer. The percentage of occurrence of GTH cells during different reproductive phases were measured calculating the number of GTH cells per hundred cells at five different sites on a total of ten different fishes and then averaging their number for a particular phase.

3. Results

The pituitary gland of *Liza parsia* is a compact, slightly oval in structure located in the sella turcica. Pituitary of *L. parsia* is of cranio-leptobasic type and the neurohypophysis has a short infundibular stalk entering the gland from the anterior side (Fig. 1). In the mid-sagittal section and based on the histological characteristics is divisible in three component parts: the frontal rostral pars distalis (RPD), the middle proximal pars distalis (PPD) and the distal lobe pars intermedia (PI). Although there is no sharp demarcation between RPD and PPD, they are recognized after the use of different tinctorial affinities. A narrow cutlas like non-cellular area demarcates PI from RPD and PPD (Fig. 1). The axonal fibers of neurohypophysis ramify into the pars intermedia. Small branches of neurohypophysis also penetrate into RPD and PPD (Fig.1).

3.1. Cell type distribution in the pituitary gland

In the present communication various cell types have been identified in the RPD, PPD and PI on the basis of their staining intensities are as follows:

3.1.1. Rostral pars distalis (RPD)

Two cell types are mainly identified in this region. The acidophilic prolactin cells (PRL) occupy the major part of the RPD which form a compact mass and stained red with Azocarmine in Azan stain (Fig. 2). The basophilic cells are found to be dispersed among the PRL cells are identified as the thyroid stimulating hormone secreting

cells (TSH) reacted positively and stained blue with Azan stain (Fig. 2). In *L. parsia* the corticotropic cells (ACTH) are found in RPD adjacent to the neurohypophysis which are faintly stained PAS-OG and negative to AF and aniline blue (Figs. 2, 13).

3.1.2. Proximal pars distalis (PPD)
In *L. parsia* the prominent cell types of PPD are of basophilic in nature and contained cytoplasmic granules stained purple colour with PAS-OG and can be recognized as gonadotrophs (GTH) or Cyanophil-I cells. TSH cells or Cyanophil-II cells are also identified in the PPD along with GTH cells and stained with PAS, AF and Aniline blue (Fig. 4). These basophilic cells manifested variable shapes and sizes and are exhibited spherical nuclei (Figs. 3, 4). The thyrotrophs and gonadotrophs in *L. parsia* show significant hypertrophy during spawning seasons (Fig. 12). The acidophilic cells are identified as the somatotrophs (STH). These cells stained orange red with PAS-OG and Heidenhain's Azan stain. They are found abundantly in the peripheral region of the neurohypophysis and showed a follicular pattern (Figs. 3, 9).

3.1.3. Pars intermedia (PI): This distal region surrounded the neurohypophysis where the maximum innervations of the axonal fibers can be observed. The cells exhibit amphiphilic reactions found in this region are identified as the melanotrophs (MSH) cells. These cells are PAS-OG and lead hematoxylin (PbH) positive, generally round in shape and have rounded nucleus (Figs. 2, 3).

Chromophobe cells
They are small in size, spherical or oval in shape with small eccentric nuclei and considered as degranulated state of various chromophil cells. These cells are located sparsely in RPD, PPD and PI regions (Figs. 4, 8, 13).

3.1.4. Neurohypophysis
The major components of this region are the neurosecretory nerve fibers and blood vessels (Fig. 2).

3.2. Gonadosomatic Index (GSI)
In our present study, it has been observed that the values of GSI in *L. parsia* followed a regular cyclical change during growth, maturation, spawning and post-spawning or resting phases. The highest GSI value (15.76 ± 1.34) has been noticed during February when the ovaries remained packed with fully mature follicles. The lowest GSI value (0.33 ± 0.06) have been noticed during the end of post-spawning phase in June. During July i.e. in resting phase the mean GSI value increased marginally to 0.52 ± 0.11. However, from the onset of growth phase in August slight increment of GSI (0.85 ± 0.14) is noticed. During the period of growth phase i.e. September and October the GSI value is found to increase gradually (1.29 ± 0.09 to 5.74 ± 0.28). Subsequently from November onwards when the ovary might have entered into the maturation phase GSI is gradually increased to 9.07 ± 0.43 and in December rose to 13.87 ± 0.87. In January the ovary was full of mature follicles and the GSI is recorded to be 12.03 ± 0.75. The GSI rose upto a peak value (15.76 ± 1.34) in February but in March it showed a declining trend (4.59 ± 0.14). In the post-spawning phase i.e. during April and May the yolky follicles are reabsorbed and the ovaries suffered from a regression state. The GSI values are recorded to 1.31 ± 0.08 and 0.42 ± 0.05 respectively (Fig. 18).

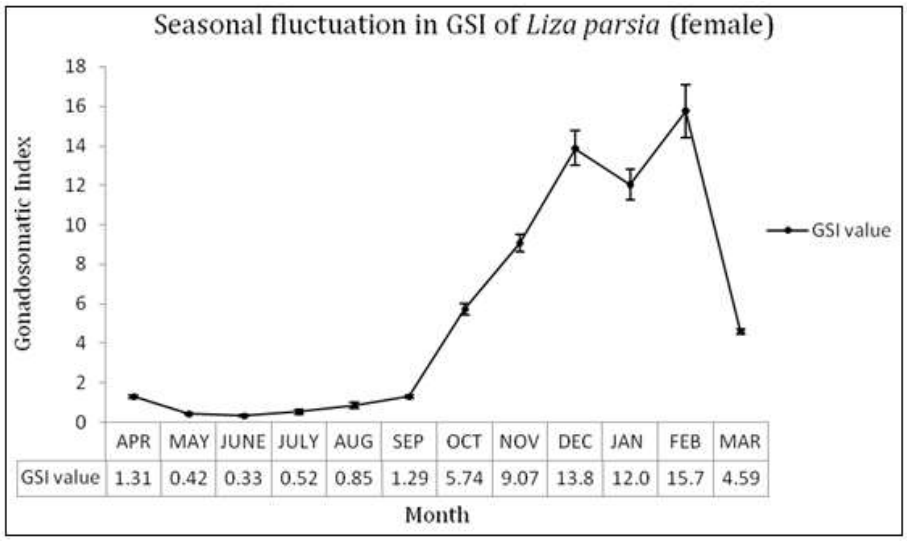

Fig 18: Variations in the Gonadosomatic index (GSI) of female *L. parsia* during different months.

3.3. Oogenesis

The ovary of *L. parsia* is a hollow sac like organ into which extend numerous ovigerous folds called ovigerous lamellae which anchor different stages of pre-vitellogenic oocytes during the course of their development. In *L. parsia* the sequence of oocyte maturation has been conveniently divided with six distinct developmental stages viz. Oogonia (Stage I), early perinucleolus stage (stage II), late perinucleolus stage (stage III), yolk vesicle stage (stage IV), yolk granule stage (stage V) and mature follicle (stage VI).

3.3.1. Oogonia (Stage I) (15-32 µm)

Oogonia are present either singly or sometimes appear in small nests within the ovigerous lamellae. The oogonium consists of large nucleus and basophilic cytoplasm (Fig. 5).

3.3.2. Early perinucleolus stage (Stage II) (35-55 µm)

This stage consists of large oval centrally placed nucleus which contains about 6 to 8 basophilic nucleoli together with chromatin materials (Fig. 5).

3.3.3. Late perinucleolus oocyte (Stage III) (55-90 µm)

This stage may also be called as the protoplasmic growth stage when the cytoplasm as well as the nuclear mass increases in size. This stage is characterized by the appearance of small vesicles in the cortical zone of cytoplasm (Fig.10). The zona radiata is gradually enlarged in thickness and a thin layer of connective tissue, the theca is found to cover the granulosa layer (Fig. 10).

3.3.4. Yolk vesicle oocyte (Stage IV) (90-130 µm)

Ova at this stage are more or less spherical in shape and the cortical alveoli cover the entire ooplasm. Most of the vesicles are empty. The oocyte is enveloped with a thick zona radiata, the middle multi-nucleated zona granulosa layer and outermost theca made up of connective tissue (Figs. 6, 10).

3.3.5. Yolk granule oocyte (Stage V) (130-190 µm)

In this vitellogenic oocyte, formation of yolk globules takes place and as a result the cell volume and diameter increases rapidly. The germinal vesicle moves towards the surface. The oocyte is enveloped with a thick zona radiata, middle multinucleated zona granulosa and outer theca (Fig. 10).

3.3.6. Mature ova or mature follicle (Stage VI) (190-260 µm)

Ova at this stage are more or less spherical in shape and yolk granules occupied the entire oocyte. The zona radiata and syncytial zona granulosa layer are greatly increased but theca layer remained unaltered (Figs. 11, 14).

3.3.7. Discharged follicle

The post ovulatory corpus luteum develops from the follicular cells immediately after the discharge of mature ovum, when the theca and follicular cells are left behind (Fig. 17)

3.3.8. Atretic oocytes

Sometimes the developing oocytes undergo resorption and fail to attain maturity are called the atretic oocytes. These atretic oocytes are characterized by irregular shaped, disintegrated nuclei and liquefied yolk granules. The granulosa cells proliferated in enormous number and invaded inside the follicle where they engulf the yolk materials by phagocytosis. These follicles are found during pre-spawning or immediately after the spawning phase (Figs. 6, 11).

3.4. Sequential changes in the pituitary gonadotropic cells and ovary during different reproductive phases

In the present observation, the seasonal changes in the gonadotropic cells in the pituitary and different germ cells in the ovary has been described on the basis of shape, size and frequency percentage of GTH cells and various oogenetic cells in the ovary. Accordingly the reproductive cycle in *L. parsia* may be grouped into growth, maturation, spawning and post-spawning or resting phases which are as under:

3.4.1. Growth phase (June to August)

The histological architecture of GTH cells of the pituitary bear a close relationship to the changes in the pre and post-vitellogenic oocytes in the ovary during the growth phase. The GTH cells are mainly distributed in the ventral portion of PPD. The rounded nuclei of the GTH cells lie at one side of the cell. These cells are positively reacted with Mallory's triple and PAS-OG stains (Figs. 3, 4). Considerable number of PRL, STH and TSH cells are discernible in the RPD and PPD zones. During this phase the average diameters of the GTH cells are calculated to be 6.23 ± 0.45 µm in June and 8.56 ± 0.16 µm in August (Fig. 3, 4). An increase in the number of GTH cells during the end of growth phase occurs at the time of propagation and development of the pre-vitellogenic oocytes.

During this phase the primary oocytes of all stages were present in the ovary (Fig. 5). However, the percentages of late perinucleolar oocytes increased during the end of this period which showed cortical alveoli stage and some yolk vesicle stages (Fig. 6). Therefore, increase in the number and activities of GTH cells correlated with the differentiation of the oocytes.

3.4.2. Maturation phase (September to November)
During this phase the GTH cells increases considerably in size and occupy almost the entire PPD zone (Fig.7) and to some extent in the border of RPD zone (Fig. 8). The increment in the cytoplasmic content and volume of GTH cells surrounding blood vessels is clearly detected at the end of maturation phase (Fig. 9). The GTH cells reached its maximum volume with secretory granules and the mean cellular diameter of the cells is 11.34 ± 0.31µm. Different stages of vitellogenic oocytes are characteristically present. However, majority of the developing oocytes were in the form of yolk granules stage having prominent zona radiata and follicle granulosa cells (Fig. 10). At the end of the maturation phase a sharp increment in number of yolk filled mature follicles are well marked (Fig.11). Subsequently with the advancement of maturity, the Previtellogenic oocytes are decreased in number (Fig. 11).

3.4.3. Spawning phase (December to February)
In the spawning phase slight decrease in the average diameter of GTH cells is noticed. The mean cellular diameter of the GTH cells is 9.39 ± 0.24 µm in December followed by 7.98 ± 0.19µm in February (Fig. 13). The GTH cells (Fig. 12) increase in number and staining intensity in the PPD region as an indication of cell granulation. During the highest ovarian activities the close association of the GTH cells with the blood cells in the PPD zone clearly indicates that the secretory products of the GTH are conveyed through the blood vessels (Fig. 12). At the end of spawning phase the cytoplasm of GTH cells is greatly reduced and the large nucleus occupied the greater part of the cytoplasm (Fig.13). The ovaries are reached in the final stage of maturity with the concomitant increase in weight and size. The predominant cell types in this period are the mature follicles at stage VI but a few previtellogenic primary oocytes are common (Fig. 14).

3.4.4. Post-spawning phase or resting phase (March to May)
The size as well as the number of GTH cells is decreased considerably from March onwards and the average size of the GTH cells are calculated to be 6.98 ± 0.3µm which further decreased to 6.12 ± 0.21µm in May. Degranulation of the GTH cells is continued. Cells in which degranulation have set in are larger in size than the granulated ones. The degranulated cells are more numerous towards the blood vessels (Fig. 15). In the PPD region the acidophils are increased in number. The chromophobes are increased in number (Figs. 15, 16). In the ovary ovigerous folds are irregular in shape and consist of mainly early primary oocytes of different sizes. Mature ova were few in numbers. Maximum proliferation of oogonia occurred and large primary oocytes also appear in between oogonia. By the end of May few developing oocytes of stage III are also found in between oogonia and primary oocytes (Fig. 17).

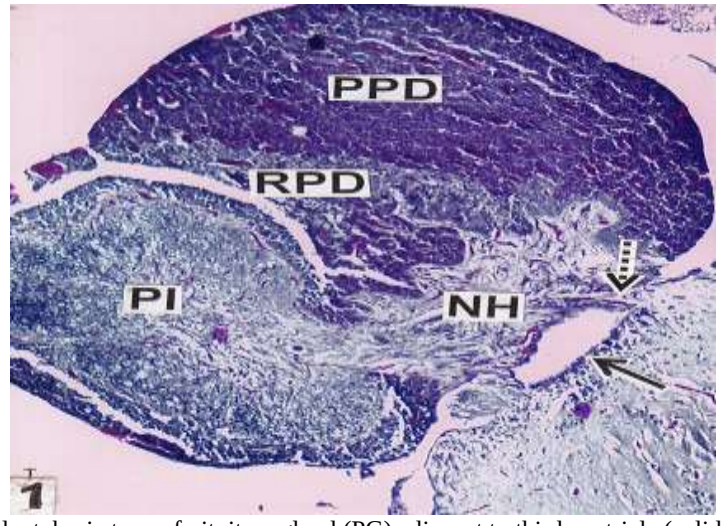

Fig 1: Showing cranio-leptobasic type of pituitary gland (PG) adjacent to third ventricle (solid arrow) with short stalk (broken arrow). Note PPD, RPD and PI of adenohypophysis and axonal fibers of neurohypophysis (NH) (CAHP) × 75X.

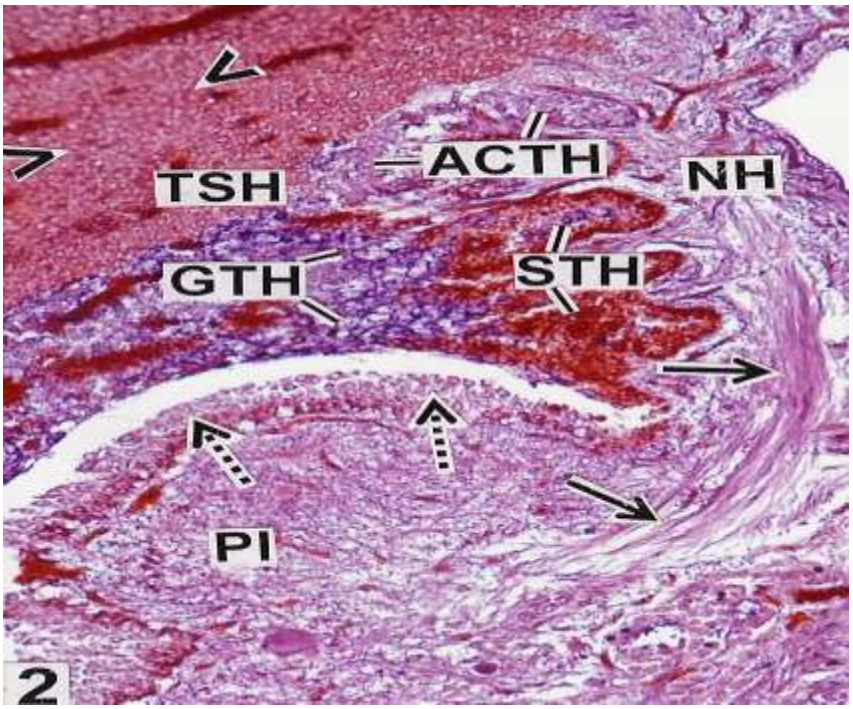

Fig 2: Showing dense population of prolactin cells (PRL) (arrow heads), thyrotropic cells (TSH) and corticotropic cells (ACTH) in RPD zone. Note gonadotropic cells (GTH) and follicular arrangement of somatotropic cells (STH) in PPD. Note also melanotropic cells (MSH) (broken arrows) along the border of PI. Solid arrows indicate axonal fiber of NH (CAHP) × 150X.

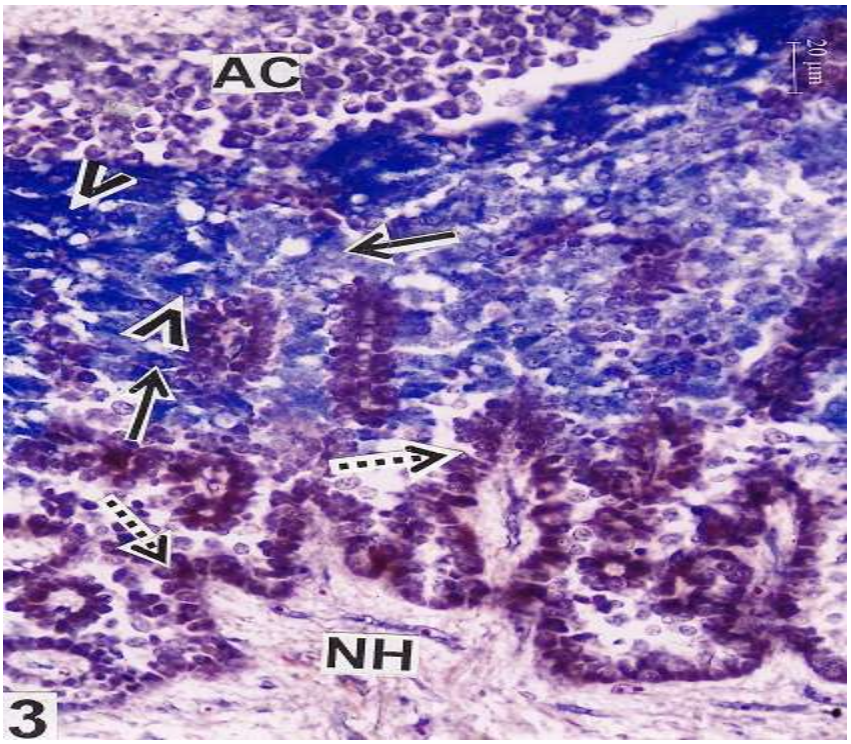

Fig 3: PPD during growth phase showing dense aggregation of GTH (arrow heads) and scattered TSH (solid arrows) in between GTH. Note the presence of STH (broken arrows) adjacent to NH and amphiphilic MSH cells (AC) in PI (MT) × 400X.

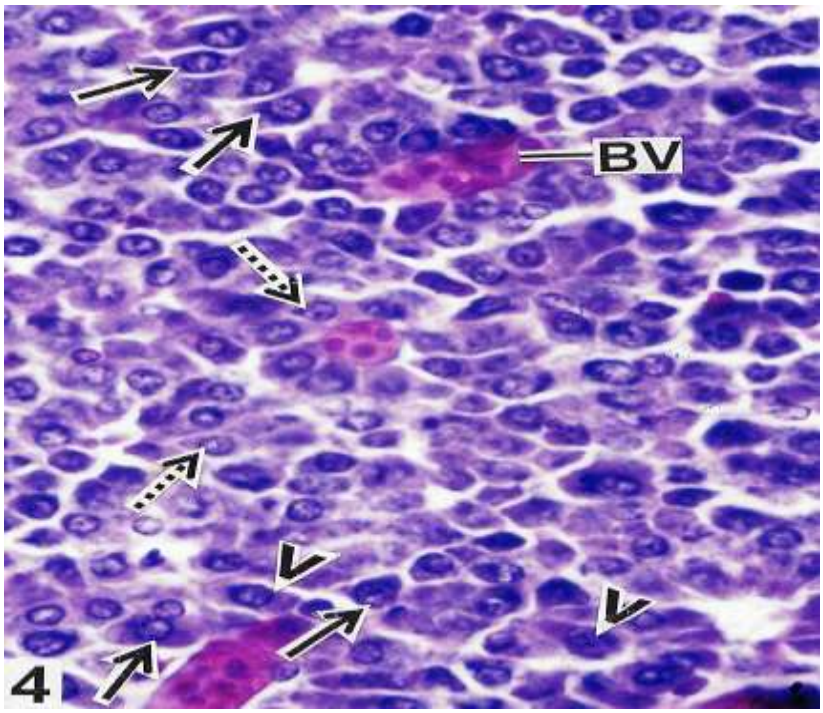

Fig 4: PPD during growth phase showing GTH cells (solid arrows), TSH cells (arrow heads) and chromophobe (CHR) cells (broken arrows) encircled in the blood vessels (BV) (PAS-OG) × 600X.

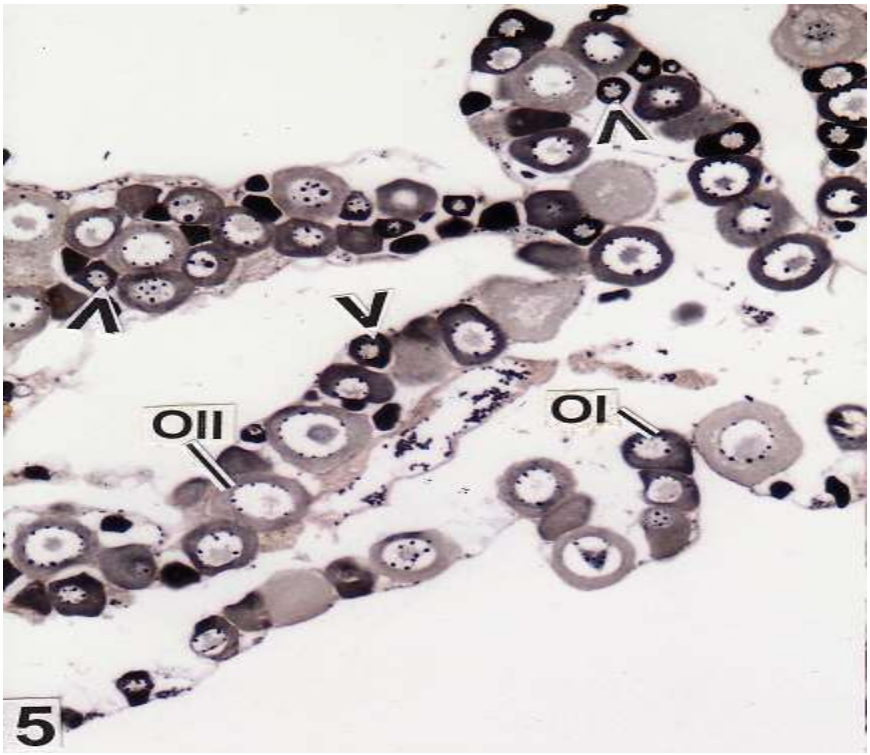

Fig 5: Showing different oogonial cells (OG) (arrow heads), oocyte I (OI), oocyte II (OII) in the ovigerous lamellae during growth phase (IA) × 150X.

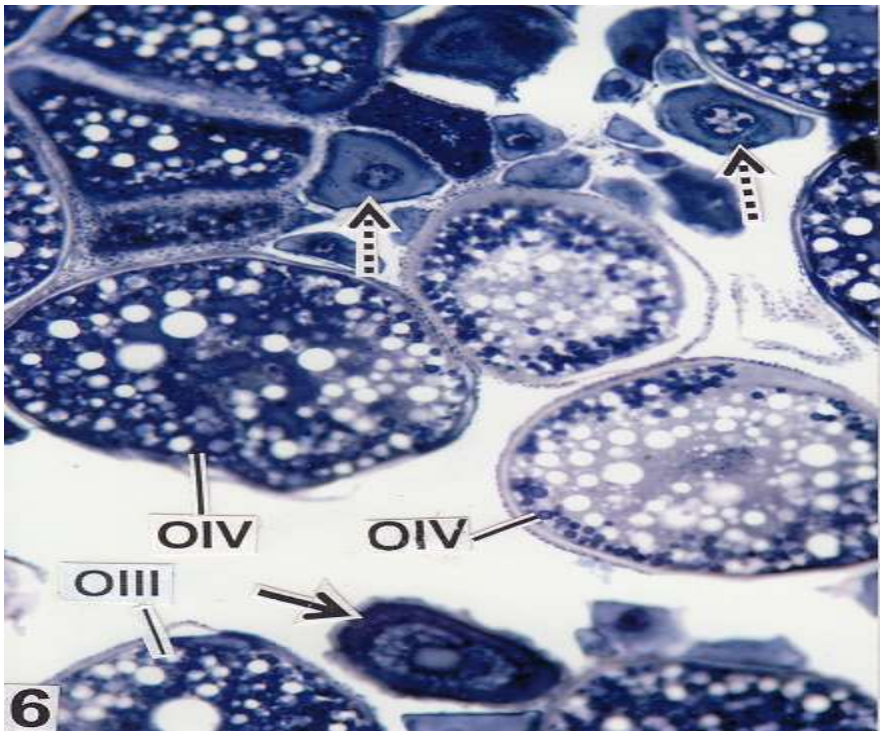

Fig 6: Ovary during end of the growth phase showing oocyte III (OIII) having cortical alveoli, oocyte IV (OIV) and few oocyte I stage (broken arrow). Solid arrow indicates atretic oocytes (IA) × 400X.

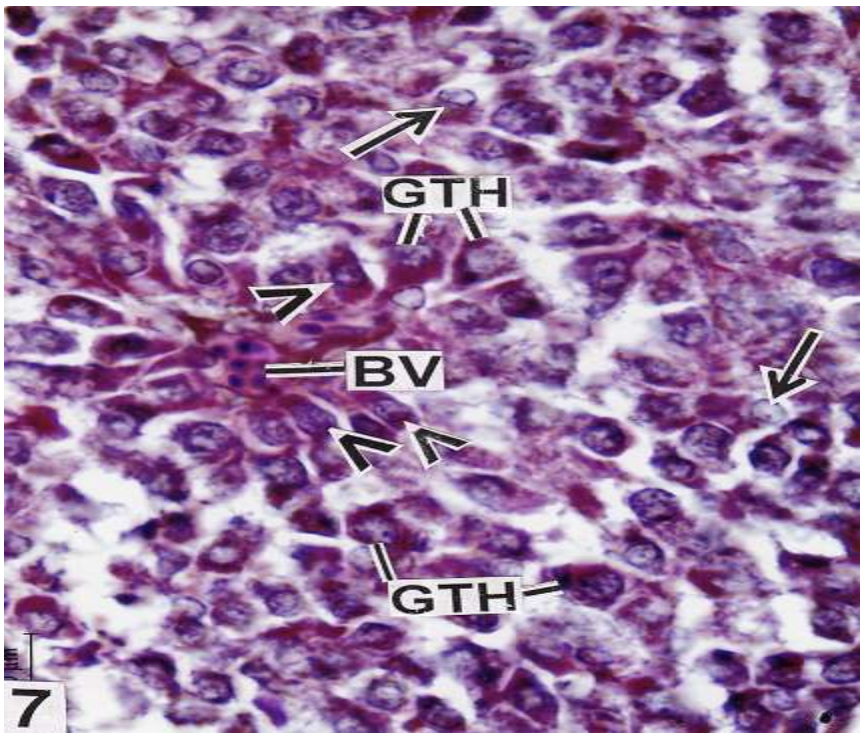

Fig 7: PPD during maturation phase showing granulation in GTH, TSH (arrow heads) adjacent to blood vessels (BV). Arrows indicate CHR cells (HE) × 400X.

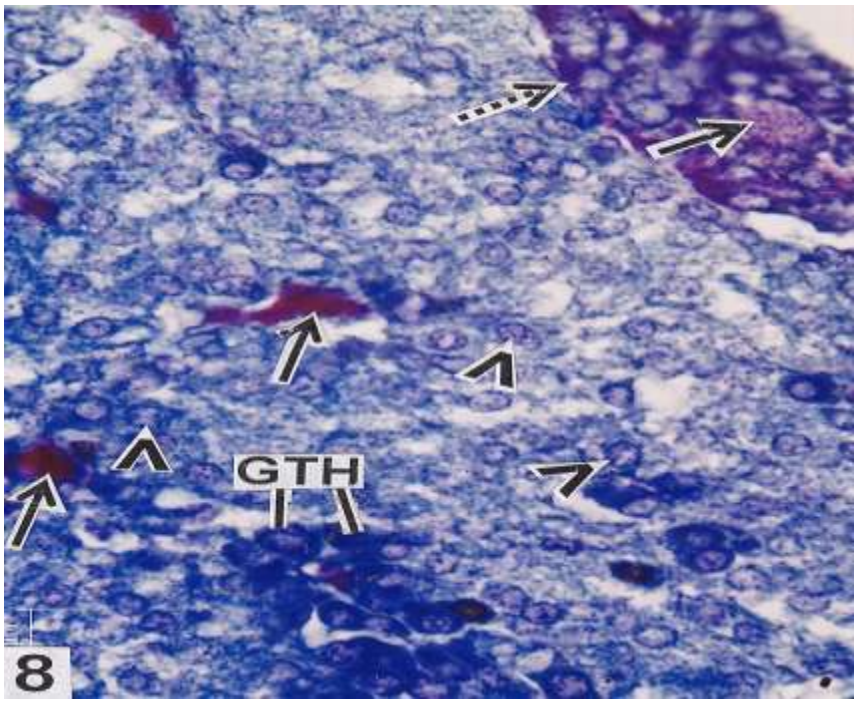

Fig 8: PPD adjacent to NH during maturation phase showing deeply stained GTH cells, TSH cells (arrow heads). Note deeply stained STH cells (broken arrow). Solid arrows indicate blood vessels (HA) × 400X.

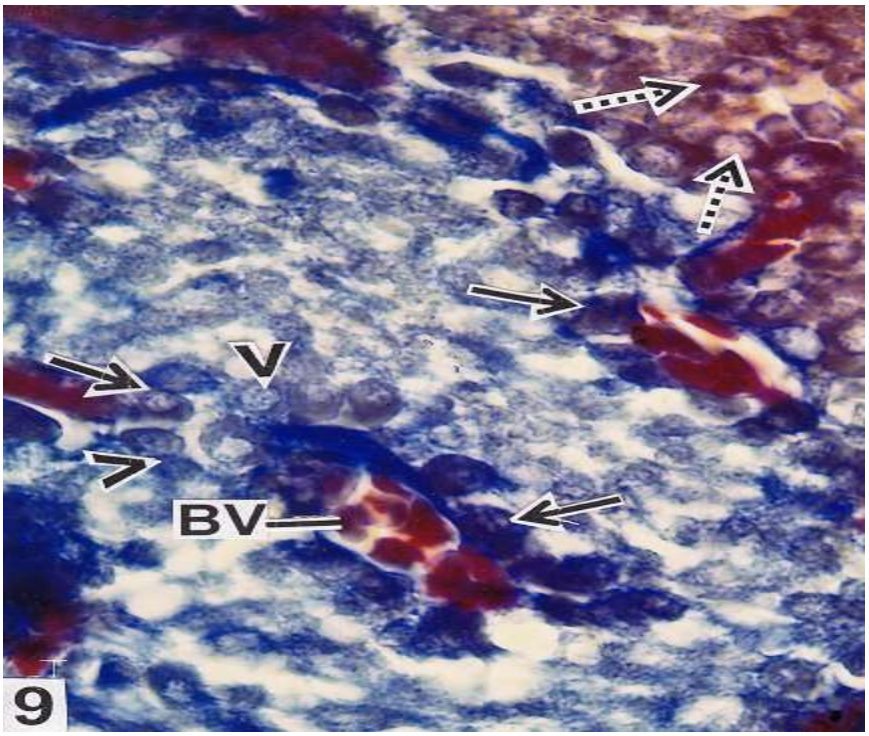

Fig 9: PPD showing densely granulated GTH cells (solid arrows) and TSH cells (arrow heads) adjacent to blood vessels (BV) during the end of maturation phase. Broken arrows indicate STH cells (HA) × 400X.

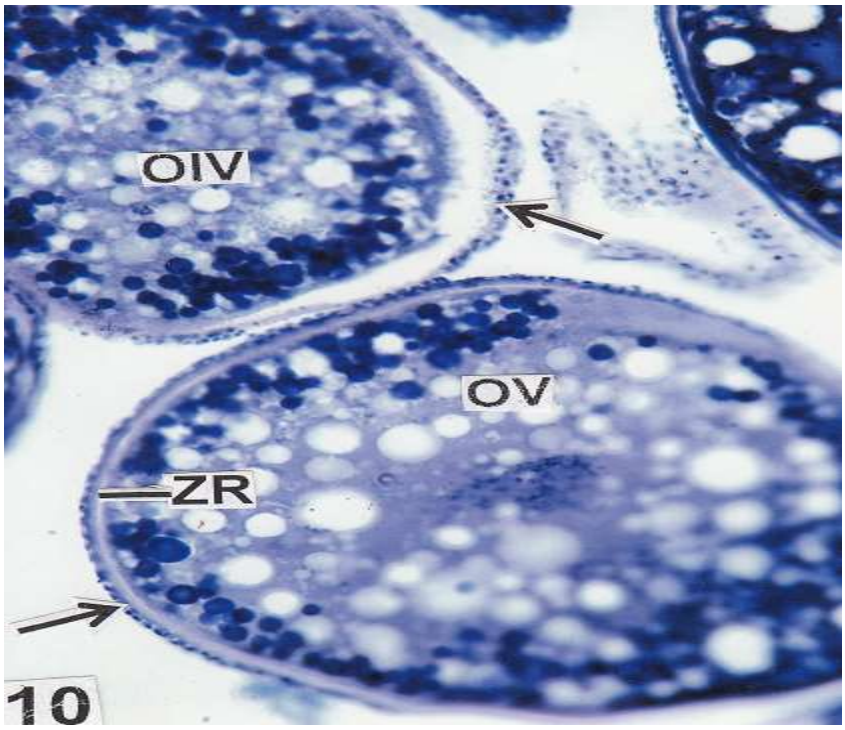

Fig 10: Showing oocyte IV (OIV) and oocyte V (OV) stages filled with yolk vesicles and yolk granules during maturation phase. Note prominent zona radiata (ZR) and prominent nucleus of zona granulosa (arrows) (IA) × 400X.

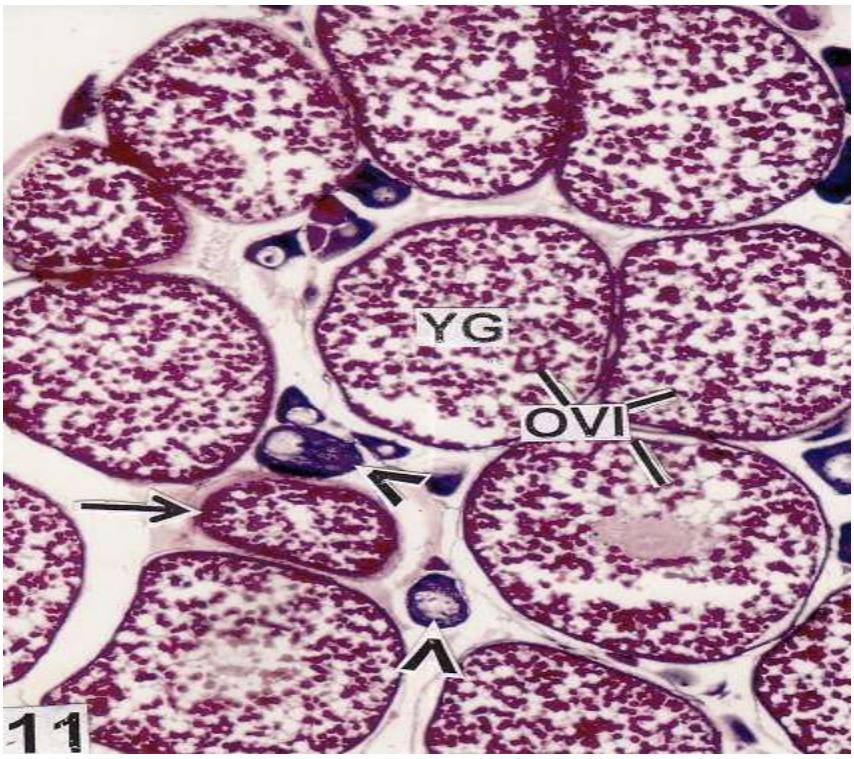

Fig 11: Mature ova or oocyte VI (OVI) stage with dense yolk granules during late maturation phase. Arrow heads indicate pre-vitellogenic oocytes and arrow indicates atretic oocytes (HE) × 150X.

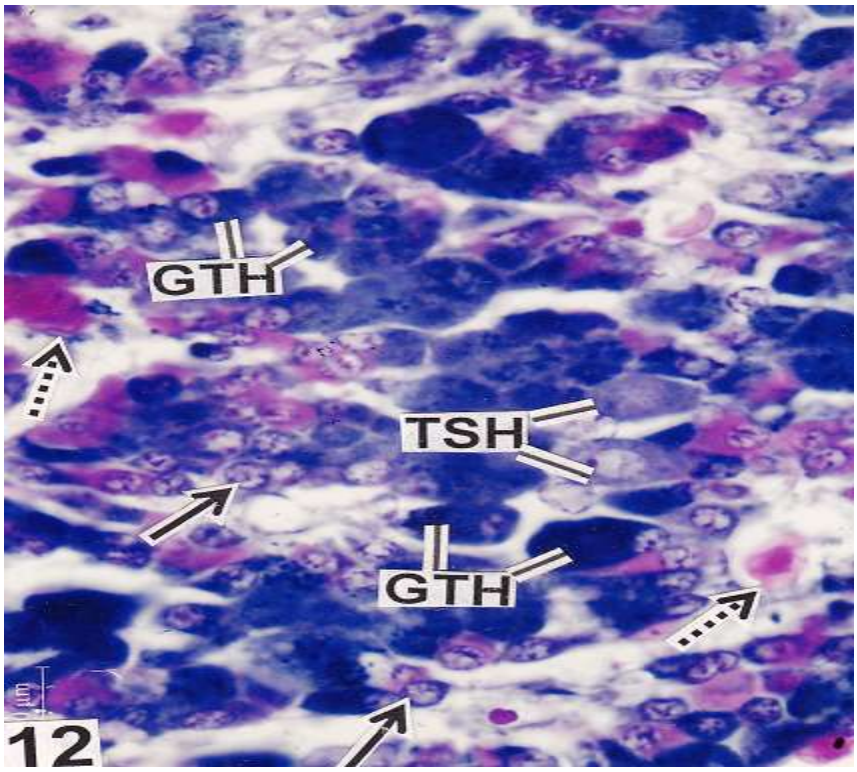

Fig 12: Showing hypertrophy of TSH and GTH cells during spawning phase. Note blood vessels (broken arrows) in between TSH and GTH. Note also few nuclei of atrophied GTH cells (solid arrows) (CAHP) × 400X.

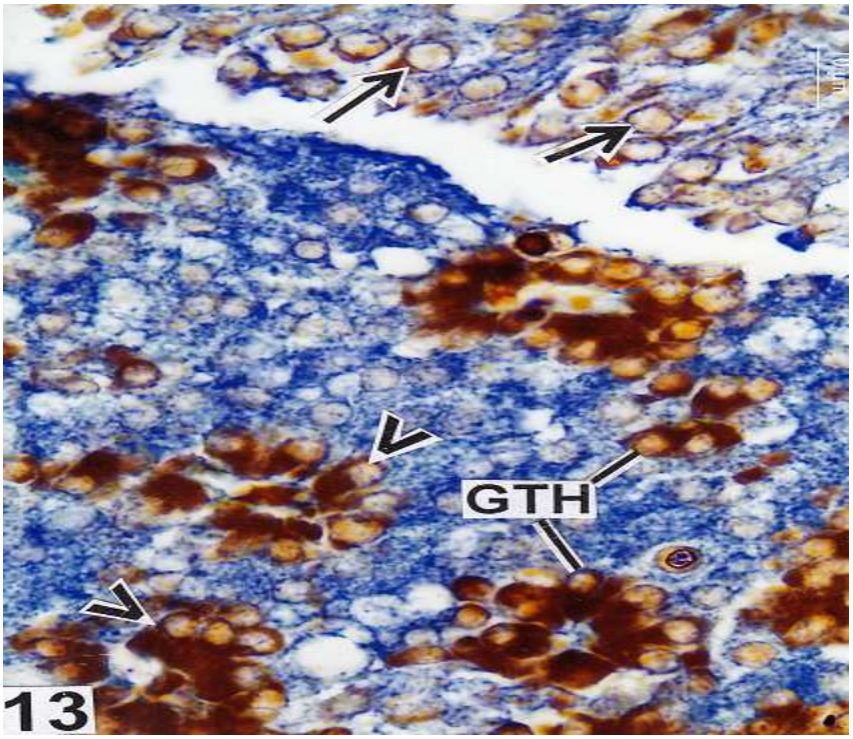

Fig. 13: Showing reduced size of GTH (arrow heads) in the PPD region during spawning phase. Note the predominant nucleus of ACTH cells (solid arrows) in between NH and PPD (AF) × 400X.

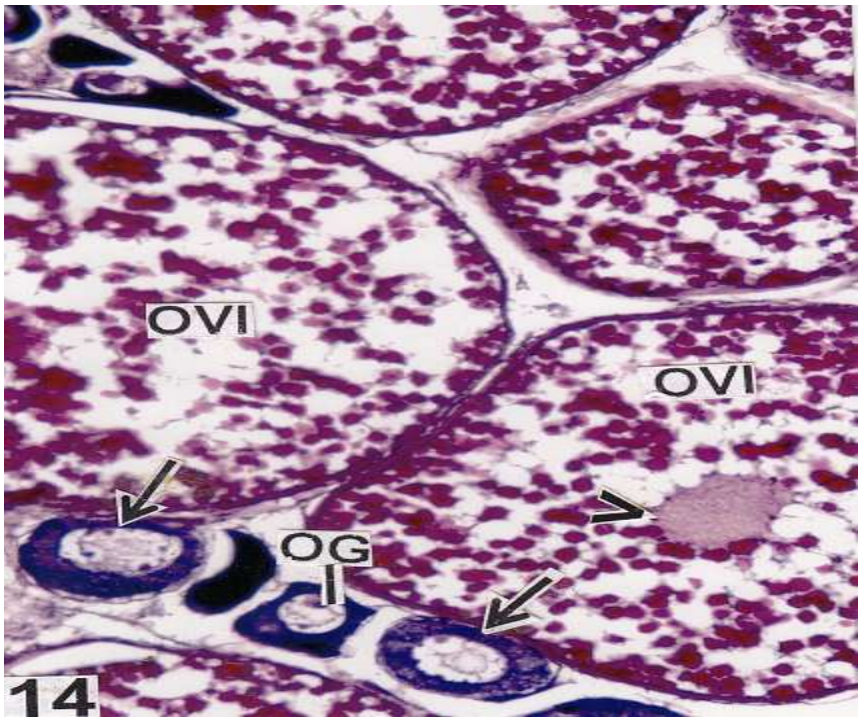

Fig 14: Mature ova (OVI) stage with eccentric germinal vesicle (arrow head) during spawning phase. Note the presence of oogonial cells (OG) and primary oocytes (solid arrows) in between mature oocytes (HE) × 400X.

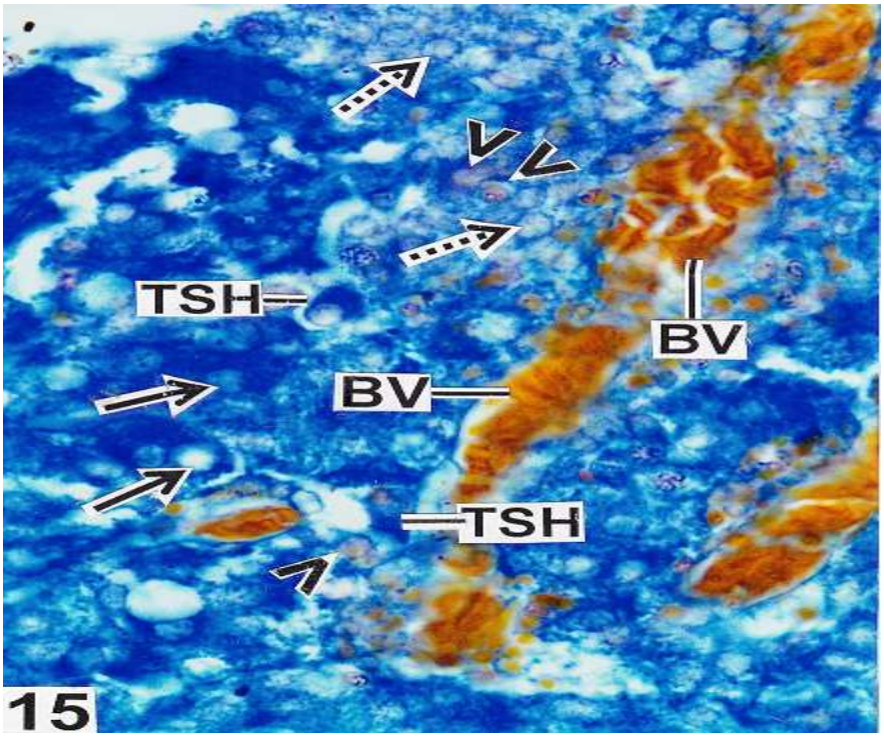

Fig 15: GTH having rim of cytoplasm encircling the nucleus (solid arrows), TSH and few acidophils (arrow heads) and chromophobe cells (broken arrows) adjacent to blood vessels during post-spawning phase in the PPD region (AB) × 1000X.

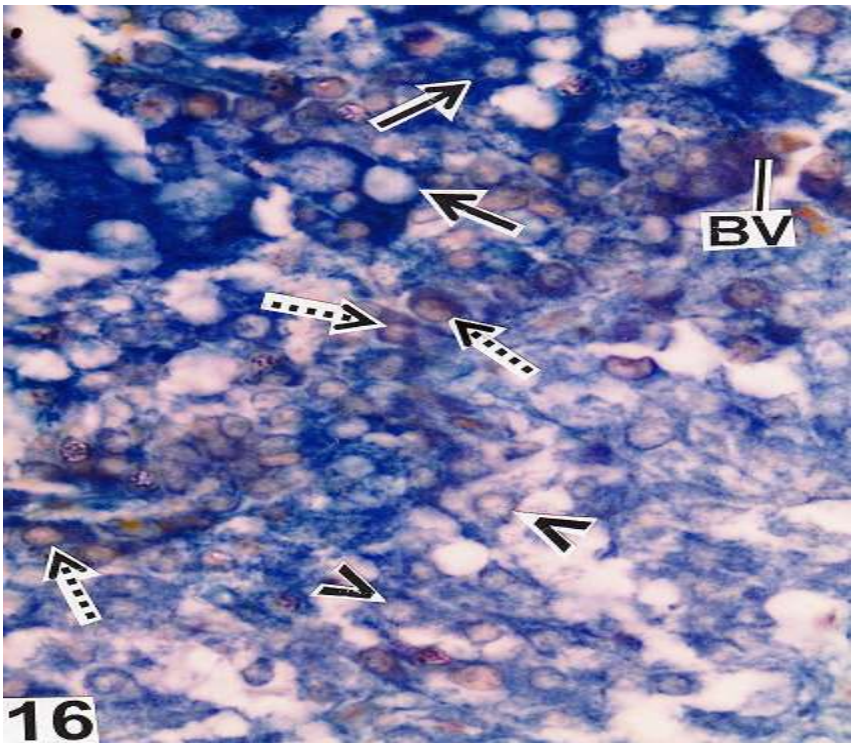

Fig 16: Showing the cytological feature of GTH (solid arrows), PRL cells (broken arrows) and chromophobes (arrow heads) in the junction of RPD and PPD during post-spawning phase (AF) × 400X.

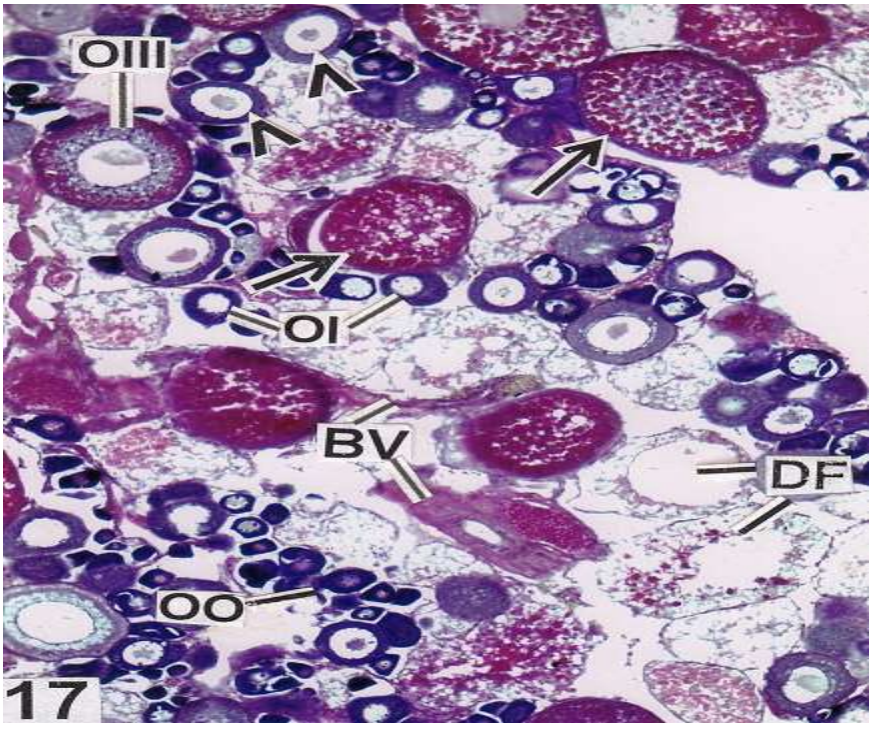

Fig 17: Ovary of post-spawning phase showing few mature oocytes (solid arrows) surrounded by blood vessels (BV), oocyte III (OIII), primary oocytes (arrow head), oocyte I (OI), oogonial cells (OG). Note discharged follicles (DF) in between oocytes (HE) × 150X.

4. Discussion

The teleost hypophysis is known to vary greatly in topography, size, and mode of attachment in nervous ramification as well as histological architecture [17]. The pituitary gland of *Liza parsia* belongs to the cranio-leptobasic type i.e. anterior dorsal attachment and was composed of adenohypophysis and neurohypophysis demarcated by a narrow strip. The same type of attachment has also been reported in the pituitary of *Valamugil cunnesius* [18]; *Oreochromis niloticus* [19]; *Mystus vittatus* [6]. Based on the tinctorial properties and distribution, adenohypophysis has been subdivided in rostral pars distalis, proximal pars distalis and pars intermedia innervated by the neurohypophysis process. In the present observation in *L. parsia* various cell types located in the constituent parts of the pituitary gland on the basis of staining reaction in the cytoplasmic content with different methods employed. Joy and Sathyanesan [20, 21] and Jafri and Ensor [22] also identified various cell types in the pituitary of a few teleosts. In the present investigation the PRL cells are provided with acid fuchsin and Azocarmine staining formed a compact mass in the RPD of *Liza parsia*. This finding is in agreement with those of Sage and Bern [23], Schreibman *et al.* [24] in different teleosts.

However, some authors reiterated the follicular arrangement of PRL cells with ovoid or spherical lumina in *Bagrus docmac* and *B. bayad* [10]; in *Clarias lazera* [25]. The prolactin like hormone was considered necessary for osmoregulation in fish [26]. This hormone is believed as a carrier for sodium ion transport in the chloride cells of the gills [27] and is essential in *L. parsia* for osmoregulation in brackish-water environment. The TSH cells stained with Azan, PAS-OG of *L. parsia* are identified between the PRL cells in the RPD and this finding was also in accordance with Olivereau [28] who also made similar observation in the RPD of *Anguilla anguilla*. In the present observation the ACTH cells were interlocated between the neurohypophysis and PRL cells which are inactively responsive to the annual cycle. This observation was similar to the findings of Zaki *et al.* [29]; Aseem and El-Boray [30] who also reported that the ACTH cells are generally found at the interphase between the PRL cells and the neurohypophysis. Mandal and Sinha [31] reported that the ACTH cells were lead hematoxylin positive and were located in the RPD bordering the neurohypophysis and occurred in groups in *Catla catla*. In the present investigation, in *Liza parsia* the Cyanophil-I or GTH cells formed the main bulk of cells of the PPD. These cells are comparatively large angular or oblong in shape and displayed purple colour in PAS-OG stain and were located in the middle part of the PPD. In *Oncorhynchus kisutch* the GTH cells were located in the PPD arranged as cords of cells [32]. It has been reported that the GTH cells of the *Mugil cephalus* stained positively with PAS-OG and Aniline blue and were negative to CAHP and acid fuchsin. In the present observation TSH cells or the Cyanophil-II cells in *L. parsia* stained navy blue colour with aniline blue and were located in the PPD, intermingled with cyanophil-I cells. Narayan *et al.* [2] also opined that in *Tilapaia mossambica* the TSH cells were located in the PPD along with the GTH cells. Both the TSH and GTH cells show a significant hypertrophy during the breeding season as also advocated by Joy and Sathyanesan [21]. In *L. parsia* the acidophils of the PPD region considered as STH cells stained positively with PAS-OG and acid fuchsin and negatively with AF. These STH cells are arranged as cords of cells dorsoventrally between neurohypophysis and GTH cells. Similar pattern of distribution of STH cells are also indicated by Narayan *et al.* [18] in *V. cunnesius*; in *O. kisutch* by Leatherland and Sonstegard [32]. The MSH cells were the amphiphilic cells located in the PI region in *L. parsia* which were tsained positively with PAS-OG and AF. PAS positive MSH cells have also been described in the pars intermedia of several teleosts [20]; in *Siganus rivulatus* [29]. In *L. parsia* the major components of the neurohypophysial region consisted of the neurosecretory nerve fibers.

In *L. parsia* the development of ovary could broadly be divided in the two phases. In the pre-vitellogenic phase growth is slow and comparatively few cytoplasmic changes occurred. The vitellogenic phase is characterized by rapid growth and deposition of large amount of yolk in the cytoplasm. The terminology and criteria of staging the oocytes were in conformity with the description of Mayer *et al.* [33].

The correlation between the GTH or Cyanophil-I cells and the gonadal cycle have been observed in a number of teleosts showing the signs of increased activity of these cells in association with the maturation of the gonads [34, 35, 36, 37, 6]. In *L. parsia* the GTH or Cyanophil-I cells of the PPD exhibited changes concomitant with the ovaries. It has been observed that the transformation of the oogonia into the primary oocyte did not seem to be influenced by gonadotrophin as the activity of the Cyanophil-I cells followed by granulation or hormone production is at its initial phase and extremely weak at this time. The GTH cells in *L. parsia* exhibited their prominence having densely stained basophilic cytoplasm with PAS, AF and Aniline blue during the maturation of the ovary. At the end of maturation phase and prior to spawning the cyanophil-I cells increased in their number. In this

phase the Cyanophil-I cells form the major component of the PPD. Degranulation and vacuolization processes of the Cyanophil-I cells appeared to be concomitant with vitellogenesis. This clearly indicates therefore, that the GTH is an essential prerequisite for the vitellogenesis. The oogenetic activity as revealed by the GSI showed an increase in the maturation stage and attains peak values in the late maturation or early spawning. During the spawning phase, most of the cyanophils, on the other hand became vacuolated although some might be continued to load with glycoproteinaceous materials. At this moment most of the oocytes are mature although a few immature oocytes might persist. It may be assumed therefore, that the entire gonadotrophin produced was utilized for the maturation of these oocytes. This is in conformity with the findings of Krishnan and Diwan [38]; Al-Absawy [5].

During the post-spawning period, extreme depletion of the GTH cells accompanied by an increase in the number of atretic follicles and oogonial proliferation suggested that a low content of gonadotrophin in the cyanophils is responsible for the oolysis of the oocytes of advanced stage but at the same time it does not inhibit oogonial proliferation in *L. parsia*. Rai [39] suggested that glycoproteinaceous gonadotrophin contents controlled the process of vitellogenesis, ovarian maturation and initiation of oviposition in *Tor* (*Barbus*) *tor*. Khanna and Pant [40] reported a poor concentration of glycoproteinaceous contents during the restitution phase in *Glyptothorax pectinopterus*. In contrary, a higher concentration is observed during the accumulation of yolk in the oocytes which later brought about the ovulation of eggs. In *L. parsia* the TSH or Cyanophil-II cells followed a more or less similar cycle as the gonadotrophs, suggested a possible synergistic association of the two in reproduction. Sage [41] believed the possibility of the thyroid involvement in reproduction in fishes.

5. Conclusion

Acidophilic prolactin cells, corticotropic cells (ACTH), somatotropic cells (STH), basophilic gonadotropic cells (GTH), thyrotropic cells (TSH) and amphiphilic melanocyte stimulating hormone secreting cells (MSH) have been found in different parts of the pituitary gland of *L. Parsia*. No significant changes of the activity of acidophilic cells were observed throughout the year. However, the basophilic GTH cells and TSH cells in PPD are reflected their changes in the cellular architecture in correlation to ovarian development during different reproductive phases.

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