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RESEARCH ARTICLE

HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDIES ON THE PITUITARY GONADOTROPIC CELLS IN RELATION TO DIFFERENT AGE GROUP OF FISH *Notopterus notopterus*

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ABSTRACT

Gonadotropic cells are distributed in the proximal pars distalis (PPD) of the pituitary in *Notopterus notopterus* and to know the structure by different age group using histochemical and electron microscopic techniques are applied for the current investigation. The PPD of pituitary gland in zero age group fish has very little number of gonadotropes. The cells are undifferentiated with indistinct boundaries. They markedly increased in number in one year aged fish, since the gonads of this age group fish shows advanced stages of growth. The gonadotropes that largely occupy the medial aspect of the PPD increased numerically as the gonads mature and become major cell type before spawning in two age group fish. The electron microscopic studies reveal that, they appear oval or elongated containing a few large granules and many small lucent secretory granules. Only one gonadotropic cell type could be identified in the PPD, although two similar cells containing different types of secretory granules are noticed. The differentiation and presence of two types of GTH needs further verification in the fish *N. notopterus*.

Key words: Gonadotropic cells; Pituitary gland; PPD; *Notopterus notopterus*

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INTRODUCTION

Among the endocrines in fishes the study of pituitary and its hormones has attracted greatest attention. The morphological structure of teleost pituitary is well established but the studies on details of its finer structure still remains open for investigation especially in this large group of vertebrates of which only a few species have been studied (Sahai, 1986). The hormones originating in the pituitary have been shown to control reproduction, growth, colour change and behaviour. These hormones originate in the cells present in the different regions of the gland. The pituitary of teleosts is known for its remarkable structural diversity and the cell types described in the adenohypophysis (AH) are found to be physiologically equivalent to those of the mammalian pituitary gland (Quesada et al., 1988). Due to the Unique organization and the specialization make the teleost pituitary an ideal model for the study of the relationship between nervous and endocrine components of neuroendocrine system.

Therefore, anatomical data obtained in the teleosts may contribute to the better understanding of hypothalamohypophysial system in higher vertebrates (Moones et al., 1988). Studies in relation to the histology of pituitary cell types and their activities, have been made on fishes of different continental waters (Pantic, 1974). Amongst the Indian tropical fishes, the histological changes occurring within the pituitary gland in relation to seasonal reproductive cycles and for the experimental allocation of different cell types has been carried out (Gopaldutt, 1989). The terminology adapted to describe various regions of the teleost pituitaries has been reviewed, (VanOordt and Peute, 1983). It is well established that the AH of teleost pituitaries is clearly divided into three regions such as rostral pars distalis (RPD), proximal distalis (PPD) and pars intermedia (PI) situated one behind the other. The main reason for dividing the AH into three parts is on the basis of even distribution of the morphological hormone producing cell types. The identification and distribution of different cell types in the pituitary of a number of fish species have been established by histochemical and immune cytochemical techniques (Yan and Thomas, 1991; Rodriguez-Gomez et al., 2001). Hence, current study would help to understand the role of gonadotropes (GTH) in gonadal

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maturation so as to improve the efficiency of breeding on this locally available food fish. Since fecundity of a fish is directly related to potency of the pituitary glands of both donor as well as recipient (Sarkar, 2000).

MATERIALS AND METHODS

Sampling collection and management

Fresh water teleost *Notopterus notopterus* used for the present study was collected from Saradgi nala (channel) situated 10 km away from Gulbarga, Karnataka, India. More than 250 fishes of different sizes ranging from 13 to 26 cm were used for the study. The fishes collected with the help of fisherman monthly by using gill nets and catch nets. They were brought to the laboratory and kept in plastic pools having size of 90 cm diameter and 60 cm height for a period of 8 days. The fishes were fed with boiled eggs, small fishes like *Gambusia sp.* and goat liver alternatively. The water was changed alternative days during the period of acclimatization.

The experimental design

Present investigation is aimed to study the gonadotropic cells using specific staining techniques. Hence after exposing the roof and sides of the brain, the heads were fixed in Bouin's fluid, as it is not possible to remove the pituitary intact with brain, they were decalcified by keeping them for longer time in fixative (15 days). After removing the brain with intact pituitary from cranium, the brains were kept in fresh Bouin's fluid for 24 hrs and then dehydrated by passing through 70%, 90 % and 100 % alcohol. Microtome sections (five microns thick) of the brain with pituitary were taken in parasagittal, frontal and transverse planes and stained.

The following staining methods were applied for the study of gonadotropic cells

- Aldehyde fuchsin (AF) (Gomari, 1951) preceded by acid permanganate oxidation using fast green – chromotrope 2R as the counter stain.
- Lead haematoxylin (PbH) technique of Mac. Conaill, (1947) preceded by PAS.
- Periodic acid (PAS) orange G (OG) procedure for Ezrin and Murray, (1963).
- Cleveland and Wolf's Trichome method (1932).
- Aldehyde thionine – PAS – Orange G method of Ezrine and Murray, (1963).

Light and electron microscopy (EM)

Pituitary gland samples, prefixed in glutaraldehyde (IV) were postfixed with potassium ferrocyanide-osmium fixative. The samples were embedded in epoxy resin and cut into sections for light and EM study. For viewing by light microscopy, 1- μ m-thick sections were stained with 0.5 % toluidine blue. Ultrathin sections (70 nm) were stained with 5 % uranyl acetate and 5 % lead citrate by using Ultrastainer and the stained sections were scanned in Jeol-TEM 100 C X II EM for ultrastructural observations.

RESULTS

General consideration of *Notopterus notopterus* pituitary gland

The pituitary gland of the fish *Notopterus notopterus* lies in the mid ventral part to the brain behind the optic chiasm. The gland is attached to the floor of the infundibulum without a definite stalk having a wide infundibular cavity (Fig- 1).

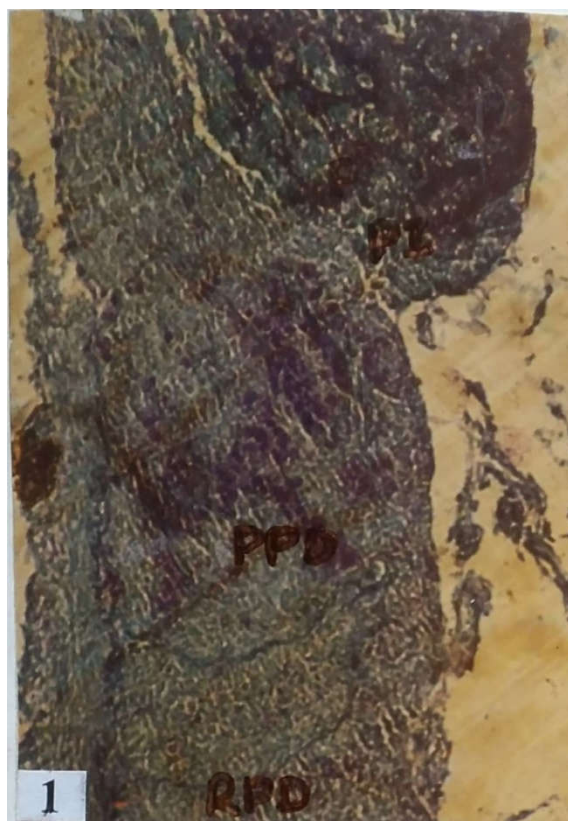


Figure 1. Showing RPD, PPD and PI of Pituitary gland the in zero age fish *N. notopterus* AF X 128



Figure 2. Section of the pituitary of zero age fish showing few AF positive gonadotropin the PPD region AF + Fast Green X 1600



Figure 3. Section of the pituitary of one year age fish (prespawning) showing AF positive gonadotropes in the PPD region AF + Fast Green X 1600



Figure 4. Section of the pituitary of two year age fish (prespawning) showing large number of AF positive gonadotropes in the PPD region AF + Fast Green X 1600

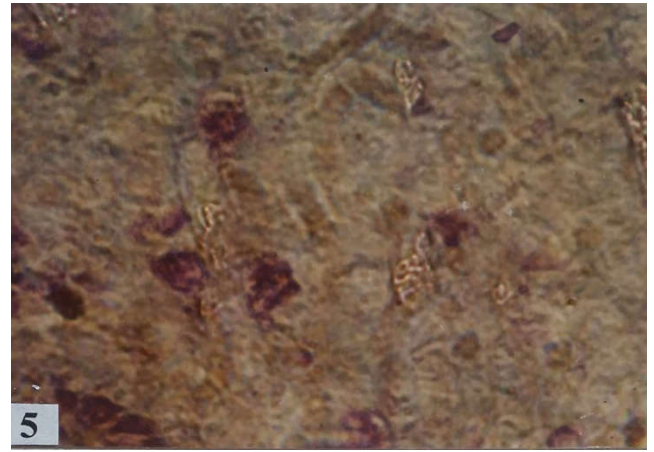


Figure 5. Section of PPD region of the pituitary of one year age fish (after spawning) showing degranulation, gonadotropes and thyrotropes AF X 1600

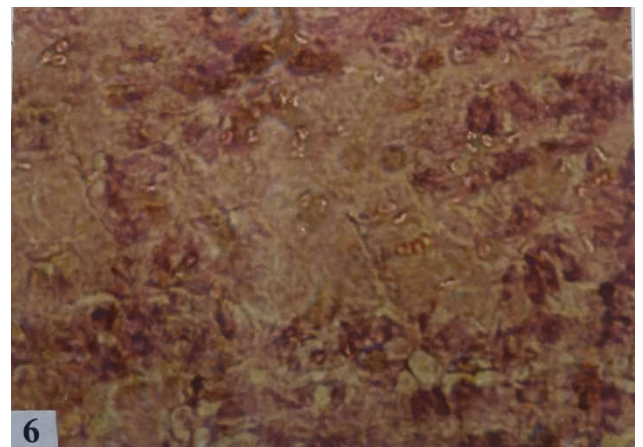


Figure 6. Section of PPD region of the pituitary two year age fish after spawning showing degranulated gonadotropes and few AF positive thyrotropes AF X 1600



Figure 7. PPD of the pituitary of two year age fish showing AF positive cyanophils AF-Fast Green-chromotrope R X 210

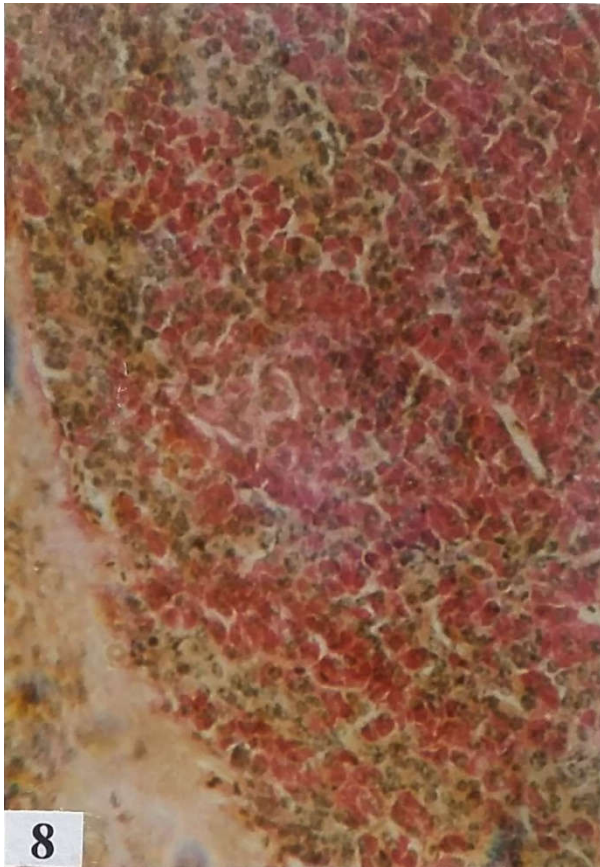


Figure 8. PPD of the pituitary in a two year age fish showing large number of PAS positive cells (Cyanophils) PbH +PAS X 336

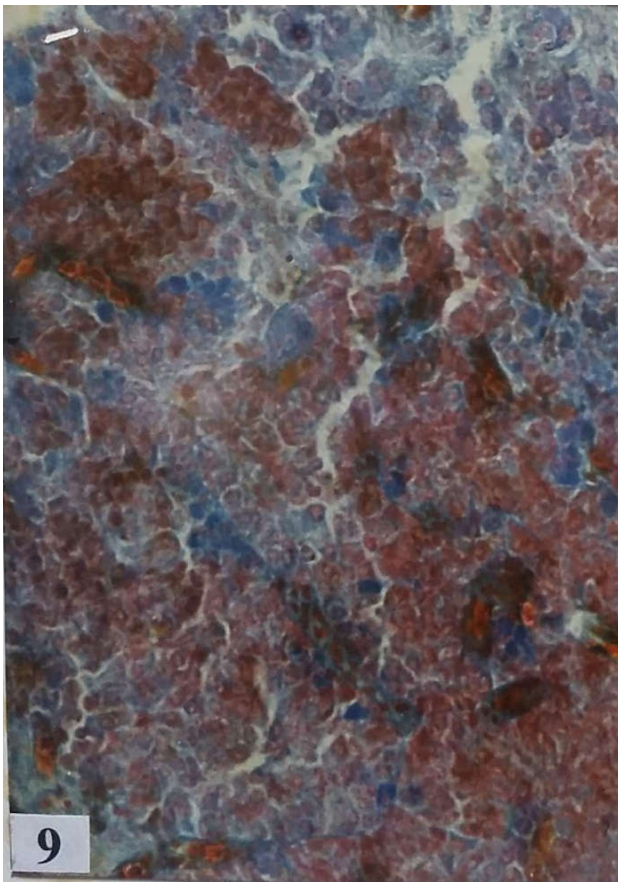


Figure 9. PPD of the pituitary in two year age fish showing erythrosine positive and orange G positive acidophils and aniline blue positive basophils Cleaveland and Wolfe's Trichome (CWT) X 336

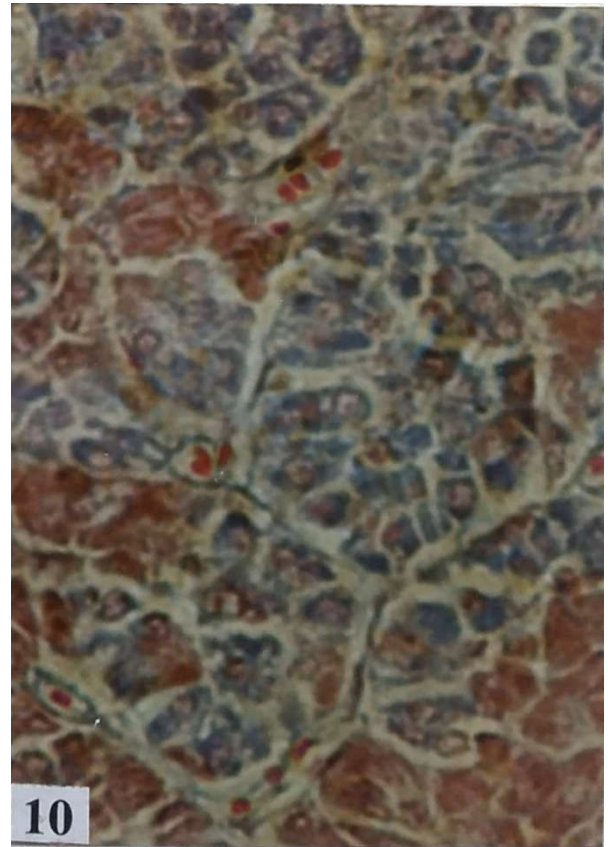


Figure 10. PPD of the pituitary in two age year fish showing cell groups of aniline blue positive basophils and erythrosine positive acidophils (CWT) X 610

In literature this type of pituitary is described as platyblastic. The pituitary gland is differentiated into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). The RPD, PPD and PI are arranged one after the other in antero-posterior direction. The RPD is made up of erythrosine positive acidophils and PbH positive cells. The acidophil cells are also positively stained to orange G. These acidophil cells are resembling the prolactin cells as described in the literature. The PbH positive cells are arranged bordering the neurohypophysis (NH) (Fig- 8) and also distributed to other parts of RPD (Fig- 9). These PbH positive cells are referred as Adrenocorticotrophic hormone (ACTH) cells. Apart from these two types a small groups or individual cells of positive cyanophils are seen in RPD resembling those of PPD (Fig- 10).

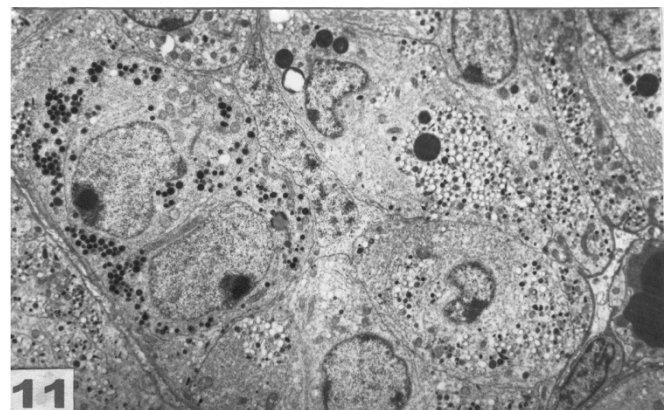


Figure 11. Ultrastructure of various types of cells secreting trophic hormones in the PPD region of the pituitary in a two year age *N. notopterus* showing A-GTH cells-II, B-GTH cells-I, C-STH cells, D-TSH cells, E-Stellate cells, F-Secretory vacuole X 6200.

The cyanophils and acidophils are two major cells types present in the PPD. The acidophils are stained with erythrosine and orange G, are arranged in the form of irregular groups (Figs 9 and 11). The acidophils are considered as somatotropes (STH) as described in literature. The cyanophils are restricted to the dorsal region of PPD and also scattered in the form of groups among the acidophils. These cyanophils are AF positive, PAS positive and aniline blue positive (Figs- 9 and 10). Which are more concentrated in dorsal aspect of the PPD, these cyanophils are less during resting phase (September-October). This suggests that those cyanophils are GTH the remaining cyanophils are considered as thyrotropes (TSH) as described in the literature. The GTH were differentiated from the TSH (as they stain alike) by comparing the pituitary gland stained during pre-spawning (Figs- 3 and 4) and post spawning (Figs- 5 and 7). The PI is recognized usually by its close relationship with pars nervosa and it is the most posterior region of the gland. The PbH positive cells are arranged around the processes of the NH (Fig- 8). These cells are considered MSH secreting cells. PI is also showing small number of PAS positive cells (Fig- 9). In all the regions of the pituitary some unstained cells are seen which are considered as chromophobes.

The gonadotropes (GTH cells)

The GTH cells in the pituitary gland of *N. notopterus* have a wide distribution in the region of proximal pars distalis (PPD). These GTH were differentiated from the TSH (as they stain alike) by comparing the pituitary gland stained during pre-spawning (Figs- 3 and 4) and post spawning (Figs- 5 and 6) period. The GTH are abundantly distributed in the medial region of PPD (Figs- 3 and 4) as these basophils stains intensely to AF during pre-spawning period. Hence, they are identified as GTH. PAS, AF and aniline blue during pre-spawning period (Figs- 3 and 4). The pituitary gland of zero age group fish has few numbers of GTH (Figs- 1 and 2) and they are undifferentiated with indistinct boundaries. The GTH markedly increase in the (PPD) region of pituitary in one year age group fish and also show advanced stages of growth (Fig- 2). Since GTH are involved in the process of growth and maturation of gonads, the elder ones (1 and 2 aged) need more GTH as compared to younger ones (0 age and 0⁺ age). Hence the GTH are abundantly distributed in the PPD of pituitary in fish having two year age (Fig- 4). The GTH are intensely stained in the prespawning period. These cells undergo degranulation with weak staining after spawning in the same age group fish (Fig- 6). In one year aged post spawned fish the GTH get degranulated (Fig- 5) indicating the hormone release during spawning activity. The ultrastructural characteristics of the GTH cells reveals that they are oval or elongated containing few large granules and many small lucent secretory granules. Large bodies are PAS positive, AF positive and aniline blue positive and presumably are lytic granules (Figs- 11 and 13). The cells have well developed RER and Golgi complex the nucleus is crescent shaped with prominent nucleolus. The nucleus is situated centrally or eccentrically depending upon the position of the cell. The GTH cells have complete or partial loss of glycoprotein secretory granules and development of large vacuoles in conjunction with them, indicating synthetic and secretory activity. The pituitary gland of *N. notopterus* was processed during August in which the fish had ripe spawning gonad, the ultrastructural details of GTH during this period shows characteristic of two different cellular inclusions.

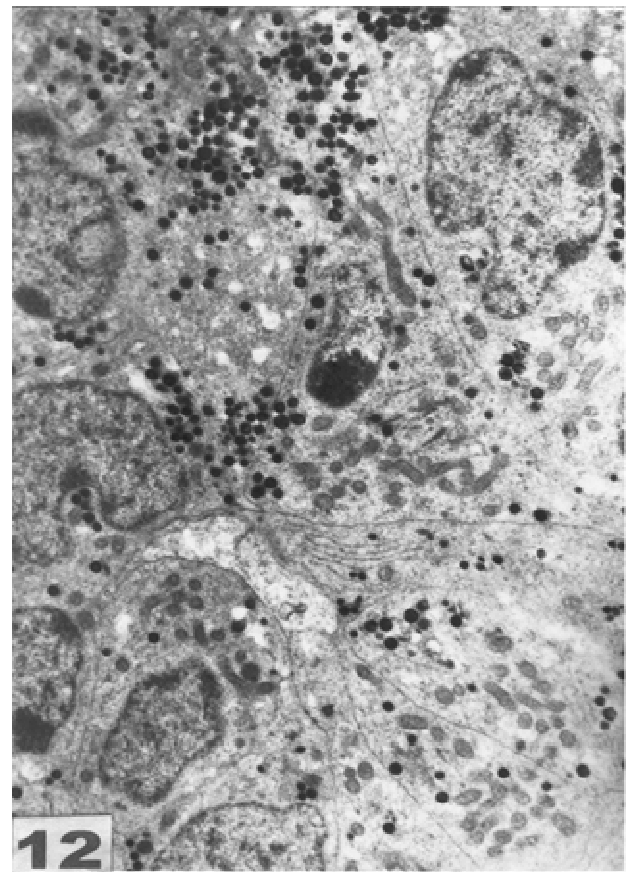


Figure 12. Ultrastructure of the GTH-I (B) and STH (C) cells in the PPD region of the pituitary of two year aged fish X 8000

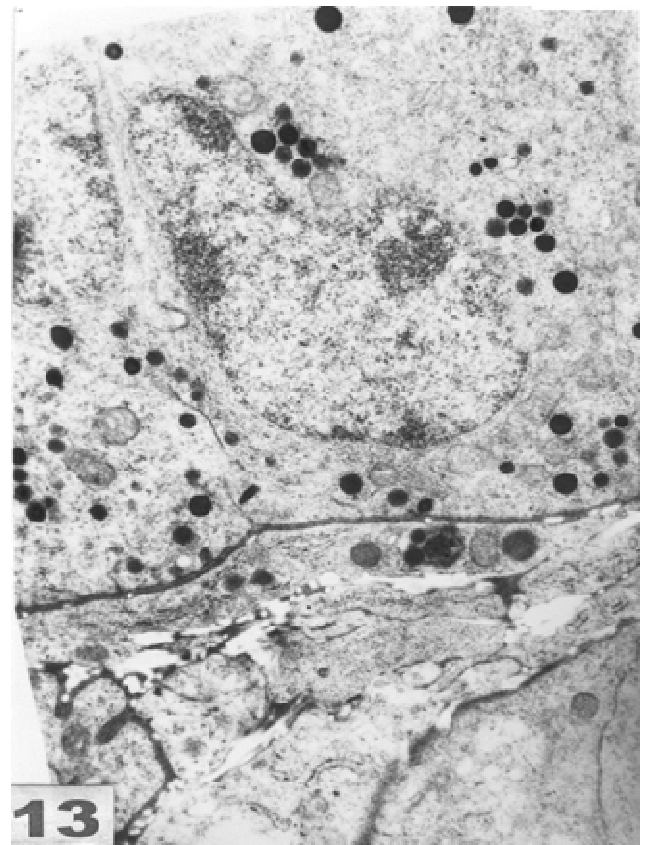


Figure 13. Electron microscopic picture of a cell (STH) in the PPD region of the pituitary showing relationship to capillaries. Squared showing the process of exocytosis of granules in to pericapillary space X 13400

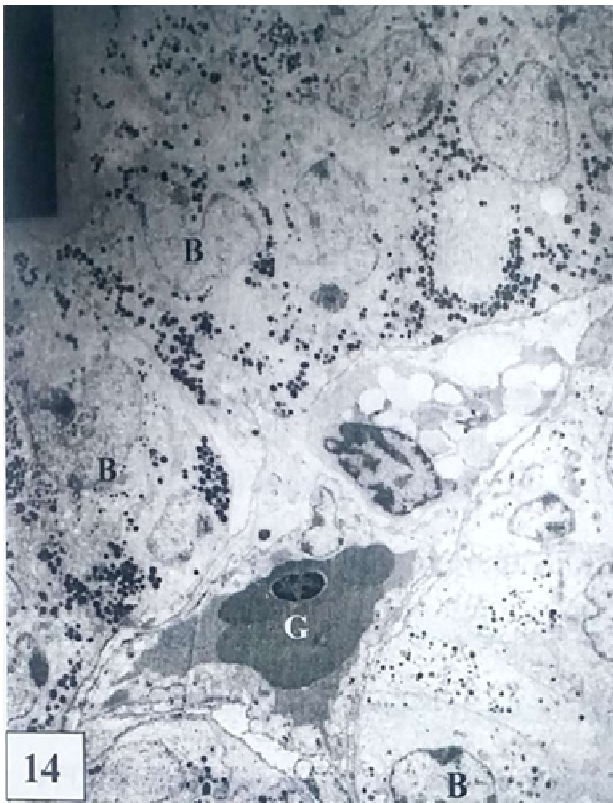


Figure 14. Electron micrograph of a section of the pituitary in the region of PPD showing GTH II cells having different granules associated with blood vessel X 5000

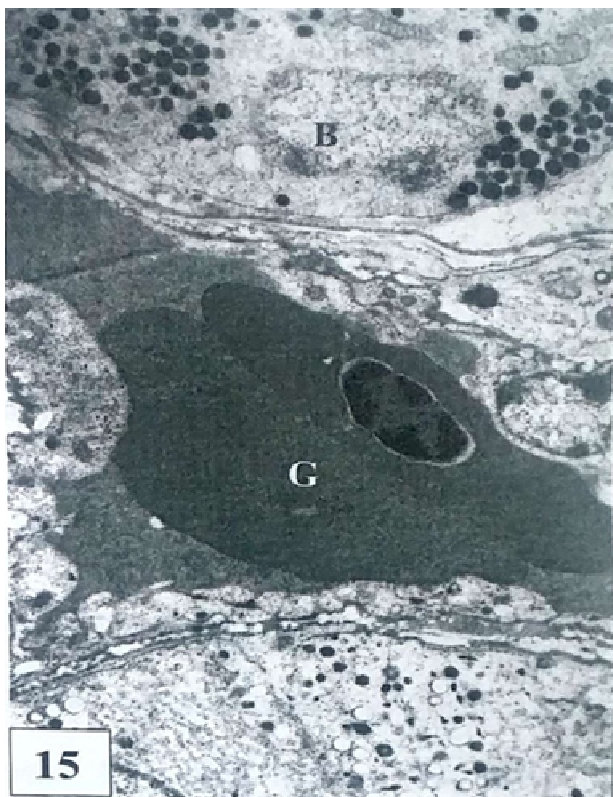


Figure 15. Higher magnification of Figure 14. In the region of blood capillary showing secretory granules of GTH cells in association with endothelium of the capillary X 10000

One types of GTH containing numerous dilated cisternae of the Endoplasmic reticulum (ER) and a small type of secretory granules (The diameter may be around 100-300 nm) whereas another type of GTH although contain similar dilated cisternae

of the ER and small type of secretory granules, they also contain large globules of diameter around 500-4000 nm. The first type of cell described above may be GTH-I and the second type may be GTH-II GTH. However, there is overlapping of each other cells due to changes in number and size of the granules, globules and cisternae of the granules endoplasmic reticulum (GER). A clear distinction to make on the existence of two types of GTH in this species (*N. notopterus*) needs a study on the immuno cytochemistry using EM. Interspread among the GTH cells in the rostral follicles of *N. notopterus* are few small cells lying adjacent to fine channels which connect the follicular lumen with the intervascular space between the follicles. Because of their position they have been called "neck cells", and the suggestion was made by altering their size and shape they could regulate the passage of substances along the channels as cited in the reviews. These cells in all probabilities belong to the stellate system, which are general features of the AH. These cells occur in all regions of the AH of teleosts, their fine processes penetrating between the endocrine cells as revealed by literature. The other cell types, which are identified, based on the size of the secretory granules are STH and TSH, which are closely associated with GTH.



Figure 16. Electron micrograph of a GTH cells the PPD showing coarse granules, Mitochondria (J), Endoplasmic reticulum (I), Glial elements (K) and LTH cells (L) X 8000

The STH have membrane bound secretory granules with clear no indication of RER or Golgi complex, the cells have few mitochondria (Figs- 14 and 17). The granules varies in diameter observed based on the morphology and the granules are well developed. The Golgi complex showed stages in the elaboration of secretory granules. The other type of cell which are associated with GTH are TSH, these cells are present in the central region and are intensely positive to AF and PAS distributed in the central region of the RPD.

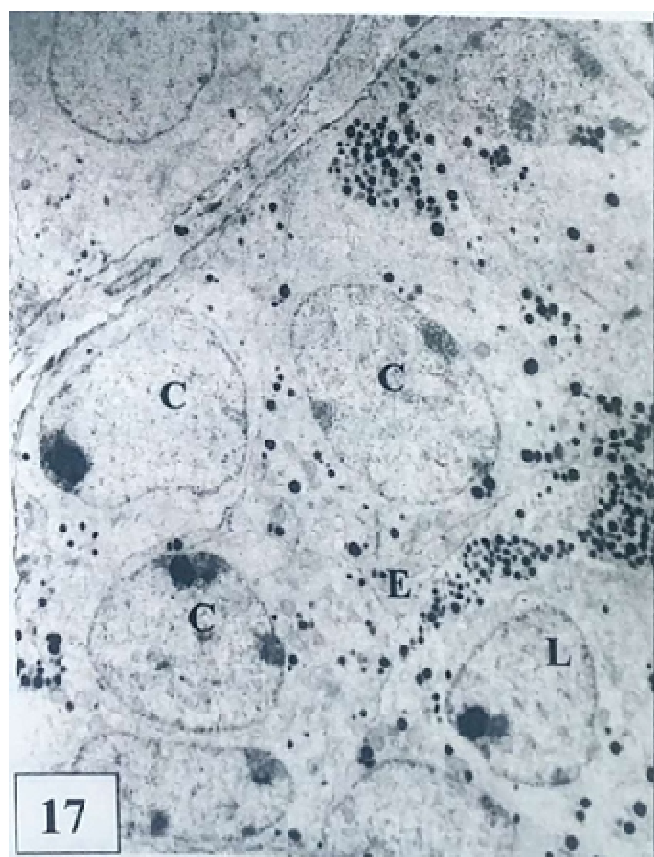


Figure 17. Electron micrograph of the cells in the region of RPD showing intense granules of various sizes (STH and LTH cells) with intracellular spaces X 6000

The ultra structural characteristic of the TSH cells are dilated RER cisternae and varying sizes of the weekly stained smaller secretory granules (Figs- 11 and 13).

DISCUSSION

Cyclic changes in secretory activity of the GTH cells during annual reproductive cycle is the most conspicuous event in the pituitary gland and are taken as a basis for their identification. The GTH cells were differentiated from TSH by comparing their distribution and intensity of staining during pre-spawning and post spawning periods of gonadal cycles. These two types of basophils stained alike with PAS, AF and aniline blue (Ball and Baker, 1969). In the post spawning phase fish pituitary only some of the cells, which are present at the basal region of PPD exhibit positive staining response while those present in the medial PPD are weak to staining. Thus the basophils of the basal PPD are considered as TSH, and the basophils present in the medial PPD are GTH. Such studies on the pituitary have been reported and those cyanophils of the middle glandular region are GTH (Gopaldutt, 1989). The distribution pattern, staining response and cytological stages of basophils during different phases of reproductive cycle of matured fish is available in the literature (Joy and Sathyanesan, 1980). The basophils distributed in the region of PPD are GTH, which are abundantly seen during pre-spawning phase of the reproductive cycle of *N. notopterus*. The pituitary of zero age group although have the GTH stainable, are very few in number and undifferentiated. These cells increased in number in one-year age group fish. They are found to be intensively positive to staining reaction and are numerically high in two-year age group fish.

Thus indicating the increase in GTH cells along with their staining intensity suggest their active involvement in the development of gonads of a matured fish (Joy and Sathyanesan, 1979). In eel and pacific salmon (Jose and Sathyanesan, 1977) two types of GTH are reported based on their colour differences and granular, size, distribution, position and vascularisation. In several other teleosts only one type of GTH are reported (Kagawa *et al.*, 1997). The ultrastructure, of pituitary GTH has two different types, identified based on the presence of secretory granules sizes, they seems to be similar in *N. notopterus*. Gonadotropic (GTH) cells were very heterogeneous with regard to their size and shape. These are oval at the medial region of PPD and elongated near the basement membrane. The GTH cells found with a few round large electron dense (Lucent and Solid) secretory granules in agreement with studies of Garcia-Ayala, (1998) on the mediterranean yellow tail *Seriola dumerilii*. These large bodies are PAS positive, AF positive and aniline blue positive and presumably are lytic 'R' granules. They occur in many teleosts (Ball and Baker, 1969). The cells have well developed RER and Golgi complex (Garcia-Ayala *et al.*, 1998). The ultrastructural characteristics of GTH cells have been described for some catfishes (Peute *et al.*, 1986) including Indian catfish *Clarias batrachus* (Joy, 1997).

According to these authors the most important characteristic feature of these cells is the presence of heterogenous secretory inclusions, they are either round or elongated, round globules with irregular masses identified based on their size and shape. The GTH cells of *C. batrachus* have the rough endoplasmic reticulum (RER) consisting of small dilated cisternae among the secretory inclusions and mitochondria. The Golgi complex is generally inconspicuous and is found near the nucleus. The GTH cells of *N. notopterus* also exhibit similar organisation under ultrastructural studies having heterogenous secretory inclusions RER with small dilated cisternae, mitochondria and Golgi complex usually appeared near the nucleus. The differentiation of two types of GTH could not be made in the histochemical studies. However, ultrastructural studies using EM indicates that the pituitary GTH cells of *N. notopterus* exhibit two different cell type, are having smaller secretory granules and whereas the other having similar smaller secretory granules in addition to large globules. Such distinction of GTH has been made in the pituitary gland of rainbow trout *Oncorhynchus mykiss* by Naito *et al.* (1993) describing that the salmon pituitary produces two chemically distinct gonadotropins and ultrastructural characteristic of GTH-I and GTH-II studied though immuno cytochemistry using antisera against salmon GTH-I beta and GTH-II beta subunits, those cells have dilated cisternae of the granular ER and a small number of I-beta positive granules (diameter 100-300 nm) whereas GTH-II beta immuno reactivity was found as ranules (diameter 200-400 nm) and large globules (diameter 500-400 nm) in apparently differently cells (GTH-II cells). Distinct cellular distribution of GTH-I and II were maintained during gametogenesis, although morphological characteristics of GTH-I and II cells overlapped each other due to changes in number and size of the granules, globules and cisternae of the GER, interestingly the globules in the GTH-I beta although in the GTH-II cells, they were always stained with GTH-II beta anti serum and confirming their results reputed that GTH-I and GTH-II beta are synthesised in distinctly different cell types in the salmonid pituitary. Although such differentiation is possible in *N. notopterus*, the existence of two types of cells cannot be confirmed and on the basis of the study further

verification using immunohistochemistry along with EM studies is needed. Although the question of existence of two types of GTH cells has been an issue in other teleosts and all evidences at the moment indicate that there is single type of GTH cells (Joy, 1997). This is also supported by GTH purification data in the African catfish that only a single GTH is characterized (Koida *et al.*, 1992). The GTH are distributed in the dorsal region of the PPD in *N. notopterus*, the gonadotropic cells are associated with some STH and TSH. This has been identified based on the tinctorial affinities, the GTH of the PPD exhibit hypertrophy accompanied with degranulation during spawning period while they exhibit intense staining during prespawning phase. The cells decrease in number with weak staining response when spawning is completed (during post spawning phase). The GTH exhibit changes with respect to growth and maturation of gonads has been studied by fixing the gland in different periods of the reproductive cycle. The region of the PPD when observed under EM in the pituitary of breeding fish has cells exhibiting unique pattern of organisation, which facilitates identification of this cell and is possible to differentiate from the other associated cells such as STH and TSH. The STH are identified based on the type of granules as described in the literature and published data (Holmes and Ball, 1974). The STH in *N. notopterus* are usually large and rounded and sometimes elongated and typically filled with fine secretory granules, often so densely packed.

The ovoid nucleus is frequently placed eccentrically at one pole of the cell and nucleus size seems to vary within the gland in different regions. A prominent nucleus and Golgi forming ring shaped with no clear indication of rough endoplasmic reticulum and having few mitochondria. The secretory granules of STH are well developed and Golgi complex showing elaboration of secretory granules such observation has been made in teleosts such as *Anguilla* (Knowles and Volrath, 1966), *Gasterosteus* (Leatherland, 1970), *Perca* (Follenius and Porte, 1961). Holmes and Ball, (1974) described in *P. latipinna*. The STH shows a tendency towards sexual dimorphism, in the adult female, they are numerous and are very active, while adult male usually has relatively few STH and poorly granulated or entirely agranular, with nucleus, nucleolus and Golgi image indicating low activity. In the female the continuous cup shaped mass of STH is easily visible in the living gland, the granules being a dense white in contrast to the more translucent ventral GTH. In the male fish, however, the STH cell mass is thinner and discontinuous, and generally not visible in the living gland. *Anguilla*, when sexually mature, displays a similar dimorphism, the female having larger and more active STH than the males. In the present study on the ultrastructure of the STH, the female fish pituitary gland is processed, the STH exhibit similar kind of cytoplasmic components as described by Holmes and Ball, (1974) above in the pituitary STH of female *N. notopterus*. It is likely that such a sexual dimorphism may exist. The TSH are another kind of cellular components present along with GTH, they are identified based on their intense staining to PAS and AF during non-breeding phase, they are usually present at the ventral region of PPD and some are mixed with GTH of the dorsal region of the RPD such type of distribution has been noticed in some Indian teleosts such as *Mystus vittatus* (Kulkarni and Sathyanesan, 1982). *Clarias batrachus* (Joy and Sathyanesan, 1978) and in other teleosts (Rodriguez *et al.*, 2001). The ultrastructure of these cells (TSH) in the teleosts studied (Holmes and Ball, 1974) indicate that the diameter of

secretory granules vary in size from 100 to 800 nm. The granules in *Oncorhynchus nerka* measure 100-200 nm and in *O. keta* it is 200 nm and often found within the dilated RER cisternae - a characteristic feature of TSH (Nagahama and Yamamoto, 1969). The gold fish pituitary TSH contain granules 60-220 nm in diameter. In *Cymatogaster*, TSH appear to release granules into the basement membrane adjoining the NH (Leatherland, 1970). In *N. notopterus* in the present study on the ultrastructural studies also indicate that the granules vary in size and the RER is dilated which is characteristic feature of TSH as reported in other fishes (Nagahama and Yamamoto, 1970). In preparatory phase cells increase in number and size, the cytoplasm is gradually filled with secretory granules. As a result, stainability is increased. Ultrastructurally, cytoplasm is filled with granules and globules regular masses are sparse (Peute *et al.*, 1986). The cytologic features indicate the intense synthesis and storage. In pre-spawning phase the cells become further hypertrophied with variable amounts of stainable granules. This phase marks strong synthesis as well as release of GTH. Because of increased cell activity, the PPD preponderates over other regions of the pituitary.

As a result release of the hormone (degranulation) the cytoplasm is vacuolated. In spawning phase the cells are maximally hypertrophied and vacuolated, and finally vacuolated. Ultrastructurally, the granules and globules decrease and vacuoles increase in the cytoplasm. Interspread among these cells in follicles of *N. notopterus* are few small cells lying adjacent to the fine channels, which connect the follicular lumen with the intervascular space between the follicles. Because of their position, they have been called "neck cells" and the suggestion was made that by altering their size and shape they could regulate the passage of substances along the channels (Knowles and Volrath, 1966). In all probability these neck cells belong to the system of stellet (Follicular) cells, which are general feature of the AH stellet cells have been illustrated in the elver pars distalis (Volrath 1966), and apparently occur in all regions of the adenohypophysis of teleosts, usually with their fine processes penetrating between the endocrine cells (Leatherland 1969, 1970; Abraham, 1971). In this fish they lie between the GTH cells and thyrotropic cells. Although hypothalamo-hypophysial portal system of circulation has been reported in addition to teleostean type of neuroadenointerface vasculature in a number of Indian teleosts (Kulkarni and Sathyanesan, 1983), there has been controversy regarding the existence of such a tetrapodan type of portal system. Morphological studies through India ink injection revealed similar pattern of organization in teleosts (Kulkarni and Sathyanesan, 1983). In *N. notopterus* such a morphological evidence for the existence of portal circulation has been also studied (Kulkarni and Sathish, 1993). However, teleosts are unique among vertebrates that the adenohypophysial cells are innervated by axonal processes of hypothalamic neurosecretory neurons and depending upon the nature of neurohormones, the innervations is classified into type A (Peptidergic AF positive neurohypophysial and AF negative neuropeptidergic systems), type B (mostly monoaminergic - catecholaminergic and serotoninergic systems) and type C (Cholinergic) (Joy, 1997). In the African catfish, innervation of the GTH cells has been studied ultrastructurally and in conjunction with PAP or immuno gold techniques (Peute *et al.*, 1987) and the cells receive both type A and B fibres which make direct synaptic or synapse like contacts.

The type A innervations is further characterized immunocytochemically to contain Gonadotropin-releasing hormone/ luteinizing hormone-releasing hormone (GnRH/LHRH) and the type B to contain dopamine. In *N. notopterus* studied under EM although such a distribution has not been made however, the GTH cells do have such a type of innervations, further studies using specific techniques are needed to characterize the presence of different types of innervation system. It is reported that in *C. batrachus*, the PPD contains several LHRH positive fibres (Subhedar and Ramakrishna, 1988) and similarly in the same fish Joy *et al.* (1992) have demonstrated the presence of monoamines around GTH cells by histofluorescence technique. According to Holmes and Ball, (1974), the EM studies have clarified some points bearing on the precise relationship of cells to blood vessels and in the pituitary as in other endocrine glands. This relationship is clearly of great importance, since the vascular route is the only one by which the secretory products can pass to influence their target organs.

Furthermore, the various releasing factors coming from the hypothalamus travel in the portal blood to influence the secretory cells. It is also described by Holmes and Ball, (1974) that essentially pars distalis is permitted by capillaries lined by endothelium cell nuclei; it frequently shows pores, such as those described in other endocrine glands, bridged by a thin membrane. The endothelium is closely opposed to a basement membrane, which consists of two parts, an endothelial and parenchyma, which are closely applied to the cell membrane of the secretory cell. In some areas these two components are fused to form a single thick basement membrane, but often separated by perivascular space of variable width containing mucopolysaccharides, the surface of the parenchymal cells is often irregular so that thin surface area is increased and processes of the cells may project in to the pericapillary space between the two basement membranes, the release of granules from the secretory cells takes place by the process of exocytosis in to the perivascular space in *N. notopterus*. In the present study sinusoids are the vessels having large diameter the capillaries having discontinuous lining of phagocytic and nonphagocytic cells.

The blood vessels are found to be capillaries have endothelial lining which is continuous, the small vessels have relationship with cells present around the capillaries are very thin lined by endothelium containing parenchyma cells, the basement membrane closely applied to the cell membrane of secretory cells with a perivascular space. The secretory granules are released through the process of exocytosis as seen for the ultrastructural picture of the PPD. Hence all the characteristics of capillaries and their relation to the secretory cells are identical as described by Holmes and Ball, (1974). However, the existence of pituitary portal system in teleosts is still under consideration. Peter, (1990) has described notably, neurosecretory terminals have not been observed on the primary capillary plexus in the neurohypophysial tissue in teleosts, moreover, in some species in which a vestigial hypothalamo-hypophysial portal system exists and neurosecretory endings have not been found on the primary capillary plexus. Thus, the functional delivery of neurohormones to the adenohypophysial tissue is by the neurosecretory terminals that have invaded the tissue. Which also supports the findings of Sathyanesan and Haider, (1974) for *Heteropneustes fossilis*, Joy and Sathyanesan, (1979, 1980) in *Clarias batrachus*, Sathyanesan and Jose, (1975) for *Channa*

punctatus, Sathyanesan and Das, (1978) in *Puntius septore* and Kulkarni and Sathyanesan, (1982) for *Mystus vittatus* and Baskaran and Sathyanesan, (1992) for *Magalops cyprinoides*. The EM structure of blood vessels and their relationship with the secretory cells in the pituitary gland of *N. notopterus* shows that, the capillaries are lined by endothelium which is very thin. The lining of endothelium shows some pores, which is bridged by a thin membrane. The endothelium is closely applied to the basement membrane of the secretory cells. The perivascular space is clear in between the endothelium and the membrane of secretory cells. The surface of the parenchymal cells is irregular so that their surface area is increased, the processes of the parenchymal cells project into the pericapillary space. The secretory cells secreting the granules probably by the process called exocytosis in to the perivascular space. Since the capillaries contain continuous endothelial lining indicate that they are to be considered as capillaries not the sinusoids.

REFERENCES

- Abraham M. 1971. The ultrastructure of the cell types and of the Neurosecretory innervation in the pituitary of *Mugil cephalus* L. Form freshwater, the sea and a hypersaline lagoon. *General and Comparative Endocrinology*. 17, 334-350.
- Ball JN. Baker BI. 1969. The *pituitary gland anatomy and histophysiology*. In fish physiology Vol .II, Eds WS Hoar and DT Randall, Academic press, p.207-240.
- Baskarans G. Sathyanesan AG. 1992. Tetrapod-like hypothalamo-hypophysial portal system in the teleost *Megalops cyprinoides* (Broussonet). *General and Comparative Endocrinology*. 86, 211-9.
- Cleaveland R. Wolfe JM. 1932. A differential stain for anterior lobe of the adenohypophysis. *Anatomical Research*. 51, 409-413.
- Ezrin C. Murry S. 1963. *Cells of the human hypophysis in pregnancy, thyroid disease and adrenal cortical disorders*. In: Cytologie de l' Adenohypophyse (Eds. J. Benoit and C da Lage, (NRS), Paris, p.183-200.
- Follenius E. 1968. Analyse de la structure fine des differents types de cellules Hypophysariens des poissons teleosteans. *Pathology and Biology*. 16, 619-632.
- Garria-Ayala A. Garcia-Hernandez MP. Quesada JA. Agulleiro B. 1998. Gonadotrophic and Thyrotropic cells from the Mediterranean yellowtail (*Seriola dumerilli*) (Risso 1810), immunocytochemical and ultrastructural characterization. *Anatomy Research*. 250, 448-458.
- Gomeri G. 1950. Aldehyde Fuchsin, a new stain for elastic tissue. *American Journal of Clinical Pathology*. 20, 665-666.
- Gopaldutt NH. 1989. *Cyclic changes in the pituitary gland of Vertebrates in relation to reproduction*. In reproductive cycles of Indian vertebrates (Ed. Saidapur, S.K). Allied Publishers New Delhi, p.1-57.
- Holmes RL. Ball JN. 1974. *The pituitary gland: A comparative account*, Cambridge University press, p.170-220.
- Jose TM. Sathyanesan AG. 1977. Pituitary cytology of the Indian carp, *Labeo rohita* (Ham). *Anatomischer Anzeiger*. 142, 410-423.
- Joy KP. 1997. *Catfish Pituitary GTH and regulation of gonadotropin secretion*. In: Advances in fish Research, (Ed. B. R. Singh), 2: p.221-232.
- Joy KP. Manickam P. Sathyanesan AG. 1997. Parafarmaldehyde and glyoxilic acid histofluorescence

- localization of monamines in the hypothalamohypophysial system of the catfish *Clarias batrachus* (L). *Biological and Structural Morphology (Masson)*, 4, 58-67.
- Joy, K. P. and Sathyanesan AG. 1979. Functional cytology of the pituitary gland of the teleost *Clarias batrachus* (L). *Endokrinologie* 72, 9-16.
- Joy KP. Sathyanesan AG. 1978. Micro morphology and angioarchitecture of the hypothalamo neurohypophysial neurosecretory complex of the teleost, *Cirrihinus mrigala* (Ham). *Archivio Italiano Di Anatomia E Di Embriologia*. 83, 3-10.
- Joy KP. Sathyanesan AG. 1980. Pituitary cytology of teleost fish, *Talipa mossambica*. (Peters). *Zeitschrift Fur Mikroskopisch-Anatomische Forschung*. 94, 337-344.
- Kagawa H. Kawazoe I. Tanaka H. Okuzawa K. 1998. Immunocytochemical identification of two distinct Gonadotropic cells GTH I and GTH II in the pituitary of Blue fin Tuna, *Thunnus thynnus*. *General and Comparative Endocrinology*. 110, 11-18.
- Knowles F. Vollrath L. 1966. Neurosecretory innervation of the pars distalis at different stages of the lifecycle. *Philosophical Transactions of the Royal Society B*. 250, 329-342.
- Koida Y. Noso T. Schouten G. Peute J. Zandbergen MA. Bogerd J. Schulz RW. Kawachi H. Goos HJ. Th. 1992. Maturational gonadotropin from the African catfish, *Clarias gariepinus* purification, characterization, localization and biological activity. *General and Comparative Endocrinology*. 87, 327-341.
- Kulkarni RS. Sathish D. 1993. Some studies on Hypothalamohypophysial system in a fresh water teleost. M. Phil. dissertation awarded, *Gulbarga University, Gulbarga*.
- Kulkarni RS. Sathyanesan AG. 1983. Hypothalamo-neurohypophysial Neuro Secretory system and its vasculature in the freshwater catfish *Mystus vittatus* (Bloch) *Zeitschrift Fur Mikroskopisch-Anatomische Forschung*. 96, 1009-1021.
- Leatherland JF. 1969. Studies on the structure and ultrastructure of the intact and 'Methallibure'-treated mesoadenohypophysis of the viviparous teleost *Cymatogaster aggregata* Gibbons. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie*. 98, 122-134.
- Leatherland JF. 1970. Season variation in the structure and ultrastructure of the pituitary of the marine form (*Trachurus*) of the three spine stickle bialk, *Gasterosteus aculeatus* L. I. Rostral pars distalis. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie*. 104, 301-317.
- Mac Conaill. 1947. The staining of the central nervous system with lead Heamatoxylin. *Journal of Anatomy*. 81, 371-372.
- Moons L. Cambre M. Marvoet S. Batton TFC. Vaderhaegan JJ. Ollevier F. Vandesande F. 1988. Peptidergic innervation of adrenocorticotrophic hormone (ACTH) and growth hormone (GH)- producing cells in the Pars distalis of the Sea bass (*Dicentrarchus labrax*). *General Comparative Endocrinology*. 72, 171-180.
- Nagahama Y. Yamamoto K. 1969. Fine structure of glandular cells in the adenohypophysis of the Kokanee *Oncorhynchus nerka*. *Bulletin Faculty of Fishery Hokkaido University*. 20, 159-168.
- Nagahama Y. Yamamoto K. 1970. Morphological studies on the Pituitary of the Chum salmon, *Oncorhynchus Keta* (I): Fine structure of the adenohypophysis. *Bulletin Faculty of Fishery Hokkaido University*. 20, 293-302.
- Naito K. Suzuki SP. Nozaki M. Kawauchi H. Nakai Y. 1993. Ultrastructural characteristics of two GTH (GTH I and GTH II cells) in the Pituitary of the Rainbow trout (*Oncorhynchus Mikiss*). *Fish Physiology and Biochemistry*. 11, 291-246.
- Pantic VR. 1975. The specificity of Pituitary cells and regulation of their activities. *International Review of Cytology*. 40, 153-195.
- Peter RE. Yu KL. Marchant TA. Rosenblum PM. 1990. Direct neural regulation of the teleost adenohypophysis. *The Journal of Experimental Zoology Supplements*. 4, 84-89.
- Peute J. Zandbergen MA. Goos HJ. Th. De R. 1986. Pituitary gonadotropin contents and ultrastructure of the GTH in the African catfish *Clarias gariepinus* during the annual cycle in a natural habitat. *Canadian Journal of Zoology*. 64, 1718-1726.
- Queseda J. Lozano M.T. Ortaga A. Agullerio B. 1988. Immunocytochemical and ultrastructural characterisation of the cell types in the adenohypophysis of *Sparus aurata* (L.) (teleost). *General and Comparative Endocrinology*. 72, 209-225.
- Rodriguez-Gomez FJ. Rendon-Uncecta MC. Pinuela C. Munoz-Cueto JA. Jimenez-Tenorio N. Sarasquete C. 2001. Immuno cytochemical characterization of pituitary cells of blue fin tuna, *Thunnus thynnus* L. *Histology and Histopathology*. 16, 443-51.
- Sahai S. 1986. Localization of cell types in the adenohypophysis of the pituitary gland of *Puntius ticto* (Ham. Buch), teleostei. *International Journal of Academy Ichthyology*. 7, 11-15.
- Sarkar SK. 2000. *Morphohistology of the Pituitary gland, Their seasonal variation and role in spawning of Indian major carps*. In Compendium of lectures on Reproductive Physiology of carps and catfishes, p. 20-25.
- Sathyanesan AG. Das RC. 1978. Hypothalamo-hypophysial vascularization of the teleost, *Puntius sophore* (Ham) with special reference to its tetrapodan features. *Anatomischer Anzeiger*. 143, 110-119.
- Sathyanesan AG. Haider S. 1970a. Hypothalamo-neurohypophysial complex of the teleost *Heteropneustes fossilis* (Bl.) with severe experimental evidence on the regeneration of neurosecretory tract. *Indian Journal of Experimental Biology*. 8, 174-178.
- Sathyanesan AG. Jose TM. 1975. Structure of the neurohypophysis and the hypothalamo-hypophysial vascularization in the teleost *Channa punctatus* Bloch. *Zeitschrift Fur Mikroskopisch-Anatomische Forschung*. 824-838.
- Subhedar N. Ramakrishna NS. 1988. Immunocytochemical localization of LH-RH in the brain and Pituitary of the Catfish, *Clarias batrachus* (Lin). *General and Comparative Endocrinology*. 72, 431-442
- Van Oordt PGWJ. Peute, J. 1983. *The cellular origin of pituitary gonadotropins in teleosts*: In Fish Physiology, 9A: WS Hoar, DJ Randall, EM Donald (Eds.).
- Vollrath L. 1966. The ultrastructure of eel pituitary at the elver stage with special referent to its neurosecretory innervation. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie*. 73, 107-109.
- Yan HY. Thomas P. 1991. Histochemical and immunocytochemical identification of the pituitary cell types in three sciaenid fishes; Atlantic croaker (*Micropogonias undulatus*), spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*). *General and Comparative Endocrinology*. 84, 389-400.