Ovarian Cycle and Estrogen Production in Snow Trout *Schizothorax niger* Heckel

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ABSTRACT

Estradiol-17 β (E_2) levels in the ovarian tissues of *Schizothorax niger* Heckel in relation with various events of its ovarian development were studied during the annual reproductive cycle on month-wise basis. Maximum E_2 values, recorded during August, September and October corresponded to the long maturing phase of vitellogenesis. During over-wintering months of November, December and January, E_2 values recorded were very low, corresponding to quiescent stage of winter diapause which is marked by low gonadal activity. The rise of temperature in late February, accompanied by abrupt increase in E_2 concentration, marked the breaking away of winter dormancy. In March, the oocyte nuclei had become eccentric and the nuclear membranes indistinct. These are indications of oocyte maturation. The rise in E_2 values in May could be attributed to activities like spawning and breeding during this period.

Keywords: Estradiol-17 β levels, ovarian cycle, oocyte maturation, vitellogenesis, spawning.

INTRODUCTION

The steroid hormone, E_2, plays an important role in the female teleost reproduction (Mones *et. al.*, 1989). The endocrine events encompass the release of gonadotropic hormone (Bromage and Cumaranatunga, 1988). Gonadotropic hormone (GTH) in turn stimulates ovarian follicles to produce the female sex hormone E_2. E_2 induces hepatocytes of the liver to synthesize the female specific plasma protein, vitellogenin (VTG) which is sequestered from the maternal blood stream by developing oocytes in the ovary and is deposited as yolk (Tata, 1976).

It has been successfully demonstrated that when defolliculated oocytes of rainbow trout, *Salmo gairdneri* (Jalabert, 1976) and Japanese medaka, *Oryzias latipes* (Iwamatsu, 1980), were cultured with trout and salmon gonadotropin respectively, the maturation of oocytes did not occur. It indicated that gonadotropins are not the final maturation inducing substances (MIS) but rather initiate steroidogensis in the follicular layer of oocytes to produce MIS which induces final matural (Haider and Inbaraj, 1989a).

In vitro experiments using immature granulose cells recovered from diethylstilbestrol-primed hypophysectomised or intact immature rats have demonstrated that estradiol enhances many FSH-mediated responses including induction of aromatase activity (Adashi and Hsueh, 1982; Daniel and Armstrong , 1983), P_4 accumulation (Welsh *et al.*, 1983), cyclic AMP (cAMP) formation (Richards *et al.*, 1979), and cell membrane receptors for both luteinizing hormone (LH) and FSH (Richards and Midgley, 1976; Ireland and Richards, 1978 and Rani et al., 1981), thereby clearly establishing a stimulatory autocrine role of E_2 in the rat ovary (Shaw and Hodges, 1992). In contrast, estrogen treatment has been shown to increase the incidence of follicular atresia in rhesus and cynomolgus monkeys (Hutz *et al.*, 1986; Koering, 1987) and to inhibit P_4 accumulation and aromatase activity *in vitro* in both humans and rhesus monkeys (Veldhuis et al., 1983; Ollsson *et al.*,1987; Yuen et al., 1989 and Hutz *et al.*, 1989).

The snow trout, *Schizothorax niger* Heckel has shown considerable decline in its population over the years. This decline has been attributed to a number of features like over exploitation of local fish, eutrophication and pollution of aquatic environment and competition with common carp, *Cyprinus caprio*. In order to restore the aquatic systems with this prized food fish, culture techniques including induced breeding have become inevitable. For such culture techniques, it is very important to understand the hormonal milieu in relation with ovarian development. Whereas a number of reports (Malhotra, 1970; Raina, 1976 and Qadri *et al.*, 1983) regarding the ovarian development of schizothoracine fish are available, no attempt has been made to correlate the ovarian development with the hormonal milieu of the ovaries. In order to clarify our knowledge of endocrinological and histological events, present study was undertaken with the objective of :

i) studying various ovarian developmental phases;

ii) estimating E_2 in the ovarian tissues during the annual reproductive cycle of *Schizothorax niger* Heckel; and

iii) correlating the ovarian developmental stages with various E_2 concentrations.

MATERIAL AND METHODS

Live specimens of adult female *Schizothorax niger*, ranging from 100 to 250 g, were procured from Hazratbal fish landing center of Dal Lake, Kashmir, which lies outside the Kashmir University Campus. The fish were checked for presence of oocytes by squeezing the abdomen. The live fish were killed in the laboratory by a sharp blow on the head.

Morphometry

The weight of the fish (in grams) and other morphometric data including total length, standard length (in mm) were recorded. The fish were dissected open, the ovaries removed by holding the posterior ends of the gonads and cutting along with mesentry. The weight of the ovaries was immediately recorded by weighing on monopan balance. Eviscerated weight of fish was also recorded.

Histological Studies

Ovaries from selected fish of same weight and length were used for histological studies and hormone assay. The ovaries were fixed in Bouin's fluid for 24 hours and then processed for histological studies. After embedding in paraffin wax, serial sections, 5-7μ in thickness, were cut and stained in Mallory's triple stain (MTS) using the technique of Gray (1964) and examined under microscope. Various events were recorded by photomicrography.

Extraction

Crushed ovaries were homogenized with twice the volume of diethyl ether. The ovarian homogenates were subjected to centrifugation (in a refrigerated centrifuge) at 3000 rpm for 15 minutes. The supernatant was decanted into assay

tubes and the residue was discarded. The ether extract was evaporated in water bath maintained at 37° C. The dried extracts were preserved at – 70 ° C till the assay was started.

Steroid Assay

Ovarian E_2 levels were determined by RIA using reagents and protocols according to World Health Organization Method Manual (1986). The scintillation counting was performed using LKB 1215 Rackbeta II Wallac Liquid Scintillation Counter (Finland) with a scintillater of PPO in sulphur free toluene. Mean extraction efficiency (determined by recovery of $_3H$ – labeled E_2 added to recovery sample tubes) was 82%, Assay values were corrected accordingly. For evaluation of final E_2 concentrations, dilution factor was also taken into account, Berthold statistical counter software package (RIA service), USA, was used to calculate the hormone concentrations of unknown samples using a logit/log transformation.

RESULTS

E_2 concentrations were detectable through the annual reproductive cycle of *Schizothorax niger* but changed with various events of ovarian development (Fig. 1). The period of August, S eptember and October marked the phase of maximum E_2

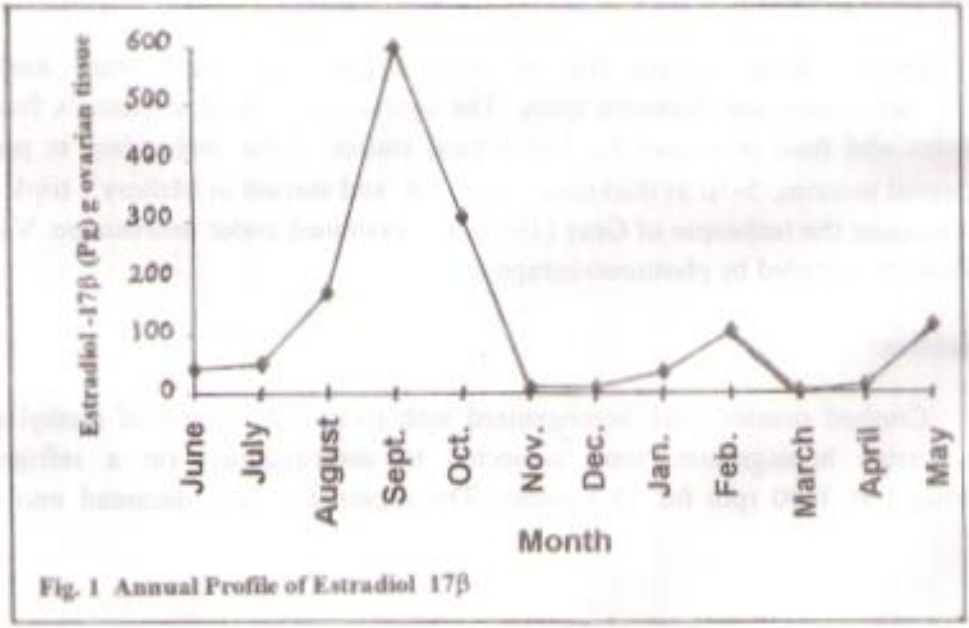

Fig. 1 Annual Profile of Estradiol 17β

concentrations viz. 175.05, 598.98 and 305.41 pg/g ovarian tissue respectively. This period exactly corresponded with the long maturing phase of vitellogenesis. During September, the vitellogenic activity was at its peak with zonation of cytoplasm into inner and outer zones. The yolk nucleus of Balbiani was seen towards the periphery (Fig. 2A). The oocytes showed an increase in size and the egg membranes were fully developed. The nucleus was centrally located.

November, December and January marked the period of very low E_2 concentrations being 7.75, 10.40 and 38.04 pg/g ovarian tissue respectively. The ovary had become fully developed with its wall becoming very thin through which glistening ova could be seen with the naked eye. The yolk almost completely filled the oocytes with no free cytoplasm being observed. The differentiation of yolk into perinuclear and peripheral regions was conspicuous. The peripheral yolk gets arranged into larger yolk plates and represent cortical alveoli staining blue with MTS.

The perinuclear or inner zone of smaller yolk plates stained yellowish brown with MTS (Fig. 2B). The ovaries during the over-wintering months of December and January did not show any remarkable change over the condition maintained in November or early December. This stage of ovarian development corresponded to the period of low gonadal activity or quiescent stage of winter diapause restricted by low winter temperatures.

With the rise of temperature in February, E_2 values abruptly increased to 109.63 pg/g ovarian tissue. The clear-cut peak marked the breaking away of quiescent phase of winter dormancy and the beginning of pre-spawning phase. The vitelline membrance had got completely separated from the follicular membrane (Fig.2C).In March, the nuclei of the oocytes had become eccentric (Fig. 2D) and the nuclear membranes indistinct. These are indications of oocyte maturation. The fish were ready to spawn. E_2 levels recorded were lowest being 2.47 pg/g ovarian tissue. The fish were fully gravid with bulging abdomen. The ova oozed out with slight pressure on the abdomen. E_2 values recorded in April were 15.32 pg/g ovarian tissue. Majority of fish come to spawn in April, though in some cases, spawning was noticed in March. The process usually continues up to the end of May. A significant rise in E_2

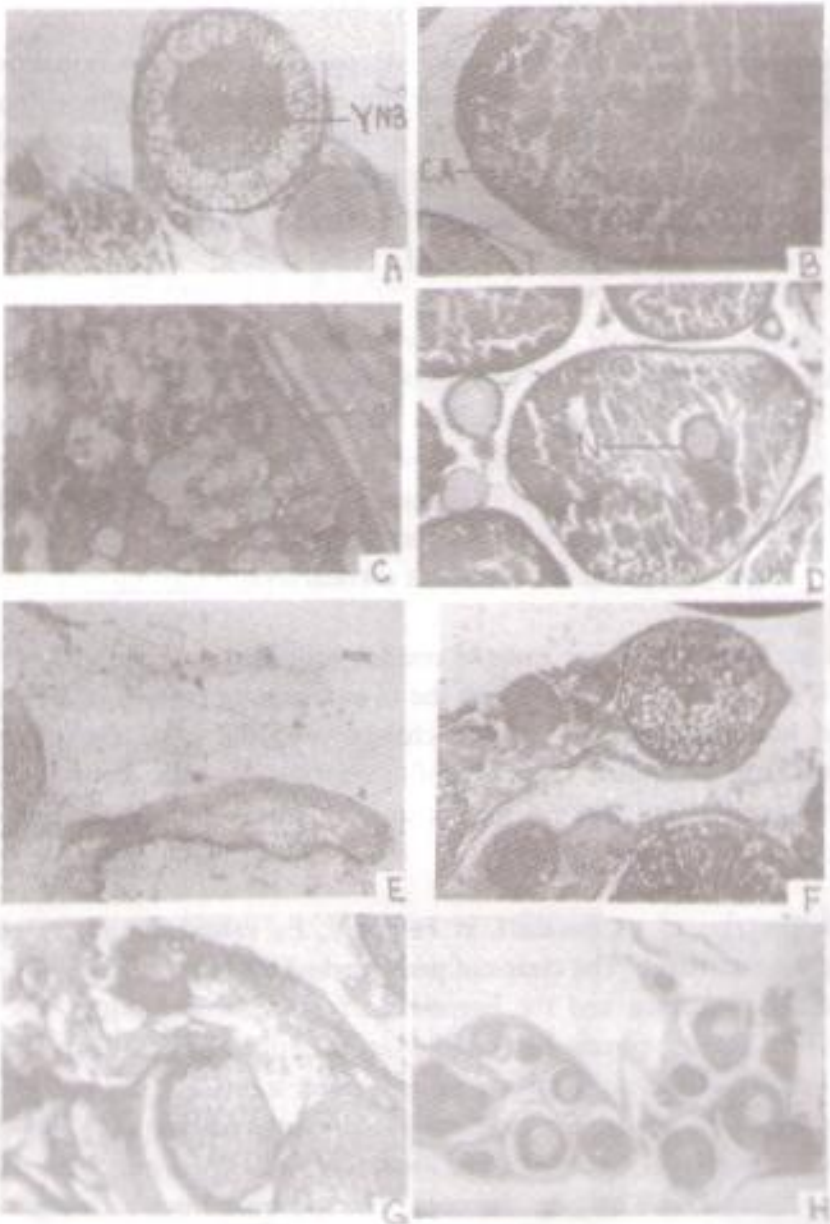

Fig. 2 Photomicrographs of sections through the ovary of *Schizothorax niger*. A: Zonation of cytoplasm into inner and outer zones and yolk nucleus of Balbiani (YNB) towards the periphery (Scale Bar = 20 μm). B: Peripheral yolk arranged into large yolk plates representing cortical alveoli (CA) staining blue with MTS and outer zone of smaller yolk plates staining yellowish brown with MTS (Scale Bar = 20 μm). C: Vitelline membrance (VM) separated from follicular membrane (Scale Bar = 100 μm). D: Nucleus (N) eccentric (Scale Bar = 20μm). E: Ovary after extrusion of eggs (Scale Bar = 10μm). F: Atretic oocytes undergoing regression and resorption (Scale Bar = 20μm). G: Recovery phase of ovary with proliferation of oogonia (Scale Bar = 100 μm). H: New crop of oocytes (Scale Bar = 10 μm).

concentrations (~ 120.40 pg/g ovarian tissue) was noticed in May, which could be attributed to the events of spawning and breeding at this stage.

Following extrusion of eggs during spawning period, the ovary was seen as a limp and greatly reduced structure with its wall frequently folded but highly vascularised (Fig. 2E). During spent and recovery stage, the values of E_2 in the months of June and July were 41.80 and 52.47 pg/g ovarian tissue respectively. A number of mature eggs that had failed to spawn undergo a gradual regression and resorption in the form of atretic oocytes (Fig.2F). In addition , a large number of follicles from which the ova have extruded could be seen in different stages of shrinkage. These become transformed into corpora lutea which are responsible for the secretion of progesterone. During June and July, peripheral vacuoles had started appearing in the cytoplasm. However, formation of yolk was not visible under light microscope. This stage, therefore, corresponded to previtellogenic phase (Fig. 2G&H).

DISCUSSION

Ovarian development and E_2 production were followed throughout the annual ovarian cycle of *Schizothorax niger* to draw some conclusions based on a simplistic interpretation that high concentrations of E_2 in ovarian tissues may be indicative of some biological function as also convincingly suggested by Campbel et al.(1980). Ovarian development was evaluated using different criteria viz. GSI, morphological and histological observations using MTS. GSI could neither be correlated to the histological ovarian development nor to the endocrinological criterion confirming its unreliability for assessment of gonadal development as has been shown in several species (Delahunty and de Vlaming, 1980; de Vlaming *et al.*, 1982 and Mones *et al.*, 1989).

Schizothorax niger is an annual breeder. The ovaries are cystovarian and the rhythm of ovarian development is typically characterized by group synchronism. Maximum spawning occurs from beginning of April to end of May. However, spawning may sometimes start from mid of March and may extend up to June.

E_2 induces hepatocytes of the liver to synthesize the female specific plasma protein; VTG. VTG is actively sequestered from maternal blood stream by developing oocytes in the ovary where it is deposited as yolk. Through accumulation

of yolk, the oocytes increase enormously in size and cause the ovary as a whole to grow (Van Weerd and Richter, 1991). Sundararaj and Nath (1981) showed that repeated injections of E_2 evoke an amplification of vitellogenin synthesis stimulation. A transient rise in E_2 could prime the liver for vitellogenin production. In the present investigation, the highest concentrations of E_2 were recorded during August, September and October which marked the stage of active vitellogenesis as confirmed by histological studies. The present studies are quite in conformity with the observations made by aforesaid workers. E_2 is also responsible for the formation of cortical alveoli (Fig. 2B). This is in agreement with Khoo (1979) who showed that oestrogens control the formation of cortical alveoli (which he called yolk vesicles) in hypophysectomized goldfish.

The steep fall in the E_2 values during the over-wintering months of November, December and January (when the vitellogenesis has not come to an end) can be attributed to quiescent phase of winter diapause restricted by low winter temperature and decrease in GtH concentrations. The fall in E_2 levels before the end of vitellogenesis has also been reported by Breton *et al.*, (1983) from female brown trout, *Salmo trutta*. Decrease in E_2 values could be explained by the drop of GtH levels (Jalabert and Breton, 1980 and Fostier *et al.*, 1981) as there has been observed positive correlation between GtH and E_2 values during early phase of reproductive cycle (vitellogenesis) and negative correlation during late reproductive cycle. Moreover, there occurs a distinct shift from the secretion of predominantly E_2 to the secretion of 17α-20β-dihydroxy-4-pregen-3-one(17α,20β-di OH prog)- Sakai et al. (1987). 17α-20β-di OH prog has been observed to be the naturally occurring most potent MIS as also convincingly suggested by Nagahama and Adahi (1985), Suzuki et al. (1987) and Haider (1990).

It is generally accepted that estrogens are not active in inducing oocyte maturation in fish (Goetz, 1983). Haider and Inbaraj (1989b) have also shown E_2 to be ineffective in inducing oocyte maturation during the culture of folliculated oocytes of *Labeo rohita* and *Cirrhinus mrigala*. However, E_2 in *Oryzias latipes* (Hirose, 1972 and 1976) and estrone in Brachydanio *rerio* (Van Ree *et al.*, 1977) were found to be effective in inducing oocyte maturation. During the present investigation, a minor peak of E_2 with a concentration of 109.63 pg/g ovarian tissue was observed in February. This corresponded to a period when nuclei of the oocytes had become eccentric indicative of oocyte maturation. It is as such suggested that E_2 at this stage may be involved in oocyte maturation. Inbaraj and Haider (1985) have also found in

Cyprinus Caprio that a small percent of oocytes did undergo GVBD when incubated with E_2.

Pheromones are indeed involved in teleost reproduction, not only by affecting the phase of ovarian development (Van Weerd and Richter, 1991), but also by affecting advanced reproductive phase (Lam *et al.*, 1978; Colombo *et al., 1982; Liley and Stacey*, 1983; Lambert, *et al*, 1986; Stacey *et al., 1986,87; Resink*, 1988 and Stacey, 1989). All teleost sex pheromones are sex hormones or derivatives (Stacey, 1989). In order to synchronize gamete maturation and facilitate spawning, there occurs interaction between sexes in teleosts. Pheromones are part of this interaction (Van Weerd and Richter, 1991). High concentrations of E_2 recorded in May during the present study, may act as releasing pheromones signaling attraction of prospective mates and triggering spawning behaviour and release of gametes. This is in agreement with Van Den Hurk and Lambert (1983) who noticed that releasing pheromones from ovulated female zebrafish (*Brachydanio rerio)* attract male conspecifics and that releasing pheromones of male origin attracted females in the goby (*Gobius jozo). Resink* (1988) noticed that releasing pheromones of male origin attracted ovulated females in *Clarias gariepinus*. Before the act of spawning, E_2 contributes in ovulated egg survival in the abdominal cavity as also reported by Lam *et al.* (1979).

E_2 produced early in the months of June and July during the spent and recovery phase of ovarian development, could act at several levels to favour oogenesis. The *in vivo* positive effect of oestrone on oogonia proliferation has been noticed by Bullough (1942) and Mackay (1973). Involvement of steroids in oogonia proliferation has also been suggested by Khoo (1975). However, such an effect is most probably indirect since E_2 did not stimulate oogenesis in goldfish ovarian explants (Remacle *et al*, 1976).

It is evident that the application of hormone assays especially ovarian steroids viz. , E_2 is a powerful tool when added to other methods of monitoring the sexual condition of schizothoracine fish. This together with accumulating data and improving understanding of other aspects would influence productivity and support future surveillance of fish stock of this group.

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