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DISTRIBUTIONAL PATTERN OF DIFFERENT CELL TYPES WITH SPECIAL EMPHASIS ON SEASONAL CHANGES OF GONADOTROPHS IN THE HYPOPHYSIS OF INDIAN RIVER SHAD *Gudusia chapra* (HAMILTON, 1822) IN RELATION TO OVARIAN MATURITY

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Reproduction is a natural phenomenon and it performs under the influence of various external factors which mediate their effect through the hypothalamo-hypophyseal-gonadal subsystem operating as an organic axis bridging the ecological influences and gonadal maturation. Information is collected through the hypothalamus and is passed on to the hypophysis. Hypothalamus releases GnRH which exerts its effect on hypophysis/pituitary to release gonadotrophin hormone from gonadotrophs of Pituitary. Gonadotrophic hormone helps in gonadal maturation and sex steroid production which plays a very important role in oogenesis, final maturation of oocytes. The Pituitary gland of *G. chapra* is cranio-leptobasic type. It is generally composed of neurohypophysis and adenohypophysis. The adenohypophysis is divided of three parts viz., rostral pars distalis (RPD), middle proximal pars distalis (PPD) and massive pars intermedia (PI). The major part of the RPD occupied by the acidophilic prolactin cells (PRL) stained with acid fuchsin. Fewer adrenocorticotropic cells (ACTH) were scattered among prolactin cells. The basophilic gonadotrophs (GTH) and thyrotrophs (TSH) reacted positively to aniline blue and periodic acid Schiff's (PAS) were distributed in the anterior and middle part of PPD. The somatotrophs (STH) were the only acidophils that identified. Melanocyte stimulating hormone (MSH) stained with aniline blue and melanocyte concentrating hormone (MCH) stained with acid fuchsin were identified in PI. During growth and maturation phase both GTH and TSH cells were distinguished by intense staining and dense homogenous granules with maximum cellular diameter. During post-spawning phase both cells showed low staining intensity. The seasonal changes in the ovary of *G. chapra* have been described on the basis of variation in GSI value and frequency percentage of the different female germ cells. The gradual change in the ovarian cycle has been correlated with changes of GTH and TSH cells in pituitary of *Gudusia chapra*.

Keywords: *Gudusia chapra;* gonadotrophs; GSI; hypophysis; ovarian cycle.

1. INTRODUCTION

The pituitary has a key position in the endocrine orchestra and plays a decisive role in reproduction. The function of pituitary is mostly controlled by the hypothalamus through the synthesis and release of GnRH, therefore, acting as a major initiation of the hormonal cascade controlling the reproductive axis. The development of the gonads and the process of reproduction are motivated by hormones of the

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pituitary gland which are known to mediate between the external environment and the reproductive organs [1]. The pituitary gland of *G. chapra* is small and lodged in a shallow depression of sella turcica. It is attached ventrally to the infundibulum by a short stalk (Fig.1); hence the gland is cranio-leptobasic type. Nerve fibres pass through the stalk of the pituitary and thus connecting the neurohypophysis with the brain (Fig.1). Based on histological characteristics and its cell types, the adenohypophysis is divisible into three component parts viz., antero-dorsal rostral pars distalis (RPD), the middle part proximal pars distalis (PPD) and posterior massive the pars intermedia (PI). Peculiar feature of oro-hypophysial duct is noticed in between lobes of RPD (Fig. 2). Various cell types have been identified in the RPD, PPD and PI on the basis of their staining intensities with various staining methods. Based on staining affinities the adenohypophysis contains either acidophilic or basophilic cells. Most of the investigators opined that the pituitary is required for gonadal maturation; in its absence vitellogenesis is suppressed with atresia of the larger developing oocytes, spermatogenesis is blocked at the spermatogonia-spermatocyte stage, steroidogenesis does not occur in the gonadal endocrine tissues [2,3,4]. The present studies were undertaken with a view to determine the seasonal environmental influence on the cytology of the pituitary gonadotrophs in relation to gonadal maturation of clupeid fish *Gudusia chapra* by using various modern staining techniques. These studies would help to ascertain the proper maturity stages of gonads and the knowledge of control mechanisms of hypothalamus and pituitary in reproduction. It is commercially important freshwater clupeid and this species fetch high price.

2. MATERIALS AND METHODS

Twenty adult healthy female (average length 10-15.5 cm and mean body weight 30.0 gm) of *Gudusia chapra* were collected throughout the year from a particular area of Panchet reservoir (23°40'41"N 86°44'49"E), Jharkhand, India.

2.1 Gonado-Somatic Index (GSI)

20 adult fishes were collected during the 2nd week of every month from January 2017 to December, 2018. Total body weight of fishes taken separately and then ovaries were taken out to measure separately to calculate Gonado-somatic-index (GSI) from following formula:

$$GSI = \frac{GW}{BW-GW} \times 100$$

(GW=total weight of gonads, BW=total weight of fish body)

2.2 Histological Methods

To study the seasonal changes of the pituitary and ovaries the fishes were sacrificed following the guidelines given by the institutional ethical committee. As the pituitary of *G. chapra* is lodged inside the shallow sella turcica, it is difficult to dissect out the pituitary intact along with the brain. The entire brain was exposed by dissection from the dorsal aspect and then dipped into 10% neutral formalin for 30 minutes. Then the brain with hypothalamus and pituitary gland were dissected out from the cranium and fixed in Bouin's fixative for 18 hours. For ovarian histological studies, fishes were sacrificed; ovaries were dissected out and were cut into pieces and immediately fixed in Bouin's fluid for 18 hours. Fixed tissues were placed in 70% alcohol and passed through ascending series of graded alcohol for the purpose of dehydration, finally cleared in xylene. The tissues were embedded in paraffin wax (56-58°c melting point). Both the ovary and pituitary (mid-sagital section) were cut at 4 µm thickness in Leica RM 2125 microtome. After dewaxing the sections were brought to water by decending series of alcohol starting from xylene then various staining methods were adopted which are stated below:

Mallory's Triple stain (MT) (Mallory, 1936) [5] to demonstrate gonadotrophs.

Chrome-alum-Haematoxylin-Phloxine (CAHP) (Gomori, 1941) [6] to demonstrate corticotrophs, thyrotrophs and somatotrophs.

Alcian blue-Orange G-Acid Fuchsin (AB-OFG) (Slidders, 1961) [7] for demonstration of somatotrophs, gonadotrophs and thyrotrophs.

Periodic acid Schiff's (PAS) technique (McManus, 1948) [8] using orange G (OG) as the counter stain (PAS-OG) for demonstration of gonadotrophs, thyrotrophs and somatotrophs.

Heidenhans Azan (HA) for basophil and acidophil cells.

Lead-haematoxylin-phloxine (PbHP) for the demonstration of gonadotrophs and thyrotrophs.

Delafield's haematoxylin and Eosin (HE)

Iron-alum haematoxylin (IAH) and

Romies Azan (RA) for identification of different ovarian cells.

After staining the sections were dehydrated through ascending series of ethanol, cleared in benzene,

mounted with DPX and examined under binocular microscope. From the prepared slides diameter of GTH cells were calculated by reticulo-micrometer and ocular micrometer. The diameter of the GTH cells was measured from a total of 10 cells per fish. The diameter of the various oocytes was measured in a total of 10 cells per fish.

3. RESULTS

The pituitary gland of *G. chapra* is cranio-leptobasic type as it is attached ventrally to the infundibulum by a short stalk (Fig. 1). Based on histological characteristics and its cell types, the adenohypophysis is divisible into three component parts viz., antero-dorsal rostral pars distalis (RPD), middle proximal pars distalis (PPD) and the pars intermedia (PI) posteriorly. Based on staining affinities the adenohypophysis contains either acidophilic or basophilic cells. A considerable amount of neurosecretory materials have been found to be scattered in the neurohypophysis (Fig. 6). The peculiar feature of oro-hypophysial duct is noticed in between lobes of RPD (Fig. 2). The various cell types have been identified in the RPD, PPD and PI on the basis of tinctorial activities which are as below:

3.1 Cell Types Distribution in Pituitary Gland

3.1.1 Rotral Pars Distalis (RPD)

This zone is located in the antero-dorsal position of adenohypophysis and is packed closely with acidophilic cells mostly (Fig. 2). The RPD zone appears to contain two types of chromophilic acidophils which shows a strong affinity for acid dyes like azocarmine, orange G and acid fuchsin. The acidophilic prolactin cells (PRL) found to be attached with blood vessels occupies the major part of the RPD and stained red with acid fuchsin (Fig. 4). Other types of acidophilic cells which are numerically fewer and generally dispersed among the PRL cells are fuchsinophilic (Fig. 4). These cells are known as corticotrophic (ACTH) cells.

3.1.2 Proximal Pars Distalis (PPD)

This glandular part of the adenohypophysis occupies the central area of the pituitary gland. Three types of chromophilic cells can be distinguished in the proximal pars distalis. The only acidophils present in the PPD are generally identified as somatotrophs (STH). The anterior and middle part of PPD region is provided with basophilic GTH cells and TSH cells stained with lead haematoxylin, aniline blue, alcian blue and PAS. The TSH cells are elongated cells. Both types of cells show a regular granulation-degranulation process correlated with the gonadal cycle (Fig. 3).

3.1.3 Pars Intermedia (PI)

It comprises the ventral portion of adenohypophysis. The branches of the neurohypophysis ramify in the PI maximally than RPD and PPD. The PI contains two types of cells, the larger cells provided with homogenous cytoplasm which stained with aniline blue and are identified as melanotroph (MSH) cells. The comparatively smaller cells are provided with scanty cytoplasm and stained with acid fuchsin and identified as MCH cells. The various sizes of neurosecretory materials (NSM) which stained with acid fuchsin have been found to be scattered in between MSH and MCH cells (Fig. 6).

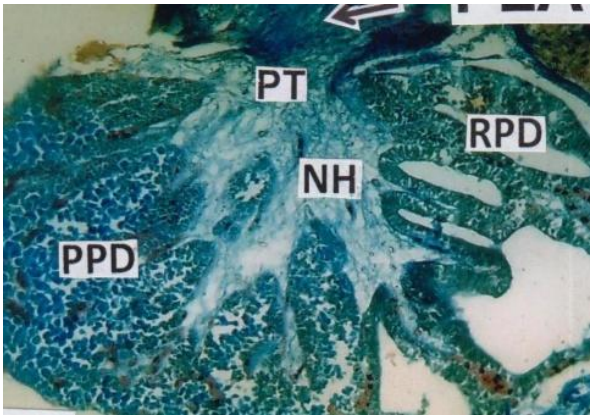


Fig. 1. Cranio-leptobasic pituitary gland of *G. chapra* having a short stalk (arrow). The adenophysis is divided into tubular arrangement of RPD and massive PPD. Note the ramification of axonal fibres in neurohypophysis (NH) (AB-OFG) X 50

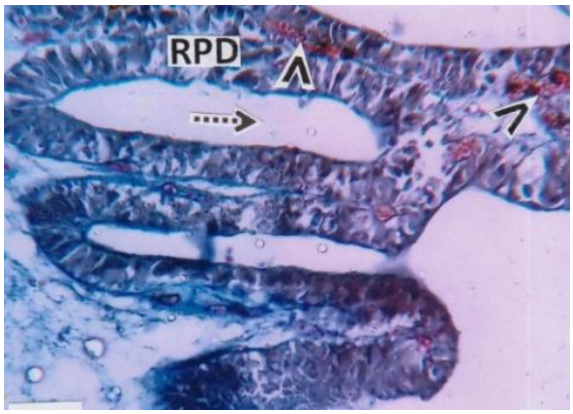


Fig. 2. Enlarged view of RPD lined with acidophilic cells adjacent to blood vessels (arrow heads). Note the pecuiliar feature of orohypophysial duct in between lobes of RPD (broken arrow) (CAHP) X 400

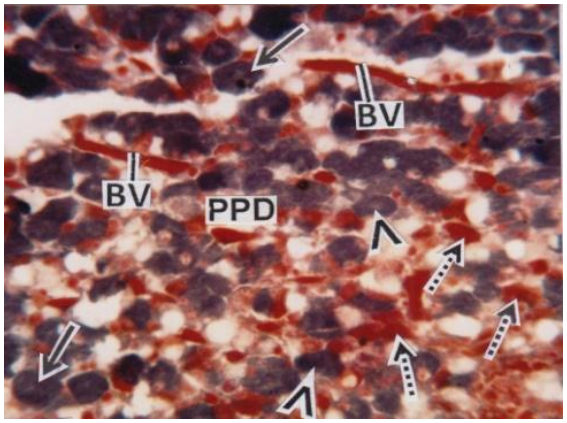
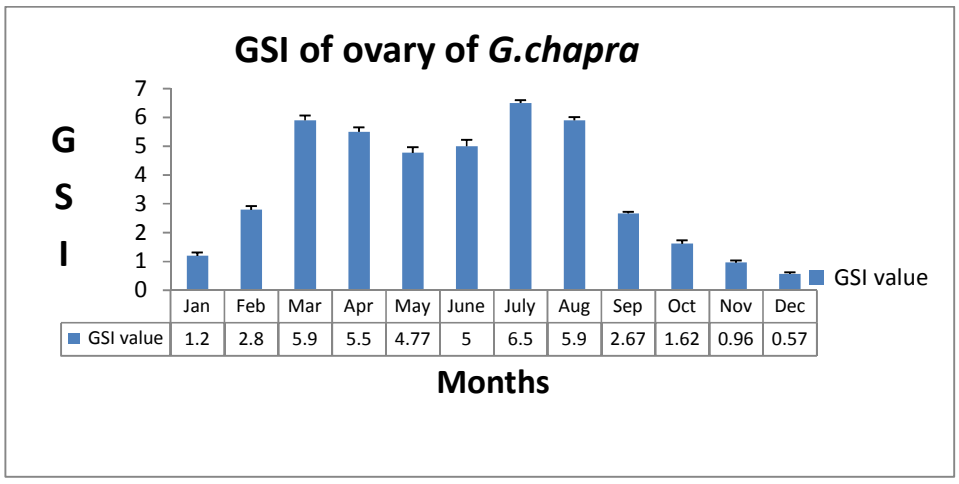


Fig. 3. PPD showing maximum number of GTH (arrows), few TSH (arrow heads) and scattered acidophilic STH (broken arrows) (PbHP) X 600



Graph 1. GSI of ovary of *G. chapra*

3.1.4 Neurohypophysis

The neurohypophysis arises from the infundibulum downwards and enters the body of the pituitary gland. It is composed of loose connective tissue fibres, nerve fibres and neuroglial cells. The neurohypophysis is highly vascularised and the neurosecretory materials are stained with CAHP (Fig. 6).

3.2 Gonado-Somatic Index (GSI)

During the present investigation, it has been observed that the values of GSI in *Gudusia chapra* follow regular cyclical changes during growth, maturation, spawning and post spawning phases due to variations in different ecological factors and availability of food. However, the highest GSI value (6.5±0.10) has been noticed during July and late March (6.0±0.20) when the ovaries remained packed with fully mature follicles. The lowest GSI value (0.57±0.60) has been recorded during the end of resting phase in December. During January and February when the preparatory and first maturation phase begin the mean GSI value increases and the values recorded to 1.2±0.12 and 3.8±0.15 respectively. During early March i.e. onset of first maturation phase the GSI further increases to 4.8±0.16. However, during late March i.e. first spawning phase the GSI value rises to 6.0±0.20 but declines at the end of first spawning phase i.e. April and recorded to 5.5±0.16. With the onset of second maturation phase the GSI further reduced to 4.77±0.2 in May which further increases to 5.0±0.23 in June. However, highest GSI value is recorded during second spawning phase in July (6.5±0.10) which declines in August (5.9±0.12). In the post-spawning period i.e. September and October the GSI values decrease to 2.67±0.06 and 1.62±0.12. In November and December i.e. during resting phase the GSI value drops to 0.96±0.08 to 0.57±0.06 and the ovaries suffer from a regression state.

3.3 Oogenesis

The sequence of oocyte maturation in *G. chapra* has been divided into six developmental stages viz., oogonia (stage I), early perinucleolus stage (stage II), late perinucleolus oocyte (stage III), yolk vesicle stage (stage IV), yolk granule oocyte (stage V) and the mature follicle (stage VI).

3.3.1 Oogonia (Stage I)

Oogonia are present either singly or in small nests within the ovigerous lamellae (Fig.7). The oogonium consists of an ecentrically placed large nucleus and two or three nucleoli (Fig. 8). These oogonial cells generally range from 10 µm x 14 µm to 19 µm x 23 µm in diameter. The nucleus varies from 6.2 µm to 7.4 µm.

3.3.2 Early perinucleolus oocyte (Stage II)

These oocytes are the product of the mitotic division of the oogonium. The early perinucleolus oocytes generally vary from 28 µm x 34 µm to 40 µm x 48 µm in diameter. The centrally placed nucleus ranging from 14 µm to 16µm and the nucleoli increased to 10-15 in number (Fig. 8).

3.3.3 Late perinucleolus oocyte (Stage III)

In this stage progressive increase of cytoplasm as well as the nuclear mass found. This stage is characterized by the formation of yolk vesicles or cortical alveoli along the peripheral region of the ooplasm (Fig. 8). The size of the late perinucleolar oocyte varies from 88µm x 102 µm to 90 µm x110 µm. Nucleus is large and spherical having condensed chromatin materials and average diameter is recorded to 18 µm x 22 µm and contains about 30-40 fragmented basophilic nucleoli.

3.3.4 Yolk vesicles or late maturing oocyte (Stage IV)

The oocyte at this stage increase in size and the cortical alveoli cover the entire ooplasm. Most of the vesicles are empty but some of them are filled with homogenous materials. This stage of oocyte enveloped with a thick zona radiata, the middle zona granulosa layer and outer theca. The diameter of this stage of oocyte ranges from 145 µm x 170 µm to 180 µm x 220 µm. The size of the yolk vesicles varies from 8µm to 10 µm (Fig. 9).

3.3.5 Yolk granule oocyte (Stage V)

Formation of yolk globules takes place in this stage and as a result the cell volume and diameter increase rapidly. This stage is represented by the migration of nucleus from the centre of oocyte towards the periphery. The nucleus shows a tendency to shrink in size and becomes irregular with the advancement of the stage. The size of the yolk granule stage ranges from 240 µm x 250 µm to 255 µm x 262 µm in diameter.

3.3.6 Mature follicle (Stage VI)

At this stage, the oocytes are completely mature and ready for spawning. In these vitellogenic oocytes, the yolk granules coalesced and remain tightly packed with each other so as to form yolk mass (Fig. 12). The nucleus is ecentric in position and irregular in outline. The diameter of the mature follicle measuring approximately 300 µm x 450 µm.

3.3.7 Atretic oocytes

Sometimes the developing oocytes undergo resorption and fail to attain maturity are called atretic oocytes. They show signs of shrinkage and characterized by irregular shaped, disintegrated nuclei and liquefied yolk granules. The follicular atresia is found to be more apparent during the end of maturation and spawning phases (Fig. 1).

3.4 Seasonal Changes of Gonadotroph Cells (GTH) in Relation to Ovary during Different Reproductive Phases

3.4.1 The preparatory or growth phase (January)

During growth phase the rostral pars distalis (RPD) is dominated by acidophilic cells stained with acid fuchsin. The proximal pars distalis (PPD) is dominated by aniline blue positive basophilic cells and scattered fuchsinophilic acidophils. The fuchsinophilic prolactin cells (PRL) in RPD are predominant during January which stain strongly with acid fuchsin in Mallory's triple stain and therefore, be also referred to as fuchsinophils (Fig.4). They range in size from 4.5 µm x 4.8 µm to 5.2 µm x 5.5 µm. The rim of cytoplasm is highly stained encircling the centrally placed nucleus (Fig. 4). Small numbers of scattered elongated corticotrophic (ACTH) cells are also present in between PRL cells which are also stained with acid fuchsin. The size of the ACTH cell varies from 2.5 µm x 4.0 µm to 3.4 µm x 5.2 µm. The nucleus is placed more or less centre of the cell (Fig. 4). During the end of growth phase the glycoprotein materials are found to be accumulated in the cytoplasm of GTH cells (Fig. 5). In the end growth phase intense stain of CAHP in GTH cells has been observed as gonadal differentiation is well marked in this phase (Fig. 6). The neurosecretory materials have been found to be closely associated with the GTH cells (Fig. 6). At the end of growth phase GTH constitute 76% and the number of STH cells reduced to 24%.

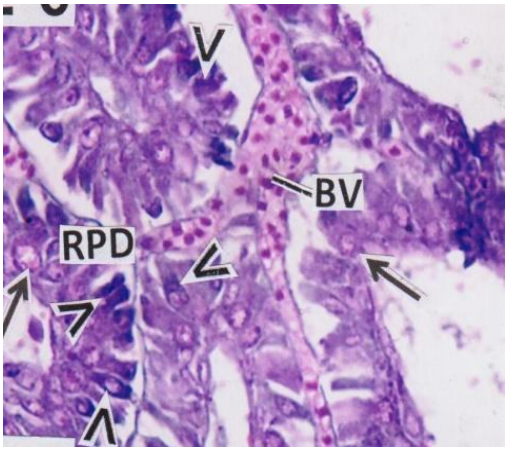


Fig. 4. RPD dominated by fuchsinophilic prolactin (PRL) cells and fuchsinophilic corticotrophic (ACTH) cells having rim of cytoplasm adjacent to BV (MT) X 400

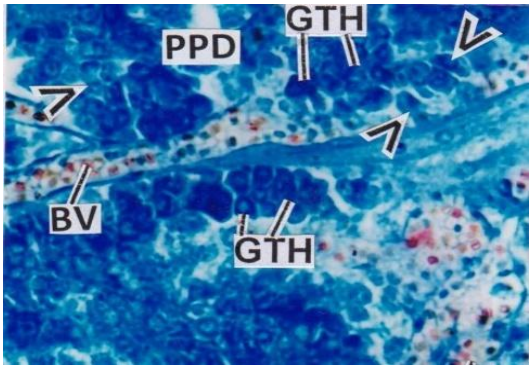


Fig. 5. End of growth phase showing alcinophilic gonadotrophs (GTH) and thyrotrophs (TSH) (arrow heads) adjacent to blood vessels (BV) (AB-OFG) X 400

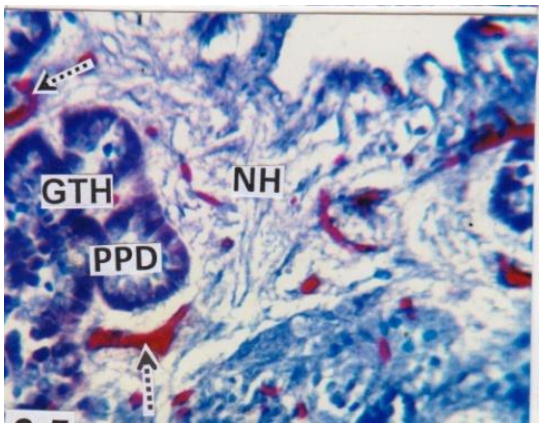


Fig. 6. PPD showing orientation of GTH at the border of neurohypophysis (NH) and surrounded by neurosecretory materials (broken arrows) (CAHP) X 400

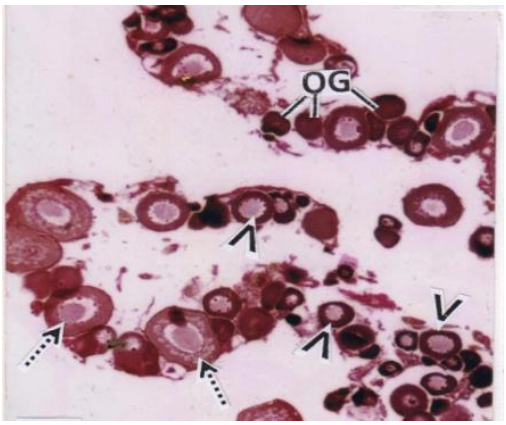


Fig. 7. Ovigerous lamellae characterized by large number of oogonia (OG), early perinucleolar oocytes (arrow heads) and late perinucleolar oocytes (broken arrows) (HE) X 100

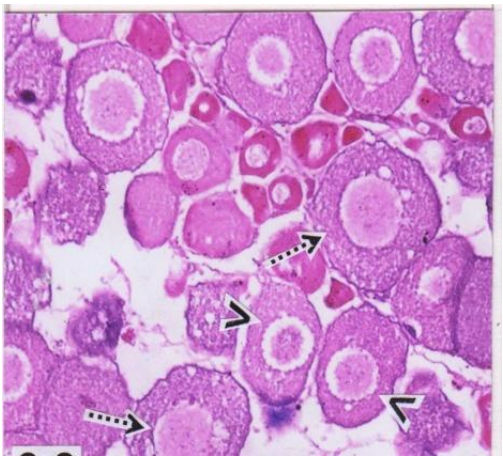


Fig. 8. Late growth phase showing oocyte III (broken arrows) and late perinucleolar oocytes (arrow heads) (HE) X 100

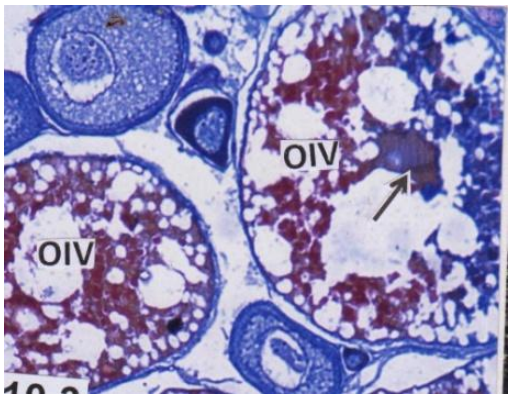


Fig. 9. Oocyte IV (OIV) showing migration of germinal vesicle (arrow) towards the periphery of oocyte (MT) X 600

The pars intermedia (PI) during growth phase comprises of two types of cells. The comparatively large and the cytoplasm with intensely stained with Orange G encircling a central nucleus which was identified as MSH cells. The other cells are more or less round or oval in shape and the rim of cytoplasm weakly stained with Orange G encircling the nucleus known as MCH cells.

During early growth phase numerous ovigerous lamellae extend into the lumen of ovary which anchors different stages of immature oocytes (Fig. 7). From middle of growth phase the ovigerous lamellae consists of oogonia and late perinucleolar oocytes (Fig. 8). At the end of growth phase considerable numbers of yolk vesicle stages (stage IV) are found (Fig. 9).

3.4.2 Maturation phase (February to early March and May to June)

During the first maturation phase the size and volume of the pituitary gland considerably increased. The CAHP stain exhibits maximum number of prolactin cells in the RPD. The phloxinophilic cytoplasmic granules of ACTH cells show intense staining during this phase and the cellular diameter increased. During the end of March, May and June the Orange G positive ACTH cells in the RPD considerably increased in size. In the PPD region the fuchsinophil STH cells are found to be intermingled with the GTH cells (Fig. 10). The aniline blue positive GTH cells provided with dense cytoplasmic mass and the diameter ranges from 14.8 µm x 15.6 µm to 16.2 µm x 16.4 µm respectively. The oval TSH cells are also exhibit strong aniline blue reaction and the size varies from 5.8 µm x 6.8 µm to 7.8 µm x 8.2 µm. During end of March the PPD comprises 70% GTH cells, 25% TSH cells and 5% STH cells. The cytoplasm of GTH cells increased considerably and strongly stained with Azan, the nucleus is acentric in position. During later period of March, May and June in the middle part of the PPD the concentration of cytoplasmic mass with predominantly glycoprotein materials in the GTH cells are found to be progressively increased. In the pars intermedia no significant changes have been encountered in two types of acidophils during this period.

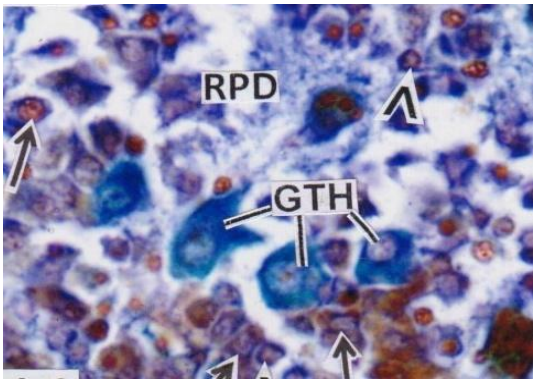


Fig. 10. PPD showing increasing cytoplasmic mass of GTH (solid arrows) and TSH (broken arrows). Note acidophilic STH cells (arrow heads) in between. (MT)X400

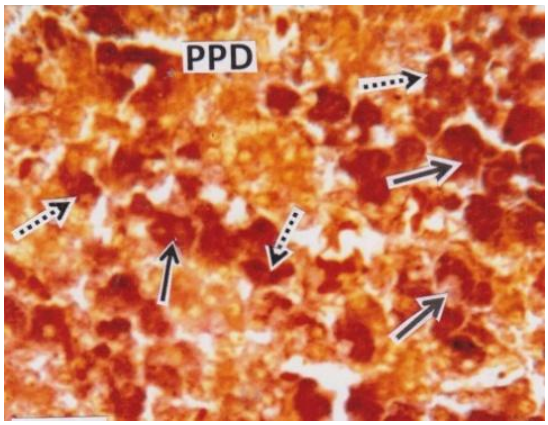


Fig. 11. PPD showing intense PAS positive GTH (solid arrows) and TSH (broken arrows) cells during end of maturation phase. (PAS-OG) X 400

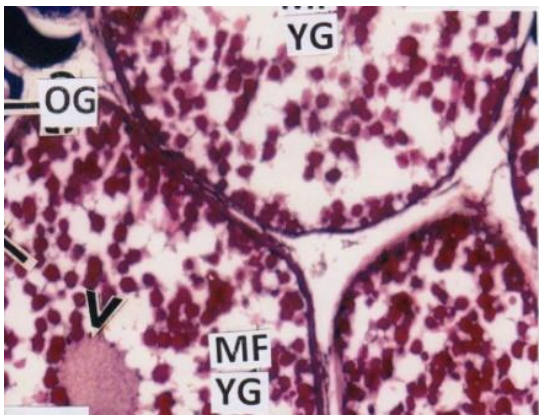


Fig. 12. MF with accumulation of yolk granules (YG) and acentric position of germinal vesicle (arrow head). (HE) X 600

During maturation phase the ovary increase in volume and the weight of the ovary begin to increase from early March. From late February and May onwards the yolk begins to deposit in the cortical and central regions of the developing oocytes and considerably increased in stage Voocyte (Fig. 15). Vitellogenesis progressed in early March. At the end of early March the number of mature follicles (MF) attain maximum with dense yolk granules. The mature follicles are provided with acentric germinal vesicle (Fig. 12).

3.4.3 Spawning phase (Late March to April and July to August)

During first spawning phase the size and volume of the pituitary gland increased. The RPD at this phase is represented by fuchsinophilic scattered prolactin cells, aniline blue positive ACTH cells having diameter ranges from 5.6 µm x 5.8 µm to 6.0 µm x 6.2 µm repectively. However, the posterior part of RPD the aniline blue positive GTH cells increased considerably. During spawning phase the anterior part of PPD is densely populated with aniline blue positive GTH and TSH cells encircling blood vessels interspersed with few STH cells. The diameter of TSH cells range from 10.0 µm x 10.4 µm to 9.4 µm x 9.8 µm. However, at the middle part of the spawning season the GTH cells increased considerably having richly stained with alcian blue and closely associated with blood vessels (Fig. 13). The size of the GTH cells ranges from 18.8 µm x 20.2 µm to 20.8 µm x 22.4 µm.

During the month of April the rich condensed glycoprotein deposition in the cytoplasm of GTH cells which are still exist as evidenced by PAS-OG stain (Fig. 14).

During this phase the ovaries are orange yellow in colour and they almost occupy the entire body cavity. The ovary is full of translucent mature ova, most of them belonging to stage V and mature follicles (Fig. 15). Few immature oocytes and atretic follicles are also encountered during this phase (Fig. 15).

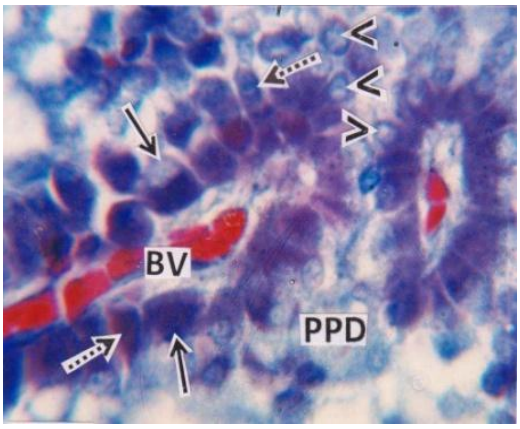


Fig. 13. PPD showing heavy accumulation of aniline blue positive cytoplasmic mass in GTH (solid arrows) and TSH (broken arrows) encircling BV. Arrow heads indicate feeble reaction in STH. (MT) X 600

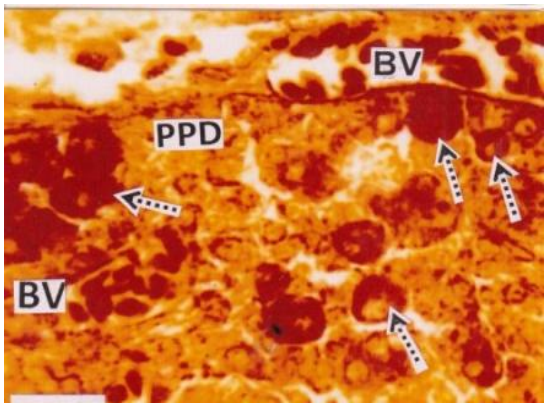


Fig. 14. Orientation of PAS positive GTH cells (broken arrows) along with the wall of BV during spawning phase. (PAS-OG) X 600

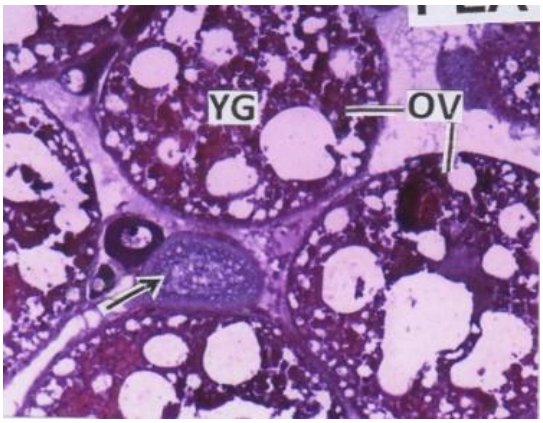


Fig. 15. Ovarian tissue having full of oocyte V (OV) having dense aggregation of Yolk granules (YG). Arrow indicates occasional presence of atretic follicle. (HE) X 400

3.4.4 Post-spawning phase (September to October)

The size and volume of the pituitary gland is comparatively reduced. In the RPD the phloxinophilic prolactin cells are more or less same diameter to that of maturation and spawning phase. The phloxinophilic and aldehyde fuchsin positive ACTH cells still larger in size. In the PPD the alcian blue

positive GTH cells and TSH cells are comparatively reduced in size and the cells range from 12.4 µm x 12.6 µm to 12.8 µm x 13.0 µm and 8.4 µm x 8.6 µm to 8.8 µm x 9.2 µm respectively. At the end of post spawning phase some of GTH cells in PPD is provided with granular cytoplasm and acentric position of nucleus but majority of the GTH cells are found to be collapsed leaving rim of cytoplasm. The TSH cells also exhibit elongate in shape having scanty cytoplasm (Fig. 17). During resting phase i.e. in November and December in the PPD zone gradual accumulation of Azan positive cytoplasmic mass in GTH cells have been encountered adjacent to blood vessels (Fig. 18). In the pars intermedia dense aldehyde fuchsin MSH cells and feeble reaction in dispersed cytoplasm of MCH cells have been encountered.

During post-spawning phase the ovary becomes flaccid and the weight and volume decreases with the progress. The deformed mature follicles begin to reduce in number. The oogonia and early stages of oocytes begin to increase in number in between deformed mature follicles (Fig. 18).

4. DISCUSSION

The basic structure of piscine pituitary is more or less similar, but differs minutely from species to species on topographical arrangement. The hormones produced by pituitary gland of teleosts regulate directly/indirectly some basic physiological activities like growth, development, reproduction and stress responses [9]. The pituitary gland in *Gudusia chapra* is small and lodged in a shallow depression of sella turcica by a short stalk; hence the gland is cranio-leptobasic type. The same type has also been reported in the pituitary of *Valamugil cunnesius* [10]; *Oreochromis niloticus* [11]; *Mystus vittatus* [12]; *Liza parsia* [13]. The pituitary gland in *G. chapra* is composed of two parts viz., adenohypophysis and the neurohypophysis. The adenohypophysis consists of rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). In *G. chapra* the neurohypophysis is rich in blood vessels and neurosecretory materials and it ramifies between RPD, PPD and PI. Various cell types have been identified in the RPD, PPD and PI on the basis of their staining intensities. Different cell types also identified precisely located in the pituitary of a few teleosts [14]. In *G. chapra* the RPD occupies the antero-dorsal position of the gland and the peculiar feature of orohypophysial duct is noticed in between the lobes of RPD. The presence of such cavities which are the diverticula of the hypophysial cavity has also been reported in *Hilsa ilisha, Pangasius pangasius* and *Engraulis telara* [15]. In *G. chapra* the acidophilic prolactin cells (PRL) occupy the major part of the RPD and stained red with acid fuchsin and are attached with blood vessels. Assem [16] also reported that the prolactin cells in the pituitary of *Dicentrarchus labrax* showed strong affinity to Azan stain. Khan [17] opined that only orangeophilic acidophils were detected in the RPD of *Colisa fasciata*. Jose and Sathyanesan [18] also advocated about the random distribution of the prolactin cells in the RPD of *Heteropneustes fossilis, Labeo rohita and Catla catla.* In *G. chapra* the carminophilic corticotrophic cells are oval or elongated in shape and generally dispersed among the prolactin cells. Mandal and Sinha [19] reported that ACTH cells were lead haematoxylin positive and were located in the RPD bordering the neurohypophysis and occurred in groups in *Catla catla*. However, the intense tinctorial reaction and activity of corticotrophic cells in *G. chapra* during maturation and spawning seasons which may triggers the secretion of steroid hormones to provoke the maturation and spawning activities. The proximal pars distalis (PPD) is perhaps the most vital part of the pituitary as it shows remarkable variations in its size as well as cellular components during different reproductive phases. In *G. chapra* three types of chromophilic cells were identified in the PPD on the basis of shape and tinctorial properties during different seasons. The basophilic gonadotrophs formed the main bulk of cells of PPD during maturation and spawning seasons. Similar observations was reported by Sathish et al. [20] in Notopterus notopterus and Nobrega et al. [21] advocated that GTH cells widely distributed in the PPD are characterized by cytoplasm with basophilic secretory granules during spermiation in *Serrasalmus maculates* and *Pimelidus maculates*. El-Gohary [22] reported single type of gonadotrophs in *O. niloticus*. The basophilic TSH cells are elongated and densely stained with aniline blue and PAS and in *G. chapra* TSH cells comparatively less in number than that of GTH cells. In the present study, thyrotrophs exhibited seasonal changes in the activity and during breeding season many cells appeared degranulation and vacuolated. In *G. chapra* the only acidophil present in the PPD region considered as somatotroph (STH) cells stained positively with azocarmine and acid fuchsin. These cells are dispersed among the GTH and TSH cells. In *G. chapra* the pars intermedia (PI) contains two types of cells, the larger cells provided with homogenous cytoplasm which stained with aniline blue and orange G and are identified as melanotroph (MSH) cells and others are identified as MCH cells. El-Sakhawy et al., [23] advocated that pars intermedia contains mainly two amphiphilic cell types, one is lead-haematoxylin positive which is the predominant cell type (melanotrophs) and the other PAS positive which

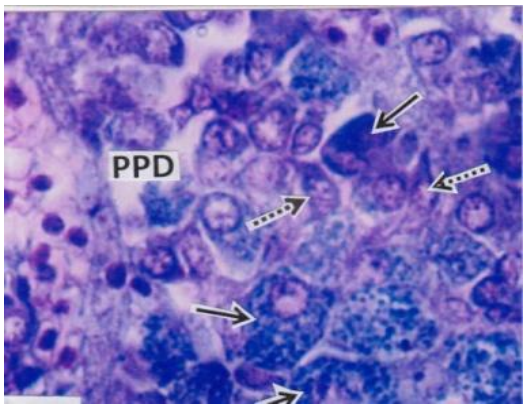


Fig. 16. Chrome-alum positive granular cytoplasm in GTH (solid arrows), comparatively feeble reaction in TSH in PPD (broken arrows). (CAHP) X 1000

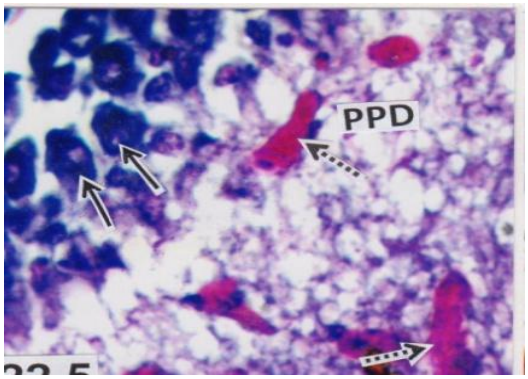


Fig. 17. End of post-spawning phase showing few active GTH cells (solid arrows) adjacent to neurosecretory materials of NH (broken arrows) in PPD (HA) X 600

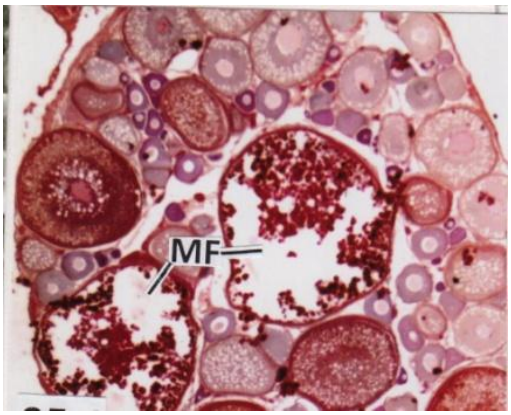


Fig. 18. Appearance of different stages of early and late perinucleolar oocytes and few deformed MF. (RA)X400

are found to be scattered throughout the pars intermedia. They further noticed that during non-breeding season the staining intensities and number of melanotrophs appeared greatly reduced and degranulated. The neurohypophysis in *G. chapra* is composed of axonal nerve fibres originating from neuronal cell bodies of the hypothalamus. These nerve fibres extend as narrow strips into the pituitary gland and found to be closely associated with the blood vessels.

The values of GSI gradually increased during the end of growth phase may be due to the fact that maximum proliferation of the oogonial cells and primary oocytes occur during resting phase which further increased during the end of growth phase along with proliferation of oocyte II and oocyte III. The enormous increase in volume of ovaries is meant for the accommodation of large number of proliferated mature oocytes within ovarian follicles opined by some authors [24]. The proliferation of pre-vitellogenic stages has also been reported by some researchers in other teleosts also [25,26,27]. The GSI value in *G. chapra* follow two peaks during late March and subsequently in July i.e. in spawning phase when the ovarian follicles are packed up with mature oocytes. Similar observations have been made by several workers [28,29,30]. This increment of GSI during spawning period is mainly due to the deposition of protein and lipid in the developing eggs [31] and also due to the vitellogenesis resulting increase in oocyte diameter [32,33]. The GSI started to decline from September onwards and this declination of GSI may occur due to discharge or resorption of yolky oocytes. This is in conformity with the findings of Abu-Hakima [28].

In the present investigation histologically the ovary of *G. chapra* is of the cystovarian type i.e. it is surrounded by an envelope and surface epithelium which at places invaginate centripetally giving rise to finger like ovigerous folds. These findings have also been confirmed in other teleosts [34,35,36]. In the present investigation, it has been observed that each oogonium passes through a number of maturation stages before it becomes a ripe ovum. These eventual processes involve complex changes in the cytology of the nucleus as well as cytoplasm [37,38,39,40]. Santos et al. [41] noticed that during exogenous vitellogenesis, a remarkable oocyte development occurs and yolk granules become larger and occupy the whole of cytoplasm. Kundu [42] also noticed that in *Puntius sarana sarana*. In the present observation during late yolk deposition stage the yolk granules occupy the entire ovum and the nuclei start to migrate to the periphery of the mature oocyte. This observation is in compliance with the findings of Agarwal [37], Subha and Meheta [39].

In the present observation it has been found that discharged follicles are more conspicuous during spawning and immediately after spawning. The atretic follicles generally found during pre-spawning and spawning period. The cause and significance of atresia is not yet completely understood. However, Barr [43] believed that the corpus atreticum is developed as a result of gonadotrophin withdrawl and therefore, it occurs usually in the post-spawning ovary when pituitary gonadotrophin content is low.

It has been observed in the present investigation that the transformation of oogonia in perinucleolar oocytes does not seem to be influenced by gonadotrophin as the activity of the GTH cells i.e. granulation and secretory phase being at its initial stage is weak during this phase. In the maturation phase dense granulation of GTH cells loaded with glycoproteinaceous materials is utilized for the maturation of oocytes. During early spawning phase most of the GTH cells exhibit degranulation which appears to be concomittant with vitellogenesis. In the spawning phase most of the GTH cells become vacuolated although some may continue to be loaded with secretory granules. Therefore, this clearly indicates that the gonadotrophic hormone is an essential prerequisite for the vitellogenesis. Rai [44] suggested that glycoproteinaceous contents of GTH cells, control the processes of vitellogenesis, ovarian maturation and initiation of oviposition in *Tor tor*. However, during late maturation and early spawning phases depletion of GTH secretion leads to atresia of some oocytes but it does not appear to interfere with oogonial proliferation. As the secretion from GTH cells act simultaneously on the maturation and spawning phases of the fish [45], it can be interfered from the above observations that the onset of gonadal maturation is activated by the secretion from the GTH cells during the commencement of the maturation phase while the secretion from TSH cells acts upon gonads via thyroid and starts acting on gonads after a phase lag from that of GTH secretion. Khanna and Pant [46] reported in *Glyptothorax pectinopterus* that there is a poor concentration of their glycoproteinaceous contents in gonadotrophs during the restitution phase, higher concentration during the period of yolk accumulation in the oocytes which later brings about the ovulation of eggs. They almost empty their contents during the post-spawning period when a good number of oocytes undergo atresia. Therefore, in the present study, the depleted condition in GTH cells during post-spawning phase suggest that early oogonial proliferation is not regulated by the pituitary gonadotrophs.

5. CONCLUSION

RPD is occupied by acidophilic prolactin (PRL) and corticotroph cells (ACTH). PPD is occupied by only acidophils somatotrophs (STH) and two basophilic cells-gonadotrophs (GTH) and thyrotrophs (TSH). PI occupied by amphiphilic MSH cells. But significant changes did not occur in acidophilic cells throughout the year. But basophilic GTH and TSH cells showed significant hypertrophy during maturation and

spawning phases. A regular change of both GTH and TSH cells and ovarian activities were correlated well during growth, maturation and spawning phases of *Gudusia chapra.*

ETHICAL APPROVAL

To study the seasonal changes of the pituitary and ovaries the fishes were sacrificed following the guidelines given by the institutional ethical committee.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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