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OOGENESIS IN A FRESHWATER LARGE MURREL, CHANNA STRIATUS (BLOCH.)

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RESUMO - Em *Channa striatus*, a oogênese foi dividida em três fases: 1) fase primária de crescimento - caracterizada por aumento mitótico do número de oogônias, 2) fase vitelogênica que envolve dois tipos de deposição de vitelo; vesículas vitelínicas (contêm substâncias glicoprotéicas) e glóbulos vitelínicos (contêm substâncias lipoprotéicas) e 3) fase de maturação - inclui a formação de oócitos maduros. O significado funcional de diferentes estágios em outros teleósteos também foi discutido.

ABSTRACT - In *Channa striatus*, oogenesis has been divided into three phases: 1) primary growth phase - characterized by mitotic increase in the number of oogonia, 2) vitellogenic phase involves two types of yolk deposition: yolk vesicles (contains glycoprotein materials) and yolk globules (contains lipoprotein materials) and, 3) maturation phase - includes formation of mature oocytes. The functional significance of different stages described in others teleosts have also been discussed.

INTRODUCTION

The process of oogenesis and the reproductive physiology of female fishes have been well described by many workers (recent reviews by Dodd, 1972; Reinboth, 1972); Donaldson, 1973; de Vlaming, 1974; Fontaine, 1976; Lam *et al.*, 1978; Tokarz, 1978, Billard and Marcel, 1978; Peter and Crim, 1979; Stacey, 1981; Wallace and Selman, 1981). However, informations on the oogenesis of sub-tropical fishes are rather scanty in comparison with that of the temperate fishes. The present study is mainly concerned with the events which occur in the oogonia while it converts into mature oocyte in a freshwater large murrel, *Channa striatus*.

MATERIAL AND METHODS

Ovaries of adult *Channa striatus* were fixed throughout the year in Bouin's solution. After routine embedding in paraffin wax sections were cut at 6 μm and stained with hematoxylin/eosin. To test the chemical nature of yolk vesicles and yolk globules, sections were treated with periodic acid Schiff (PAS), mercuric bromophenol blue (Hg-BPB)and Sudan black B. The ovaries of each month were studied for the histological and cytological details of oocytes. The oocyte measurements were taken with the aid of ocular micrometer and their average values were calculated.

OBSERVATIONS

General morphology, histology and cyclical changes in the ovaries of *Channa striatus* have already been described elsewhere (Srivastava and Swarup, 1979). In *Channa striatus*, during the process of oogenesis three phases have been recognized:

1. Primary growth phase (previtellogenic phase)
2. Vitellogenic phase
3. Maturation phase

1. *Primary growth phase:* It involves two main stages:

a) *Chromatin nucleolar stage:* The oogonia measuring 11-12 μm in diameter are observed embedded in clusters (Fig. 1) in the ovigerous lamellae. An oogonium consists of an eccentrically located large nucleus containing a single basophilic nucleolus and chromatin threads with a little amount of cytoplasm (Fig. 2) This stage passes through pre-synaptic, synaptic and post-synaptic stages. The end of chromatin nucleolar stage is marked by the appearance of small nucleoli variable in number (Fig. 3)

b) *Perinucleolar stage:* Concomitant with oocyte growth, the nucleus increases in size and many nucleoli appear. In the early stage, nucleoli are generally arranged at the periphery of the nuclear membrane although during subsequent developmental stages they may lie randomly within the nucleus ; and chromatin threads are distributed within it. The cytoplasm surrounding the nucleus increases in relative volume and shows marked affinity for hematoxylin (Fig. 4) At this stage, oocytes measure 92 μm in diameter. Later on, the oocyte volume increases and the cytoplasm gradually loses its affinity for hematoxylin. The nucleoplasm contains chromosomes with evenly distributed minute granules. The oocyte at this stage measures 129 μm in diameter. Extruted nucleoli of various sizes and yolk nucleus of Balbiani are also observed in the ooplasm (Fig. 5) In the early phase, they lie close to the nuclear membrane and then move to the periphery of the ooplasm with the growth of the oocytes and finally disintegrate. A thin follicular layer appears around the oocyte.

2. *Vitellogenic phase:* It involves two stages depending upon the types of yolk; yolk vesicle stage and yolk globule sta - ge.

a) *Yolk vesicle stage:* Increase in the size of oocyte (195 µm in diameter) is followed by the appearance of yolk vesi - cles (intra-vesicular yolk) in the peripheral region of the ooplasm. Initially, small vacuoles are observed to develop at the periphery (Fig. 6) Gradually, they increase in size and number and move through the ooplasm towards the nucleus and finally occupy the entire ooplasm (Fig. 7) These vesi - cles exhibit a strong positive reaction to PAS and bromophe nol blue (BPB). At this stage oocytes measure 375 µm in dia- meter. In the beginning of this phase, the nucleus is spheri cal and contains many nucleoli situated on its periphery. In the late phase, the nuclear membrane exhibits irregular out- line. The zona radiata now differentiate between follicular layer and cytoplasm.

b) *Yolk globule stage:* This stage is characterized by the ap pearance of yolk globules in the cortical region of the matu ring oocyte (600 µm in diameter). They appear as small granu les at the periphery of the cytoplasm (Fig. 8) During subse quent stages of development their number increases considera bly and they are distributed throughout the cytoplasm. Later on, the yolk granules accumulate and coalesce into large and dense granules and fuse with the pre-existing vacuoles so that granules and vacuoles predominate in the cytoplasm(Fig. 9) These granules show marked affinity for hematoxylin, Su- dan black B and bromophenol blue (BPB) The oocyte at this stage measures 800 µm in diameter. Simultaneously, with vi - tellogenesis the changes in the nuclei are more pronounced , the nuclear membrane becomes irregular and the number of nu- cleoli is decreased. The oocyte is now surrounded by inner zona radiata, middle follicular layer (zona granulosa) and an outer theca.

3. *Maturation phase:* At this stage the yolk granules fill the entire cytoplasm and the oocyte is heavily impregnated with yolk globules (Fig. 10) The mature oocyte measures 900 µm in diameter. The nucleus of the oocyte becomes eccentric in position with irregular outline and evenly distributed nu cleoli. Such oocytes are ready for subsequent ovulation.

DISCUSSION

According to de Vlaming (1974) "The transformation of oogonia into oocytes is referred to as oogenesis. In annual- ly spawning oviparous teleosts ovarian development consists of several phases. The first phase is a mitotic increase in the number of oogonia. Oogonia transform into primary oocy - tes, and enter into a primary growth phase in which the cyto plasm increases in size. The secondary growth phase of the primary oocyte is characterized by yolk accumulation. Yolk vesicles begin to appear in the periphery of the ooplasm ear ly in this phase and when this phase is completed the oocyte

is filled with yolk globules At the end of secondary growth phase, mature oocytes may be ovulated almost immediately or may remain within the follicle for some time depending on the species"

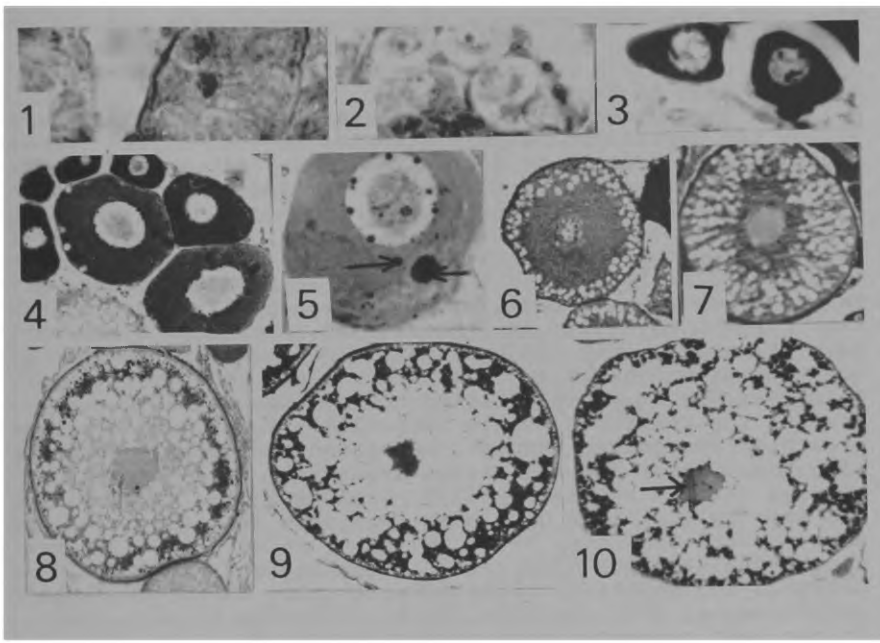
In *Channa striatus*, oogenesis is divided into three phases: 1) primary growth phase which is characterized by mitotic increase in the number of oogonia, 2) vitellogenic phase marked by the appearance of yolk vesicles and yolk globules and, 3) maturation phase includes the formation of mature oocytes.

Contradictory views are on record whether oogonia persists into adult life in all teleost species and form a reserve from which oocytes may be produced. Yamamoto (1962) observed mitotic division of oogonia in *Oryzias latipes* restricted to the spawning period only while Bullough (1942) and Yamazaki (1965) reported its occurrence mainly in the post-spawning season. In *Rhodeus ocellatus* oogonial mitosis continues throughout the year (Yamamoto and Shirai, 1962).In the present study too, the oogonial proliferation was observed throughout the year.

In *Channa striatus* during the period of primary growth phase, the major changes were observed in the cytoplasm and nucleus. The oocyte at this stage has very little cytoplasm surrounding the nucleus which contains a basophilic nucleolus. With the growth of the oocyte, cytoplasm becomes strongly basophilic. Simultaneously, nucleoli also increase in number. Similar changes have been observed by many other workers (Barr, 1963; Guraya, 1965; Guraya *et al.*, 1975) The increase in the number of nucleoli is suggested to be amplification of ribosomal genes (Vincent *et al.*, 1969; Vlad,1976)

In the present study, during the primary growth phase, extruted nucleoli were also observed which finally disintegrate in the ooplasm. There are contradictions about the role of extruted nucleoli. Their vitellogenic role has been suggested by many workers (Hisaoka and Firlit, 1962; Malone and Hisaoka, 1963; Malhotra, 1963). On the other hand, its role in vitellogenesis is obscure (Chaudhry, 1951; Guraya , 1965). According to MacGregor (1972), nucleolar extrusions in the growing oocytes contribute ribosomes to the ooplasm.

The other significant structure observed in the ooplasm during the primary growth phase, is the appearance of a basophilic body "yolk nucleus of Balbiani" which finally disintegrates and disappears in the ooplasm. Similar structure has been described previously by many workers (Guraya , 1963, 1965; Guraya *et al.*, 1975; Srivastava and Swarup,1979; Srivastava, 1980) Histochemical and autoradiographic studies have suggested that yolk nucleus contains ribonucleoprotein particles (Guraya, 1965; Riehl, 1978) Ultrastructural studies have revealed that these bodies may include any combination of mitochondria (especially), the Golgi elements , cisternae of smooth endoplasmic reticulum, multivesicular bodies, and lipid granules depending upon the species (review by Guraya, 1979) There are contradictory statements regarding the role of yolk nucleus. Its vitellogenic role has been ascribed by Chaudhry (1952), Yamamoto (1958) while Wheeler (1924) could not establish its role in vitellogenesis.



Figs. 1-10. Different stages of maturing oocytes in the ovary of *Channa striatus*. Hematoxylin/eosin. Fig. 1 - Clusters of oogonia. X 450 Fig. 2 - Oogonia containing little a - mount of cytoplasm. X 650. Fig. 3 - Oogonia with many nu - cleoli. X 650. Fig. 4 - Perinucleolar stage of oocyte.X 50. Fig. 5 - Yolk nucleus and extruted nucleoli (arrows) X 450 Fig. 6 - Early phase of yolk vesicle stage. X 100. Fig. 7 - Late phase of yolk vesicle stage. X 100. Fig. 8 - Deposi - tion of yolk granules. X 100. Fig. 9 - Oocyte in the advan - ce stages of maturation. X 100. Fig. 10 - Mature oocyte hea vily impregnated with yolk materials and eccentric position of the nucleus (arrow) X 50

Recently Guraya (1979) suggested that it acts as a centre for the formation, multiplication, and accumulation or organelles and materials needed within the oocyte prior to yolk deposition.

The vitellogenic phase is characterized by the appearance of yolk vesicles and yolk globules. In *Channa striatus* yolk vesicles exhibit PAS and bromophenol blue-positive reaction indicating the presence of glycoprotein materials. Other studies have also confirmed the presence of glycoprotein materials in the yolk vesicles (Korfsmeier, 1966; te Heesen and Engels, 1973; te Heesen, 1977; Khoo, 1979)

In the present study, vitellogenic stage is characterized by the deposition of yolk globules in the oocyte. These globules are Sudan black B and bromophenol blue-positive indicating its lipoprotein character. Similar properties have been reported for other fishes (Malone and Hisaoka, 1963; Guraya, 1965; Khoo, 1979; Srivastava, 1980) Different views have been putforth about the formation of these yolk globules. According to Wallace (1903) this is formed without any relationship with formed elements. Raven (1961) suggested that nuclear materials are transformed into yolk. Other workers (Yamamoto, 1955, 1956; Guraya, 1965) attributed the idea that mitochondria and the yolk nucleus give rise to yolk globules. Now it is established that in nonmammalian vertebrates, the principal events responsible for the enormous growth of the oocyte involve the sequestration and packaging of a hepatically derived plasma precursor, vitellogenin, into yolk protein (Wallace, 1978) Transformation of vitellogenin into yolk protein have been confirmed in fishes also by many workers (Wallace, 1978; deVlaming *et al.*, 1977; te Heesen, 1977; Hara and Hirai, 1978; Shackley and King, 1978 ; Campbell and Jalabert, 1979; Idler *et al.*, 1979; Korsgaard and Peterson, 1979; Le Menn, 1979; deVlaming *et al.*, 1980) It has been suggested that teleost pituitary gonadotropins enhance the transfer of vitellogenin from the blood into vitellogenic ovaries or oocytes (Campbell and Idler, 1976; Campbell, 1978; Crim and Idler, 1978; Ng and Idler, 1978)

During vitellogenesis, in most teleosts, yolk proteins accumulate in fluid-filled yolk spheres (Grodzinski, 1954 , 1973) These yolk spheres or yolk globules may either maintain their integrity throughout oocyte growth to form non-continuous mass of yolk (Yamamoto, 1957) or fuse centripetally eventually to form a continuous mass of fluid yolk. In *Channa striatus* yolk globules form a non-continuous mass of yolk.

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