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SEASONAL CHANGES IN GONADS AND THEIR RELATIONSHIP WITH GONADOTROPHS OF THE PITUITARY IN *ETROPLUS SURATENSIS* (BLOCH)

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ABSTRACT

Detailed histological studies of the gonads of *Etroplus suratensis* (Bloch) collected from the brackish-water areas of Poothotta near Cochin during May 83 - April 84 have shown that the peak breeding season of the species is in December and January. A secondary peak is also noticed in June and July. During peak growth phases of the gonads, the gonadotrophs of the pituitary are found to be active and prominent denoting a close relationship between the activity of these cells and the maturation of the gonads of the species. The gonadotrophs are seen less prominent and inactive during non-breeding months. The details of the maturity stages of the male and the female have also been described.

INTRODUCTION

STUDIES on the development and seasonal histological changes in the gonads of teleosts have been plenty (Hoar, 1957 ; Harrington, 1957 ; Thomas and Satyanesan, 1981 ; Schreibman *et al.*, 1982). In recent years there has been more inclination of similar type of studies on cultivable varieties of fishes due to their potential prospects for aquaculture (Kuo and Nash, 1975 ; Jalbert, 1976 ; Bieniarz *et al.*, 1977 ; Hurk and Peute, 1979). Similarly voluminous literature is available on teleost pituitary describing various aspects such as morpho-histology, cytology and physiological functions (Pickford and Atz, 1957 ; Satyanesan, 1958, 1963 ; Singh and Satyanesan, 1962 ; Sage and Bern, 1971 ; Schreibman *et al.*, 1973 ; Ball and Baker, 1969 ; Joy and Satyanesan, 1980 ; Narayan *et al.*, 1985). Considerable work has been done on correlation of cyclic changes of gonadotrophs of the pituitary with that of changes occurring in the gonadal maturation of teleost fish (Atz, 1953 ; Matty and Matty, 1959 ; Satyanesan, 1962, 1963 ; Overbeeke and McBride, 1967 ; Olivereau and Ball, 1964; Van Oordt and Ekengren, 1978). However, similar studies on estuarine fishes of India are very limited. Hence in the present work preliminary investigation has been made to study the seasonal histological changes in the gonads in relation to maturity and further the maturity events have been correlated with the activity of gonadotrophs of the pituitary in *Etroplus suratensis*.

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MATERIAL AND METHODS

E. suratensis were collected every month from the brackishwater areas of Poothotta, 30 km away from Cochin city from May 1983 to June 1984. Specimens were brought to the laboratory, sacrificed immediately and required material for histological examination such as pituitary and gonads excised out and fixed in Helly's fluid and aqueous Bouin's respectively. Preserved samples were processed

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and paraffin sections were cut at 4 to 6 μ thickness. The sections of the gonads were stained with routine Ehrlich hematoxylin and eosin while pituitary sections were stained with modified Mallory's triple stain and Orange fuchsin green method.

Results

Ovary

The paired ovaries in *E. suratensis* are found in the dorsal part of the coelom. Each ovary is suspended in the body cavity along its dorsal side and surrounded by peritoneal membrane. The peritoneal membrane forms a gonoduct posterior to the ovary. Both the gonoducts end in a urinogenital sinus.

The ovary has been found to be composed of ovigerous folds transversally arranged along its longitudinal axis. The ovigerous folds contain germ cells in various developmental stages embedded in loose connective tissues. The developmental process of the ovum as observed in the present study has been arbitrarily classified into four stages *viz.* previtellogenic, vitellogenic, mature and post-ovulatory-atretic.

During previtellogenic stage (February to May and August to October) follicles are seen with primary oocytes. Initially the oocytes appeared to be very small and spherical or oval in shape measuring in the range of 20-45 μ in diameter. The spherical or slightly oval nucleus (13 μ) of each oocyte occupies greater part of the cell body within a thin layer of cytoplasm (Pl. I A). With the growth of oocytes the cytoplasm increases considerably in relative volume and becomes more basophilic. The nuclei now appeared to contain small nucleoli which migrate to the periphery of the nucleus. As the primary oocyte stage progresses (105 μ) cytoplasm of the oocyte further increases in volume and loses its basophilic nature (Pl. I B). The small yolk nuclei which are seen in early stages of some oocytes now migrate to the periphery of the ooplasm. The wall of the primary oocytes is found to be surrounded by a thin follicular layer. Differentiation of different layers of the oocyte wall in this phase of maturation was not possible.

In the vitellogenic follicles (April to June and September to November) the appearance of each oocyte has been found to be characterised by the granular cytoplasm and darker zone surrounding the germinal vesicle. The cytoplasm also appeared to be vacuolated showing the beginning of endogenous yolk formation (Pl. I C). The follicular layers of the oocyte increases their thickness and all the three layers *i.e.* Zona radiata, granulosa and theca get differentiated and are distinctly seen in larger oocytes (Pl. I F). The structure of nucleus did not change much, but chromatin nuclei are found to be distributed close to the nuclear membrane (Pl. I C). The nuclei vary in shape from spherical to elliptical. Yolk formation in the beginning is characterised by the appearance of a number of yolk globules and as the oocyte develops further, yolk globules increases in number and size (Pl. I D).

In mature stage (July, December and January) the fusion of the globules occur and finally yolk appears as a homogenous mass (diameter varied between 650-900 μ) (Pl. I E). The Zona radiata and granulosa have a thickness of 3.5 μ and 4.0 μ respectively (Pl. I F).

In the post-ovulatory stage (August and February) the follicles are found raptured resulting from evacuation of the mature eggs (Pl. I H). The oocytes in such follicles have folded granulosa.

The atretic follicles represent follicles with oocytes containing yolk and with degenerative conditions. During this stage, the granulosa cells strongly proliferate, grow into the oocyte and start resorbing its contents. Due to resorption of yolk material by granulosa cells distinct intracellular spaces are visible between the granulosa cells (Pl. I G). Further follicles

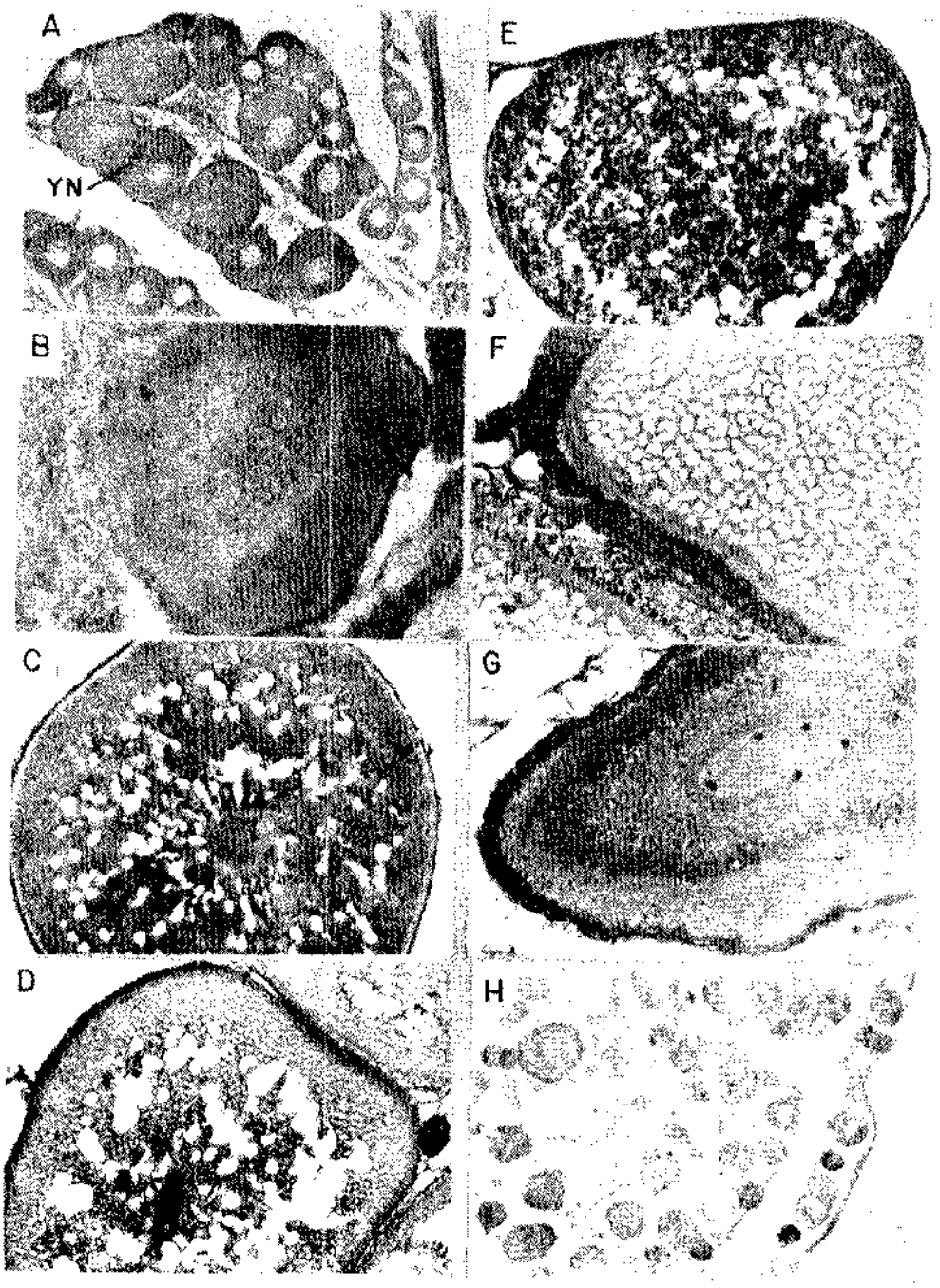

PLATE I. A, B. Oocytes in previtellogenic stage (×100) ; YN—Yolk nucleus ; C, D. Oocytes in vitellogenic stage (×100) ; E. Mature oocyte (×100) ; F. Mature oocyte wall (×400) : ZR—Zona radiata, G. — Granulosa, T—Theca ; G. Atretic oocytes (×200) and H. Post-ovulatory oocytes (×100).

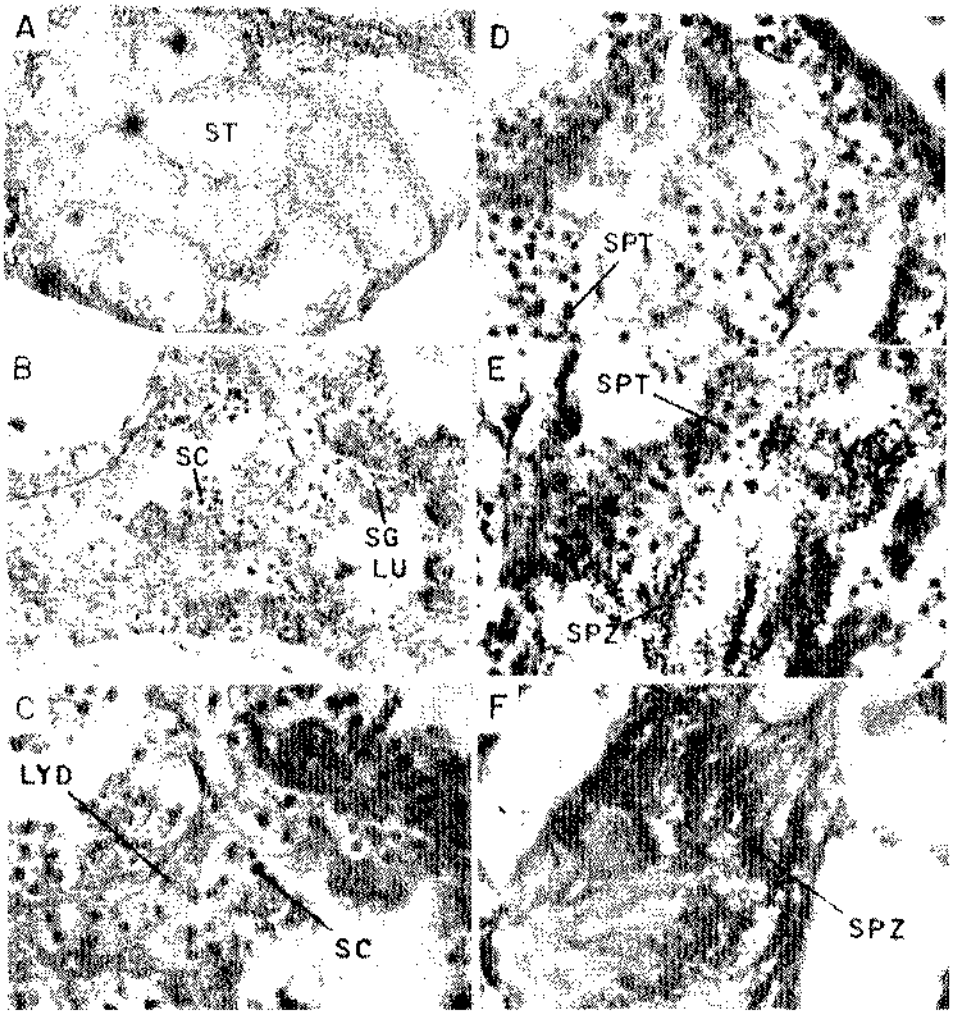

PLATE II. A, T.S. of testes — seminiferous tubules (×100) ; ST —Seminiferous tubule ; B, Seminiferous tubules with spermatogonia and spermatocytes (×200) ; SC—Spermatocytes, SG—Spermatogonia, LU —Lumen ; C, Seminiferous tubules with spermatocytes (×400) ; LYD —Leydig cells in between the tubules, SC —Spermatocytes ; D, Spermatids in seminiferous tubules (×400) ; SPT—Spermatids ; E, Spermatids and spermatozoa in seminiferous tubules (×400) ; SPT—Spermatids, SPZ —Spermatozoa, and F, Thickly packed spermatozoa bundles (×400) ; SPZ—Spermatozoa.

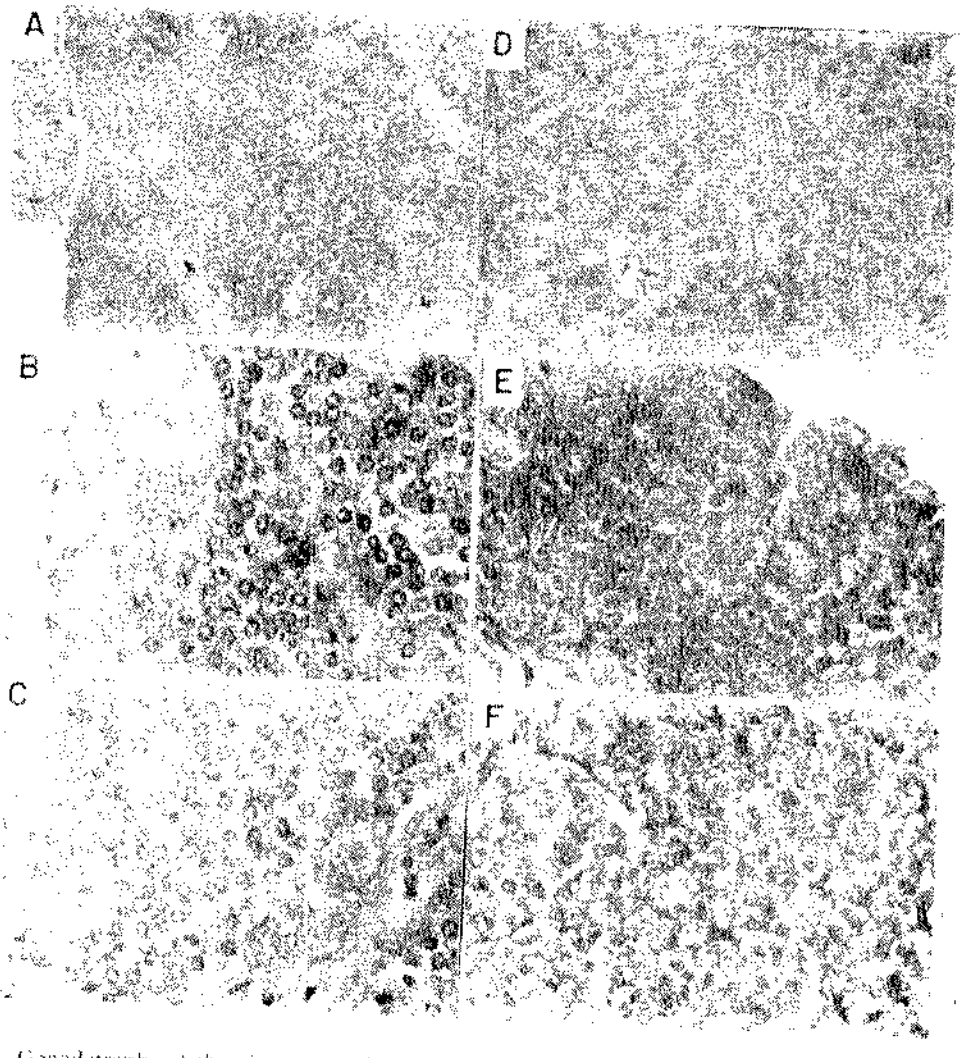

PLATE III. Gonadotrophs of the pituitary in different phases of activity : A-C. Corresponds to gonadotrophic cells (resting phase) observed during March-April and August-October (×200, ×400 and ×400) and D-F. Corresponds to gonadotrophic cells (active phase) observed during July- December and January (× 400).

become small due to reduction in size of granulosa cells. The granulosa cells finally become necrotic.

Testis

The elongated testes of the pearlspot are found in the dorsal part of the coelom. The testes appeared threadlike and pale whitish in colour in the mature fish. They originate anterodorsally below the heart and travel back posteriorly and then after making a vertical dip posterio-anteriorly pass along with urinary bladder to join and open at the urinogenital papilla.

Histologically the testes as seen under light microscope are characterised by the presence of long circular or oval seminiferous tubules (Pl. II A). Spermatogenetic cysts are observed along the walls of the tubules and surround a central lumen in which sperm cells are generally stored (Pl. II B). Different developmental stages of spermatogenesis such as spermatogonia and their proliferated products *viz.* spermatocytes, spermatids and spermatozoa are generally observed in the seminiferous tubules (Pl. II C-F). Leydig cells though not observed very clearly in the present study are randomly distributed in the interstitial tissue between the seminiferous tubules (Pl. II C).

From the detailed histological studies of the ovaries procured from monthly samples of fish, the peak breeding season for *E. suratensis* has been found to be during the months of December-January and a smaller peak in the months of June and July. But in our annual samples, males were found to be in mature condition for major part of the year. However, in the breeding season the sections of the testes prepared showed enormous amounts of fully mature sperms thickly packed as bundles in the testicular lumen (Pl. II F). During the months other than peak breeding seasons the seminiferous tubules are found in normal condition with large number of primordial germ cells in the interstitium. The tubules also contain spermatogonia and groups of spermatocytes.

Pituitary gland and Gonadotrophs

The pituitary gland is situated in a shallow pit at the floor of the skull and is attached to brain by a short stalk which is closely applied to the floor of the infundibulam. Histologically the gland as seen under the microscope has two distinct regions *viz.* adenohypophysis and neurohypophysis. The adenohypophysis is further observed to have three conventional areas namely rostral pars distalis (anterior), proximal pars distalis (middle) and pars intermedia (posterior). The demarcations between these regions are clearly seen in sections. Although major portions of neurohypophysis aborises into the pars intermedia, smaller branches also penetrate into both rostral pars distalis (RPD) and proximal pars distalis (PPD). Six conventional cell types have been identified in the pituitary. The gonadotrophs whose morpho-histological structure has been used in correlating gonadal cycle in the present study are mainly distributed in the ventral portion of proximal pars distalis. The other cells present in this region are found to be somatotrophs and thyrotrophs. The gonadotrophs reacted positively with Mallory's triple and acid fuchsin stains. A clear prominent nucleus could be seen in these cells (Pl. III B, C). Compared to gonadotrophs, somatotrophs and thyrotrophs are smaller in size and are situated mostly towards anterior portion of the PPD.

Number of authors have indicated the role of cyanophils (Gonadotrophs) in the production of gonadotropins in fish. This is identical with what is known about the source of gonadotropins in higher vertebrates. Evidence for this view consists mainly of an increase in the number and activities of these cyanophils during growth and differentiation of the oocytes. In *E. suratensis* during the process of active maturation of gonads (July, December and January) more pronounced changes are

observed in PPD of pituitary as this area is found to be occupied with more of gonadotrophs. In non-active season (March-April, August-September) PPD consisted of more of somatotrophs and thyrotrophs. Gonadotrophs could be seen, but without any secretory activity. In the resting phase, the nucleus of the gonadotrophs is generally found to be large with number of nucleoli and occupied greater part of the cytoplasm (Pl. III A-C). The cytoplasm of these cells stained blue with aniline blue of Mallory and nuclei red with azocarmine. During subsequent growth of the ovary the cytoplasm of these cells increase and become granular. Their number and size also increased and occupy large portions of PPD (Pl. III D-F). Simultaneously there is reduction in number of other cells of PPD.

Discussion

The structure of the teleostean pituitaries and their organization has been the subject of much research in recent past (Van Oord 1968 ; Ball and Baker 1969 ; Sage and Bern 1971). The adenohypophysis of the pituitary and its main role in regulating gonadal function in many teleosts is well documented. Gonadotropin secreting cells (Cyanophils and acidophils) have usually been identified in many fish by their cytological characteristics during sexual cycle and are most frequently located in the proximal pars distalis (Van Oordt, 1968 ; Ball and Baker, 1969 ; Sage and Bern, 1971), Ball and Baker (1969) have mentioned that the distribution of cells types in teleost adenohypophysis is extremely regular so that to a large extent differentiation of cell types on tinctorial grounds becomes easy. Frequently then, gonadotrophic cells have been distinguished by many workers on the basis of tinctorial features, position, cell size and other morphological features (Sage and Bern, 1971 ; Schreibman *et al.*, 1973 ; Van Oordt and Ekengren, 1978 ; Thomas and Satyanesan, 1981).

In many teleosts a correlation between the gonadotropic cells and the gonadal cycle have been observed showing the hyperplasia, hypertrophy and other signs of increased activity of these cells in association with the ripening of the gonads (Olivereau and Herlant, 1960 ; Satyanesan, 1960, 1963 ; Olivereau and Ball, 1964 ; Lehri, 1966 ; Zambrano, 1971 ; Lambert *et al.*, 1972). Number of investigators have shown that pituitary gonadotropin activity is low following spawning or during gonadal regression, but is relatively high during most other parts of the year (Satyanesan, 1962, 1963 ; Barr and Hobson, 1964 ; Singh, 1970). In an electron microscopic study Zambrano (1971) observed that the pituitary gonadotropic cells in *Gillichthys* with recrudescing gonads are large and show several characteristics of actively secreting cells including secretory granules in them. In contrast, the gonadotropic cells of fish with regressed gonads were small and contained few secretory granules (Zambrano, 1971). Our findings in *E. suratensis* on gonadotropic activity in relation to maturation are in concordance with the findings earlier reported in the literature. However, experimental approach on stimulating the gonadal activity and its response to gonadotropic cells may help in elucidating further facts on these findings.

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