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# HISTOLOGICAL AND CYTOLOGICAL STUDIES ON OOCYTE DEVELOPMENT OF THE LARVIVOROUS FISH, *APLOCHEILUS PANCHAX* HAMILTON AND BUCHANAN

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Histological and cytological observations of oocyte development in the lesser top minnow, *Aplocheilus panchax* were described. Various stages of developing oocytes of *A. panchax* also shown. The development of oocytes were classified into six stages according to the histological characters. First growth phase with two stages and three stages in second growth phase. Yolk bodies make their appearance from the beginning of second growth phase. Histochemical studies show that three types of yolk granules are found during vitellogenesis. Lipid yolk found as lipid doplets followed by protein yolk granules. the third type of inclusions are the intravesicular yolk, carbohydrate yolk which form the peripheral cytoplasm after the lipid prrotein yolk started to accumulate.

## INTRODUCTION

*Aplocheilus panchax* belongs to the family Cyprinodontidae. As early as 1972 Gunther while working on freshwater fishes of the world made a statement that the toothed carps of the genus *Aplocheilus* are mainly distributed through southern Asia and Indo-Australian Archipelago. In Indian continent *Aplocheilus* is of great interest in malaria control as it chiefly feeds on mosquito larvae. This fish can be easily reared in marsh conditions and exhibits rapid growth reaching a maximum length of 5 cm.

The present paper aims to describe the histological picture of the oocyte development in *A. panchax*. Raven (1961) used the term oogenesis for the entire growth phase from oogenesis to maturation. Similar histological studies on the development of oocytes in capelin *Mallosus villosus villosus* (Forberg, 1982) and bass *Dicentrarchus labrax* (Mayer *et al.*, 1988) are of great interest. It is a well established fact that the oocyte growth in fishes is categorised into two phases. first growth phase (FGP) includes two stages and second growth phase (SGP) three stages. Later phase involves deposition of yolk and is characterised by rapid oocyte growth.

## MATERIALS AND METHODS

*A. panchax* were obtained from freshwater streams in and around Visakhapatnam. After gross examination of the ovary the pair was fixed in Susa, formol-calcium and Carnoy fluid. The tissue was processed and 8-10 μ thick sections were cut and processed for histological and histochemical studies. All histochemical techniques used were adopted mostly from Pearse (1968) and Bancroft & Stevenson (1975).

Oocyte dimensions were taken with an ocular micrometer using fixed preparation. The oocyte diameter was taken as the greater length in the horizontal plane. The dimensions given are the average of 20-50 samples of stages of oocytes. Terminology used in the histological description of the oocyte stages is that used by Forberg (1982), and Mayer *et al.* (1988) previously adopted from Yamamoto (1956).

## RESULTS

### Histology of oocyte development

*A. panchax* shows the group synchronism type of oocyte development where the groups of oocyte grow side by side. The developmental phases were described and reviewed with reference to the literature to other vertebrates especially for the teleostean fishes.

First growth phase

*Stage I* (Fig. 1) : The oocytes at first stage are young and range between 50 to 90 μm in diameter. The oocyte is irregular in form contains a round or slightly oval nucleus and appears occupying almost the entire cell. The smallest cells of this stage contain a darkly staining nucleolus within the nucleus. In the later phases when the oocytes have increased in their size to 75 μm many rounded nucleoli are seen near the periphery of the nucleus inner to the nuclear membrane in the form of a ring. The oocyte is covered with by a very thin membrane limiting the cytoplasm. The cytoplasm of the oocytes stained red with Heidenhain's Azan and hematoxylin whereas nuccleoplasm stained faintly.

*Stage II* : The diameter of the oocytes in this stage ranges from 120 to 180 μm. This stage is characterised by a clear zonation of cytoplasm (outer light and inner dense region) and by the appearance of small vacuoles located in the circular band of the peripheral cytoplasm. The oocyte membrane becomes somewhat thicker and there is layer of flattened cells, the follicle cells. The nucleus becomes oval in shape and nucleoli which are arranged as a ring near the nuclear membrane increase in number and come close to the membrane.

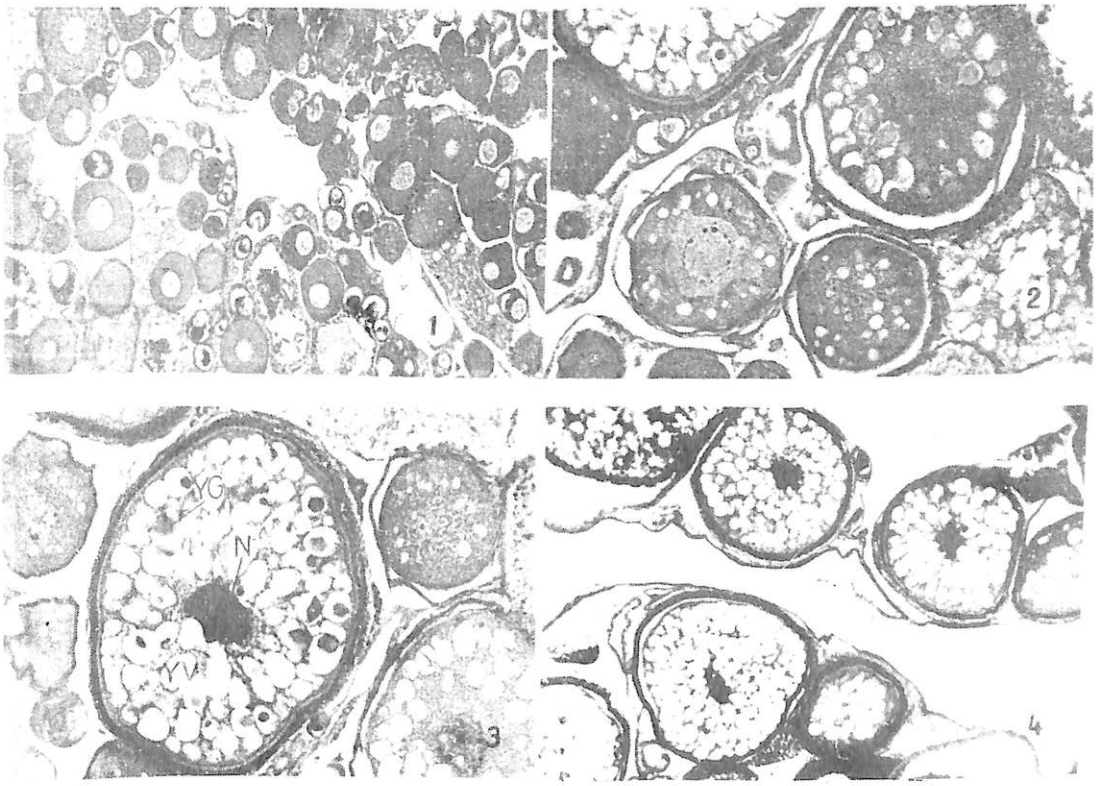
Second growth phase

*Stage III* (Fig. 2) : The diameter of the oocytes in this stage ranged from 250 to 350 μm. This is characterised primarily by the appearance of yolk globules and also by considerable change in the membrane, cytoplasm as well as the nucleus. Yolk vesicles make their appearance first in the peripheral vacuoles of the cytoplasm rather than towards centre. The oocyte membranes are distinctly seen. The cytoplasm of cells stains freely with Azan. Inner to this is a fibrillar layer, the zona radiata. this appears as homogeneous layer in the beginning but gradually becomes thick and stains darkly called the true egg membrane and contains large number of closely packed striations to give it a tough consistency. The follicular epithelim is closely apposed to this zona radiata and hence it can be presumed that the oocyte gains nutrients from the follicle cells. The number of nuclei decreases and the existing ones become larger in size. The nuclear membrane becomes thin and irregular, stains intensely with hematoxylin. The nucleus with its organelles after the stage of yolk formation in the oocyte appears as the germinal vesicle.

*Stage IV* (Fig. 3) : The eggs of this stage are considerably larger in size ranging from 390 to 550 μm. The most important event of this stage is the appearance of micropylar cells. Three to four cells of the follicular epithelium lying dorsal to the germinal vesicle get enlarged and appear differently. The cytoplasm of all the cells is thrown into follicular strands which appear in the

section as a stringed plug. The cytoplasm also showed marked increase in the amount of yolk with the granular staining much more densely than those of the previous stages. The increase in number and form a 2 to 3 layered membrane. The radial striations appear in the zona radiata. The true vitelline membrane is present inner to the zona radiata as a thin band like granular layer. The germinal vesicle moves towards one pole and reach the micropyle. The germinal vesicle and especially the spherical nucleoli stain darkly red with hematoxylin.

*Stage V* (Fig. 4) : The eggs of this stage measure on an average 600 μm. This is the stage ready to resorb or spawn. Just after attaining this stage some atretic follicles were noticed in sections. the germinal vesicle on reaching the animal pole loses its definite shape and nucleoli gradually disappear. The yolk vesicles decrease in size and are finally seen as very small spherules. During maturation of oocytes some of the follicle cells undergo resorption. When resorption begins the cytoplasm starts disintegrating although some of the yolk globules make
their appearance.




**Figs. 1-4.** T.S. of the ovary of *A. panchax*; **1.** Showing developing oocytes; **2.** Fully mature oocytes; **3.** Migratory nucleus; **4.** Ovary stained (PAS/Light green).

As atresia proceeds, the yolk granules disintegrate and appear as small dispersed particles within the degeneration follicle. A number of empty vacuoles appear in distorted follicle. The follicular cells get distorted and loose their cell boundaries. The zona radiata is deflected at some

laces for easy resorption. Numerous blood cells are seen in the cytoplasm. A large number of empty follicles are seen in a spent ovary. The follicle loses its shape and the cells may transform into interstitial cells.

**Histochemistry and oocyte development**

*Carbohydrates* (Fig. 5) : The only carbohydrate stain that gave positive result with PAS. The early oocytes show PAS positive substance which is slightly resistant to diastase digestion and is blocked by acetylation. Acid mucosubstances are present as evidenced by alcian blue reaction. All the stages of oocyte constituents gave a PAS poistive reaction. The oocyte layers which form in the later stages also gave positive reaction to PAS. So the presence of carbohydrates were found to be confirmed.

*Proteins* (Fig. 6) : The primary oocytes, the cytoplasm as well as the nucleoli stained strongly for proteins. Basic proteins are present in the cytoplasm as evidenced by bromophenol blue technique noticed in all the stages of the oocytes. With bromophenol blue the nucleus stained feebly but the nucleoli stain distinctly. The egg membrane reacts positively for protein and it is for this reason they have been previously termed as protein yolk granules. The oocytes of all the stages show positive reaction to all the protein tests showing the presence of proteins, glycoprotein, sulfhydril and disulphide groups. Same way the oocyte membranes show more intense positive reaction to all the tests given in Table I.

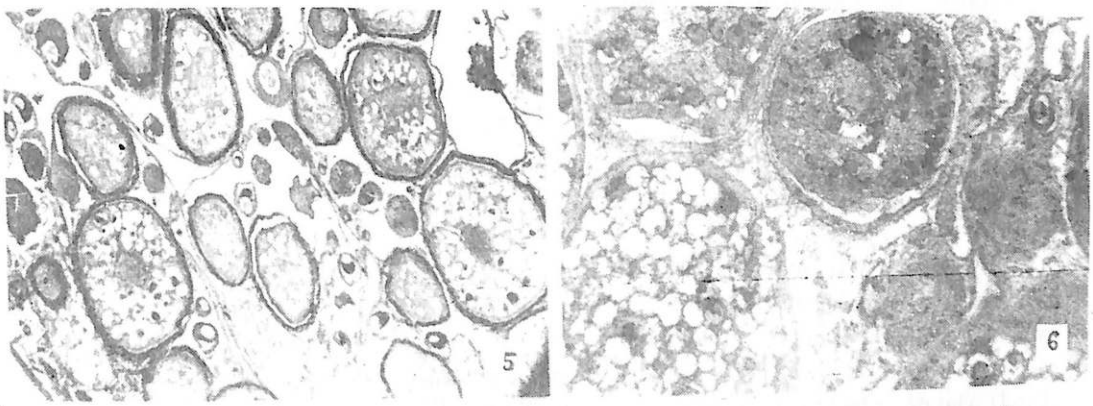
**Table I** : Cytochemistry of oocytes (I to V stages).

| S.No. | Histochemical test applied | Cytoplasm & oocyte layers | Yolk granules | Nucleus |
|---|---|---|---|---|
| 1. | Periodic acid/Schiff (PAS) | +++ | ++ | + |
| 2. | PAS light green | Magenta +++ | Magenta ++ few more green ++ | Green ++ |
| 3. | PAS/Saliva | + | ++ | + |
| 4. | Acetylation/PAS | - | - | - |
| 5. | Deacetylation/PAS | +++ | ++ | + |
| 6. | Alcian blue (AB) pH 1.0 | - | + | - |
| 7. | AB (pH 2.0) | - | + | - |
| 8. | Bromophenol blue | +++ | +++ | +++ |
| 9. | Millon's reaction | + | + | + |
| 10. | p-DMAB-nitrite | + | + | + |
| 11. | $KMnO_4$/AB | + | +++ | ++ |
| 12. | Ferric ferricyanide | ++ | +++ | +++ |
| 13. | Ninhydrin/Schiff | +++ | ++ | + |
| 14. | Congo red | +++ | ++ | +++ |
| 15. | Sudan black B | ++ | +++ | - |
| 16. | Copper phthalocyanin | ++ | +++ | - |
| 17. | Pyronin Y | +++ | ++ | - |
| 18. | Feulgen reaction | + Nuclic of follical cells | - | - |

+++ = Intensely positive; ++ = Moderately positive; + = Slightly positive; - = Negative.

*Lipids* : The primary oocytes were positive to lipid techniques to show the presence of lipids and phospholipids. With Sudan black B yolk granules stained intensely and the cytoplasm moderately. With copper phthalocyanin the yolk vesicles showed a moderate reaction. The SGPoocytes stained intensely for Sudan black B and copper phthalocyanin. Hence, the yolk vesicles are temed lipid droplets.

In all the stages the nucleus and germinal vesicle of the oocyte is Feulgen negative whereas the nucleoli stain darkly to all the protein tests. The nucleoli of the follicular epithelial cells are not only Feulgen positive but contain cytoplasmic RNA.



**Figs. 5-6**. T.S. of young ovary of *A. panchax showing*; **5**. Ovary (Bromophenol blue); **6**. Ovary (Sudan black B).

In all the stages the nucleus and germinal vesicle of the oocyte is Feulgen negative whereas the nucleoli stain darkly to all the protein tests. The nucleoli of the follicular epithelial cells are not only Feulgen positive but contain cytoplasmic RNA.

## DISCUSSION

Histological studies on oocyte development in *A. panchax* reveal a series of clear cut developmental stages. These different stages are in clear agreement with those described by Yamamoto (1956), Forberg (1982) and Mayer *et al.* (1988).

Yolk formation is of two types *i.e.* massed and non-massed in teleostean eggs. In *A. panchax* yolk deposition is initiated as minute granule appearing at the periphery in the extra vesicular cytoplasm and gradually increase in number as well as size and get confined to the peripheral zone (non-massed). Early reports of the type are those of Mas (1952) in Perca & Yamamoto (1958) in Herring.

In *A. panchax* chromatin particles form the nucleus. As growth advances the nuclei increase in number and get arranged towards the periphery. According to Yamamoto (1952) and Bara (1960) the nucleoli are formed from the chromatin particles present in nucleoplasm. The egg membrane is differently termed by different suthors but the most appropriate term 'zona radiata' as suggested by Yamamoto (1958) and Rai (1967) has been used in the present study. The formation of micropyle is common in most of the teleosts in the ripe oocyte at the animal pole. In

*A. panchax* the micropyle formed enlarges the impenges on the zona radiata as plug. This condition is in agreement with that described by Aravindan & Padmanabhan (1972).Histochemical as well as histological studies reveal the presence of 3 types of inclusion during vitellogenesis. First type of yolk inclusion is lipid yolk. this is in the form of lipid droplets. This marks the start of endogenous vitellogenesis. it is now generally accepted that lipid yolk formation is followed be protein yolk formation (exogenous) (Weigard, 1982). Generally in teleosts lipid yolkdroplets are supposed to arise in perinucleolar cytoplasm (Shackley & King, 1977; Weegard, 1982). Contrary to this lipid inclusions are first seen in the outer cortex.

Protein yolk formation is concomitant with lipid yolk formation. protein yolk granules become the predominent yolk inclusion and are densely packed in the cytoplasm in *A. panchax*. A similar observation was made by Mayer *et al.* (1988). The third type of inclusion are the carbohydrates. Histochemical techniques applied have shown that carohydrate inclusion appear after lipid protein yolk formations have started. Mayer *et al.* (1988) have made a similar observation in *D. labrax*. In some teleosts carbohydrate yolk formation occurs prior to both lipid yolk and protein formation (Khoo, 1979; Wallace & Selman, 1981; de Vlaming, 1983).

The lipid globules and the carohydrate protein granules filling the entire ooplasm were also observed by Verma (19770 in *Gambbusia affinis*. The origin of carbohydrate yolk from the follicular epithelium and the protein yolk from RNA (from cytoplasm and from extrusion of the nuclear sap) noticed in the present study is in agreement with the findings of Malone & Hisaoka (1963) who described the participation of nucleoplasmic RNA in protein yolk formation in zebra fish.

The germinal vesicle of *A. panchax* is Feulgen negative. This has been reported by Marya *et al.* (1937) in Fendulus & Verma (19770 in *Gambusia affinis*.

Verma (1977) reported in the oocyte layers of G. affinis intense positivity of carbohydrates and proteins rich in RNA and DNA. He also accepts the origin of extra-oocytal yolk from follicular layer. Therefore, the intensity of staining declines bythe end of vitellogenesis. The present finding are also in agreement with the above authors.

Before resorption stage the lipid yolk combines with protein and carbohydrate yolk forms a homogeneous mass of yolk in *A. panchax*. This condition is also observed in the eggs of *Liopsetta* sp. described by Yamamoto (1956).

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