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RESEARCH ARTICLE

HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDIES ON THE PITUITARY GONADOTROPIC CELLS OF THE FISH, *NOTOPTERUS NOTOPTERUS*

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ABSTRACT

Gonadotropic cells are distributed in the proximal pars distalis of the pituitary in *Notopterus notopterus*. Histochemical and electronmicroscopic techniques are applied for the present study. 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 gonodotropes 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 electronmicroscopic studies reveals that, they appear oval or elongated containing a few large granules and many small lucent secretory granules and based on this two GTH cell type could be identified in the PPD containing different types of secretory granules. The differentiation and presence of two types of gonadotropes needs further verification in the fish *N. notopterus*.

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INTRODUCTION

The gonadotropes of teleosts are distributed in proximal pars distalis (Ball and Baker, 1969; Rodriguez-Gomez *et al*., 2001). However the gonadotropes are spread to RPD at sexual maturity in various teleosts including eels and trouts and gonadotropes increase in number and size when the fish is having the matured or maturing gonads (Weng Youzhu *et al*., 2000). The histochemical and immunocytochemical studies were made on pituitary gonadotropes to understand their nature, structure and distribution (Fantodji *et al*., 1990). In some species two types of gonadotropes were reported and the work has been also carried out on the isolation characterization and purification of two types of gonadotropes as reported in other fishes (Garcia-Ayala *et al*., 1997; Garcia-Hernandez *et al*., 1998; Hassin *et al*., 1998). Although the functional cytology of the pituitary gland in teleosts have been carried out (Sathyanesan and Kulkarni, 1983), the studies on the cell type and their functioning in relation to different age groups of fishes are not much accounted in literature. Since the gonadal maturity depends on the pituitary gonadotropins secreted from the gonadotropes, their related changes in number, and their activity on the age at first maturity and subsequent studies in the ageing fish will furnish a clear understanding of their role in the process of maturation of gonads in the ageing process.

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Hence, in the present study on the cytology of the pituitary gland with special reference to gonadotropes is being undertaken in different age groups including their activity during different maturity stages of gonads, which will give clear information about their role in the maturation process of gonads. The ultra structural details of the gonadotropes in aged fish (two-year) has also been made on the pituitary of *N notopterus* to identify, if any differentiation on the two types of gonadotropes reported in other fishes (Garcia-Hernandez *et al*., 1997 Hassin, 1998) can be identified. In view of this the present study on the histological and electron microscopic nature and distribution of gonadotropes in relation to different age groups of locally available fresh water fish *N. notopterus* has been undertaken.The study would help to understand the role of gonadotropes in gonadal 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

Fresh water teleost *Notopterus notopterus* used for the present study was collected from Saradgi Nala situated 10 km away from Gulbarga City. More than 50 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. 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.

1.Aldehyde fuchsin (AF) preceded by acid permanganate oxidation using fast green – chromotrope 2R as the counter stain. 2. Lead haematoxylin (PbH), preceded by PAS. 3.Periodic acid (PAS) orange G (OG) procedure. 4.Cleveland and Wolf's trichome method. 5.Aldehyde thionine – PAS – Orange G method.

Electronmicroscopy

Fixation: The pituitaries were fixed in Glutaraldehyde (because its capacity to stabilize most of the protein without coagulation) following fixation in the primary fixative, the tissues are washed in 0.1 M buffer. The tissues were post fixed in 1% OSO_4 for 1-2 hours at 4^0C. The tissue is embedded in embedding medium (Epon 812/Araldite Cy212). To obtain a high resolution, the thickness of the specimen should be about 600 A (60 nm). Such thin sections are obtained on ultra microtome. The tissue is double stained with uranyl acetate followed by lead citrate. The ultra thin sections placing in copper grids observed under electron microscope at NIMHANS, Bangalore.

Observation: The gonadotropes or GTH cells in the pituitary gland of *N. notopterus* have a wide distribution in the region of proximal pars distalis (PPD). These gonadotropes were differentiated from the thyrotropes (as they stain alike) by comparing the pituitary gland stained during pre-spawning and post spawning period. The gonadotropes are abundantly distributed in the medial region of PPD as these basophils stains intensely to AF during pre-spawning period. Hence are identified as gonadotropes. The pituitary gland of zero age group fish has few numbers of gonadotropes and they are undifferentiated with indistinct boundaries. The gonadotropes markedly increase in the (PPD) region of pituitary in one year age group fish and also show advanced stages of growth. Since gonadotropes 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 gonadotropes are abundantly distributed in the PPD of pituitary in fish having two year age (Fig.1). The gonadotropes are intensely stained in the prespawning period. These cells undergo degranulation with weak staining after spawning in the same age group fish. In one year aged post spawned fish the gonadotropes get degranulated indicating the hormone release during spawning activity. Gonadotropes in two year aged fish stains positively with PAS, AB, AF, ATH and aniline blue (Fig. 7, 8, 9 and 10). They are distinguished with thyrotropes as they are positive to PAS, AF and aniline blue, but thyrotropes have less affinity for AF, AB than gonadotropes and a stronger affinity for PAS. The gonadotropes are more in number and in size and their intense staining, since the fish is having maturing gonads. The ultra structural characteristic 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 (Fig. 11, 12, 13, 14). 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 gonadotropes during this period shows characteristic of two different cellular inclusions. One type of gonadotrope containing numerous dilated cisternae of the ER (Endoplasmic reticulum) and small type of secetory granules. The diameter may be around 100-300 nm, whereas another type of gonadotrope although contain similar dilated cisternae of the ER and small type of secretory granulas, 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 may be GTH-II gonadotrope. 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 gonadotropes in this species (*N. notopterus*) needs a study on the immunocytochemistry using EM. Interspersed among the GTH cells in the rostral part of *N. notopterus* are few small cells lying adjacent to fine channels which connect the lumen with the intervascular space, 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 adenohypophysis. These cells occur in all regions of the adenohypohysis 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 somatotropes and thyrotropes, which are closely associated with gonadotropes.

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 thyrotropes 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 thyrotropes, and the basophils present in the medial PPD are gonadotrops. Such studies on the pituitary have been reported and those cyanophils of the middle glandular region are gonadotropes (Gopal Dutt, 1989).

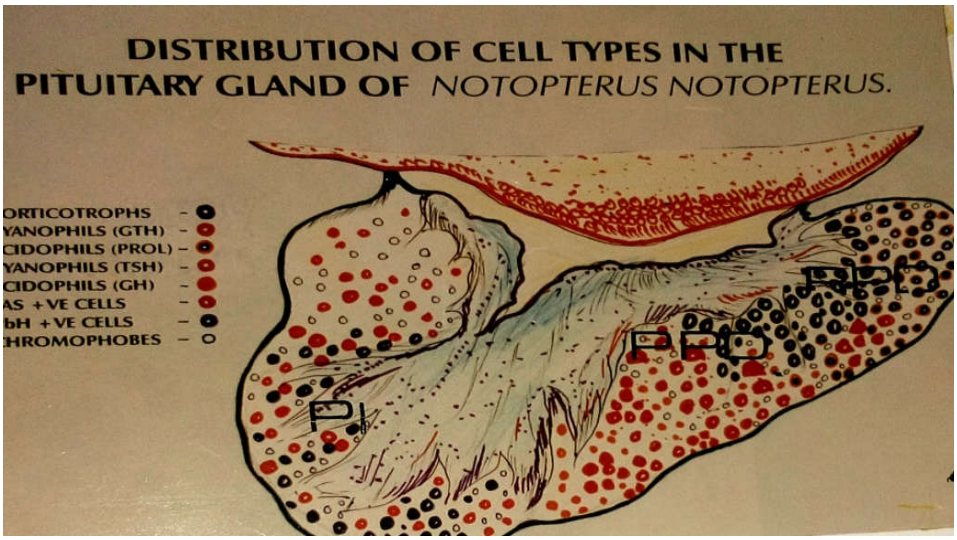

Fig.1. Diagramatic presentation of Pituitary cell type distribution in the fish *Notopterus notopterus*

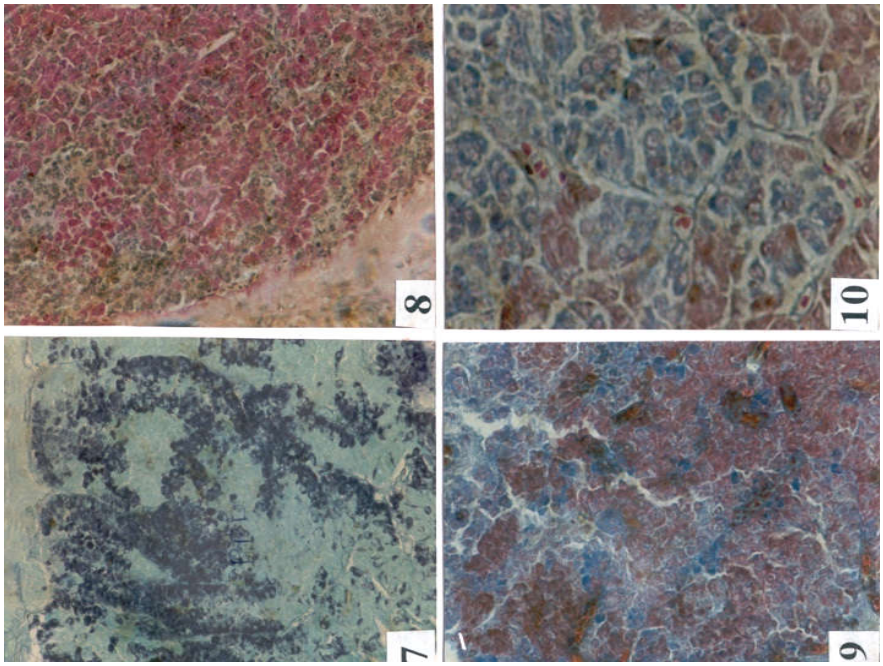

Fig.7-10. Pituitary gonadotrophs during breeding period of the fish Notopterus notopterus – PAS=PbH+Tetrachrome stains. X1200

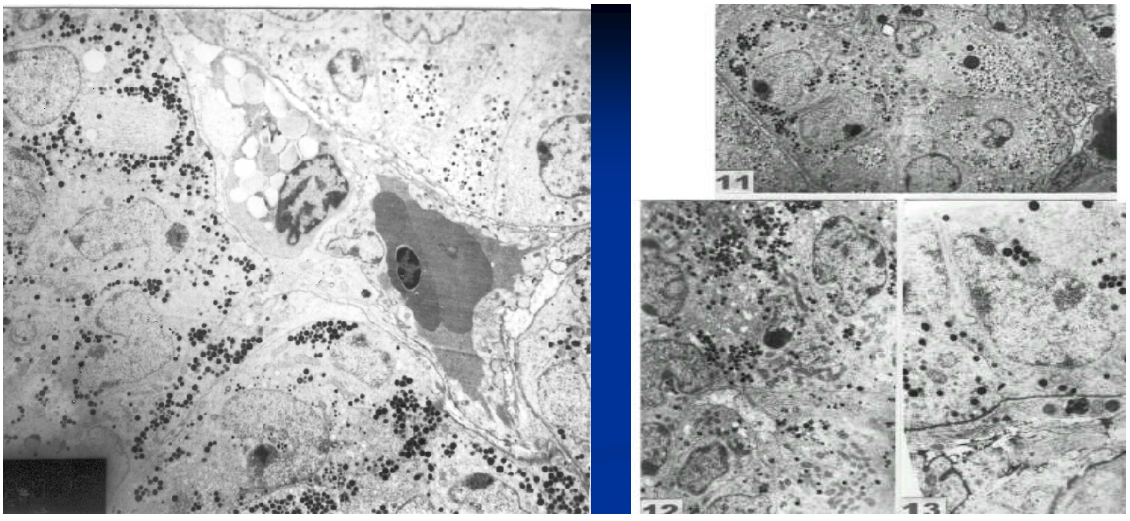

Fig.11-13. Electon microscopic photographs showing secretary granules in the gonadotrophs. EM

The distribution pattern, staining response and cytological stages of basophils during different phases of reproductive cycle of matured fish is available in the literature (Shamim Haider, 1978; Joy and Sathyanesan, 1979). The basophiles distributed in the region of PPD are gonadotrops, which are abundantly seen during pre-spawning phase of the reproductive cycle of *N. notopterus*. The pituitary of zero age group although have the gonadotropes 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 (in agreement with the reviews – Shamim Haider, 1978; Joy and Sathyanesan, 1979).In eel and pacific salmon two types of gonadotropes are reported based on their colour differences and granular, size, distribution, position and vascularisation and in several other teleosts only one type of gonadotropes are reported (Holms and Ball, 1974; Par Ekergren *et al*., 1978; Hirohiko Kagawa *et al*., 1998; Joy *et al.,* 1989). The ultrastructure, of pituitary gonadotropes 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 *etal;* (1998) on the mediterranean yellowtial *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 (in agreement with reviews - Nagahama and Yamamoto, 1969; Joy *et al*., 1989; Garcia-Ayala *et al*., 1998). The ultrastructural characteristics of GTH cells have been described for some catfishes (Van Oordt and Peute, 1983; 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 rough endoplasmic reticulum (RER) with small dilated cisternae, mitochondria and Golgi complex usually appeared near the nucleus. The differentiation of two types of gonadotropes could not be made in the histochemical studies. However, ultrastructural studies using electromicroscope 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 gonadotropes has been made in the pituitary gland of rainbow trout *Oncorhynchus mykiss* by Naito *et al.* (1993). He describes that the salmon pituitary produces two chemically distinct gonadotropins. The ultrastructural characteristic of GTH-I and GTH-II is studied through 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 granules (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 ER, 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 reporting that GTH-I and GTH-II beta are synthesised in distinctly different cell types in the salmnoid pituitary. Recently, Matan Golan et al. (2016) have reported on the anatomical and functional gonadotrope networks in two teleost fish species, tilapia and zebra fish that two types of gonadotropes based on the immunogold-lebeling and EM studies indicated that LH-producing cells were found to reside in tight clusters in peripheral parts of thePPD, whereas FSH producing cells were located more dorsall, close to the dorsal projections of the pars nervosa. In comparison to LH-cells, FSH cells were distributed more loosely throughout PPD. Similar type of differentiation and distribution is possible in the fish, *N. notopterus*, the existence of two types of cells secreting LH and FSH still needs to be confirmed using immune-histochemistry along with EM studies.

Conclusions

Two types of gonadotropic cell distribution are identified based on the staining response and electron mocroscopic studies in the fresh water fish, *Notopterus notopterus.*

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