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Oogenesis in a tropical loach *Lepidocephalus thermalis* (Cuv. & Val.)

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Abstract. The development of oocytes has been traced in detail in the loach *Lepidocephalus thermalis*. The various stages of oocytes are described under distinctive stages namely 1. chromatin nucleolus stage; 2. perinucleolus stage; 3. yolk vesicle stage; 4. primary yolk stage; 5. secondary yolk stage; 6. tertiary yolk stage; 7. migratory nucleus stage; 8. pre-ripening stage; 9. ripe egg stage. The yolk deposition is initiated as minute globules in the extravesicular ooplasm. A yolk nucleus is entirely wanting in any stage of oocyte maturation. The origin of new crops of oocytes and the formation of the micropyle are described. Nature of degeneration of unextruded ripe oocytes has been followed.

Keywords. *Lepidocephalus thermalis* (Cuv. and Val.); oogenesis; maturing stages of oocytes; yolk formation; germinal vesicle; micropyle.

1. Introduction

Lepidocephalus thermalis, a cobitid fish, inhabiting a wide variety of habitats such as annual and perennial ponds, shallow channels and conduits, paddy fields which are subject to inundation during monsoon, shallow edges of streams and rivers where the currents are not strong, is the commonly occurring loach of the Kerala State, lying in the extreme southwest of Peninsular India. Though the occurrence of this species has been reported, information on functional morphology, biology, etc., are wanting in literature. The present study dealing with the development of oocytes in the ovary of the species forms part of a detailed investigation on this species.

2. Materials and methods

Ovaries in different stages of development were collected and fixed in aqueous Bouin's fluid. Paraffin sections were cut at 6–10 μ and stained in Heidenhain's iron haematoxylin or Heidenhains azan using eosin as counter stain.

3. Observations

The ovary is an unpaired and mesially fused structure (figure 1) with a mid-dorsal groove lying in the body cavity attached by mesovarium. The posterior region of the ovary is drawn backwards into a single, short oviduct which opens to the exterior just behind the anus. A fully mature ovary of a 56 mm fish measures about 24 mm in length and is dirty brown in colour. The mature ovary fills the entire available space of the body cavity bordered anteriorly by the liver and dorsally by the kidneys. The surface of the ovary is supplied with numerous blood vessels.

The wall of the ovary consists of three definite layers; a thin and transparent outer peritoneum, a thick tunica albuginea in the middle and germinal epithelium beneath to form the inner most layer (figure 2). The comparatively thick tunica albuginea is richly supplied with numerous blood vessels. The germinal epithelium is thrown into a number of folds and the oogonia are seen budding off singly inside these folds. Based on the various histological changes taking place in the ovary as it advances to maturity, the following stages are distinguished. The terminology adopted by Yamamoto and Yamazaki (1961) in general and Rai (1967) in particular is followed here.

3.1. *Chromatin nucleolus stage*

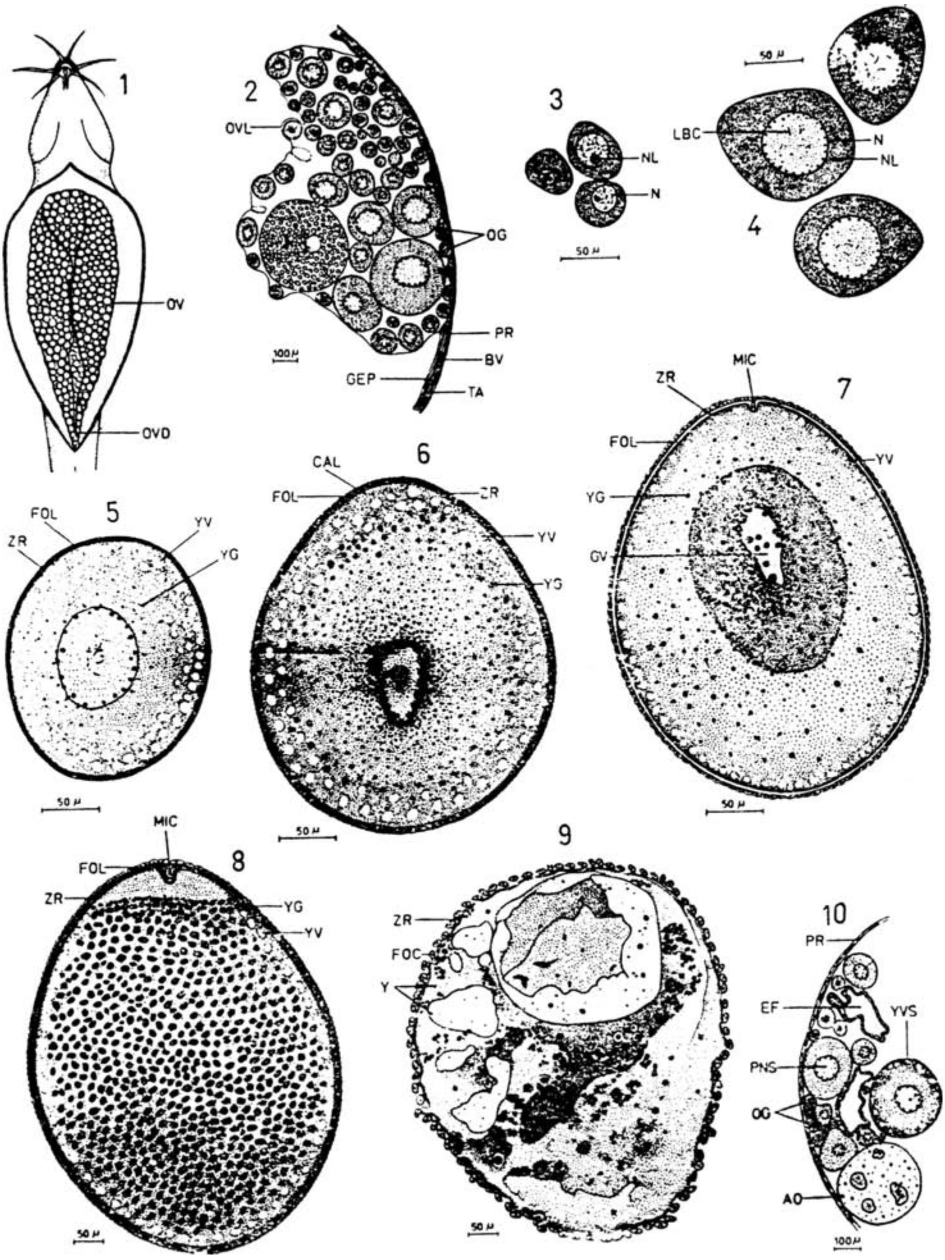
The oogonia, seen close to the germinal epithelium, are usually spherical in shape with a large nucleus in the centre. The nucleus is filled with reticulated chromatin granules with a single nucleolus (figures 3 and 11). The cytoplasm forms a thin sheet around the nucleus. An oogonium measures about 35–40 μ in diameter.

3.2. *Perinucleolus stage*

This stage is marked by the increase in number of the nucleoli. The single nucleolus present in the earlier stage divides to give rise to a number of nucleoli which gradually move towards the periphery of the nucleus (figure 4). During the later phase of this stage, numerous nucleoli are seen arranged in the periphery of the nucleus. The cytoplasm shows more affinity towards basic dyes revealing the beginning of the formation of yolk. An oocyte measures about 100–125 μ in diameter. An oocyte normally possesses 24–27 nucleoli. The nuclear membrane is slightly wavy during this stage. The yolk nucleus reported in many teleost fishes is absent in this species. The lamp-brush chromosomes characteristic of this stage in teleost oocyte were clearly noticed in the centre of the nucleus. The peculiar zonation in the cytoplasm of oocytes in this stage noticed in some fishes is not visible in this fish.

3.3. *Yolk vesicle stage*

Even in the late phase of perinucleolus stage, yolk vesicles make their appearance in the peripheral region of the oocyte. In the yolk vesicle stage, these vesicles increase in number and size and are seen even in the inner regions of the cytoplasm. The egg membrane or the zona radiata develops from the peripheral zone



Figures 1–10. **1**. Ventral side of the body opened to show the position and nature of a mature oocyte. **2**. T.S. of ovary showing the ovarian wall and the oocytes of different stages of growth. **3**. Chromatin nucleolus stage. **4**. Perinucleolus stage. **5**. Yolk vesicle stage. **6**. Primary yolk stage. **7**. Secondary yolk stage. **8**. Ripe egg stage. **9** Degenerating oocyte. **10**. Section through partially spent ovary.

(Key to lettering in figures in p. 49)

of cytoplasm. The lamp-brush chromosomes which made their appearance in the perinucleolar stage, gradually disappear in this stage. The follicular layer surrounding the oocyte consists of a single layer of small flattened cells. Small yolk globules start appearing around the nucleus and they gradually increase in number. An oocyte at this stage measures 175–200 μ in diameter (figures 5 and 11).

3.4. *Primary yolk stage*

The actual process of yolk deposition starts in this stage. The number and size of yolk globules increase and they gradually push the yolk vesicles to the periphery of the oocyte (figure 6). In the final phase of this stage, on account of the pressure exerted by the yolk globules, the yolk vesicles become confined only to the peripheral region. These yolk vesicles together with the extra vesicular cytoplasm form the cortical alveoli. However it is noticed that the yolk vesicles are devoid of any yolky material.

The nuclear membrane gradually disappears and the nucleus looses its spherical shape. An oocyte in this stage measures about 300–325 μ in diameter.

3.5. *Secondary yolk stage*

The yolk globules increase more and more in number and become crowded together around the nucleus (figure 7). The size of the nucleus becomes greatly reduced. A micropylar cell makes its appearance in one pole of the oocyte. One of the follicle cells enlarge in size and push the zona radiata in that region inwards. A depression in this membrane is thus established which represents the micropyle. The cortical alveoli gradually get reduced in size evidently owing to pressure of the yolk globules. An oocyte in this stage measures about 400 μ in diameter.

3.6. *Tertiary yolk stage*

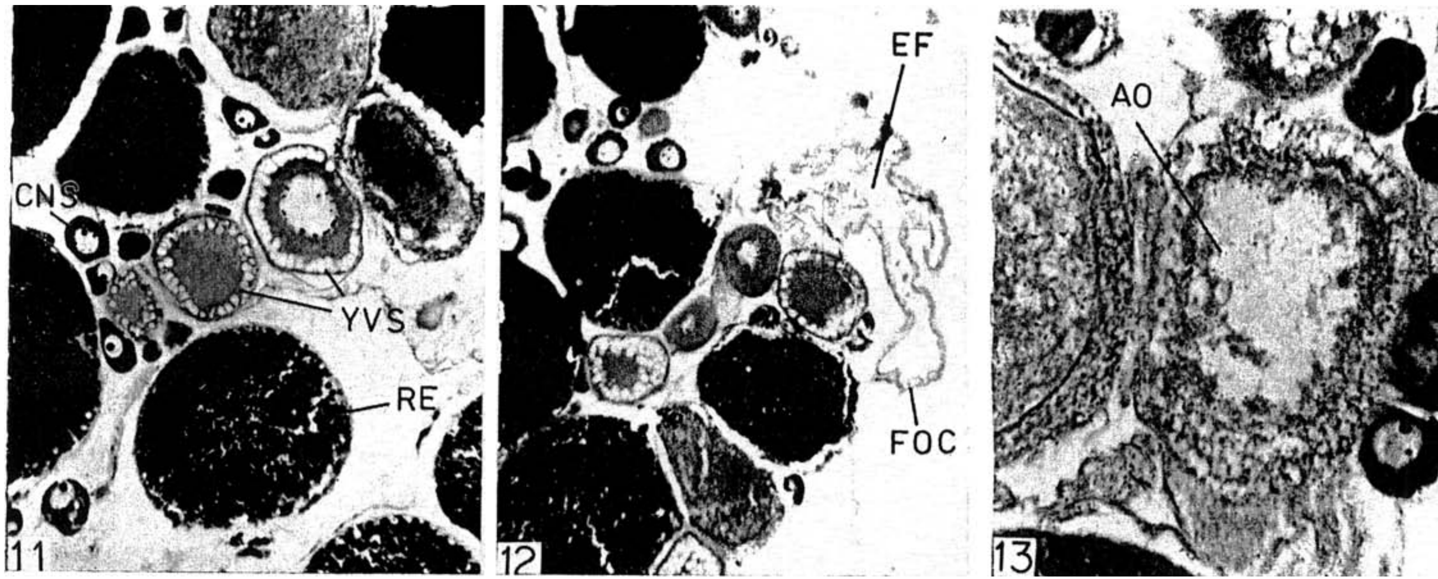
The spherical yolk globules increase in number. The cortical alveoli now consist of a very thin layer of weakly staining cytoplasm just below the zona radiata. The follicle layer becomes more pronounced with larger follicle cells. The nucleus shows signs of a shift towards the region of the micropyle.

3.7. *Migratory nucleus stage*

The nucleus or the germinal vesicle gradually moves towards the region of the micropyle. The nuclear membrane is not distinguishable and the nucleoli are now few in number. In the final phase of this stage, the nucleus reaches just below the micropyle. An oocyte in this stage measures on an average 500 μ in diameter.

3.8. *Pre-ripening stage*

As the nucleus reaches the animal pole, the nucleoli disappear completely and the nucleus looses its shape. Chromatin elements become thick and round and are found distributed in the cytonucleoplasmic mass. The micropylar opening is clearly visible in this stage. The whole oocyte is filled with dense staining and compactly packed yolk globules.



Figures 11–13. **11.** Section of ovary showing oocytes in the different stages of growth. × 88. **12.** Section of partially spent ovary showing an empty follicle. × 88. **13.** Section of spent ovary showing atretic oocytes. × 200.

Key to lettering in figures

AO, atretic oocyte; BV, blood vessel; CAL, cortical alveoli; CNS, chromatin nucleolus stage; EF, empty follicle; FOC, follicle cell; FOL, follicular layer; GEP, germinal epithelium; GV, germinal vesicle; LBC, lamp-brush chromosome; MIC, micropylar cell; N, nucleus; NL, nucleolus; OG, oogonia; OV, ovary; OVD, oviduct; OVL, ovarian lamella; PNS, perinucleolus stage; PR, peritoneum; RE, ripe egg; TA, tunica albuginea; Y, yolk; YG, yolk globule; YV, yolk vesicle; YVS, yolk vesicle stage; ZR, zona radiata.

3.9. *Ripe egg stage*

The ripe egg of *L. thermalis* is reddish yellow in colour and spherical in shape and translucent with the micropyle at the animal pole. The egg membrane or zona radiata is thick with minute radial canals. The follicle cells become extented. The average diameter of fully ripe intraovarian egg is $600\,\mu$ (figures 8 and 11).

3.10. *Resorption of the unspawned ova and empty follicles*

A few mature unspawned ova, seen in post-spawned ovary, undergo atresia and are eventually resorbed. Immature and maturing ova present in the spent ovaries do not show any signs of atresia. The first sign of atresia is shown by the liquifaction of the yolk (figures 9 and 13). Due to the liquifaction of yolk, innumerable minute ovoid bodies and spherical globules appear inside the ova. The zona radiata looses its contour and becomes wavy in appearance invaginating into the egg proper. Gradually this membrane breaks at a number of points. Through these points, a number of follicle cells enter into the liquified mass. The follicle cells which are small and weakly staining in a ripe egg become considerably larger in size and stain deep dark in atretic oocytes. The affinity of yolk to basic dyes gradually looses and after some time the yolk rarely gets stained. A number of minute blood vessels appear around these atretic oocytes and blood cells are seen all round the follicle layer. Gradually the follicle cells loose their cell membrane and get dissolved in the liquified yolk mass. The number of blood cells increase and gradually the liquified yolk matter is resorbed. The empty follicles also consist of a bag-like structure formed of a single layer of weak staining cells (figure 12). The central portion forms an empty cavity. The cells loose their cell boundaries and eventually some of them get dissolved.

3.11. *Origin of a new crop of oocytes*

The inner-most layer of the thick ovarian wall, the germinal epithelium, is thrown into numerous folds. In immature ovaries, a careful study of serial cross-sections of the ovary reveals that the folds of the germinal epithelium contain a number of small oogonia. These oogonia are seen throughout the ovarian wall. As maturation of the ovary continues, these oogonia are seen to grow and migrate towards the centre of the ovocoel. Even after spawning, the ovary contains some unspawned ripe eggs and a number of oocytes in different stages of maturity (figure 10). Ripe unspawned eggs alone undergo atresia and the remaining oocytes develop to give a fresh supply for the next spawning. During the reorganising phase, the thick germinal epithelium is found to contain a number of minute oogonia showing mitotic divisions. These again transform into oocytes and aid to replace those which have been extruded during spawning.

4. Discussion

4.1. *Formation of yolk*

The yolk deposition starts in *L. thermalis* as minute globules in the extravesicular ooplasm. They first appear in the periphery in the yolk vesicle stage and move towards the centre. When the oocyte reaches the secondary yolk stage, these globules increase in number and size and become confined to the peripheral zone owing to the pressure exerted by the yolk. This layer of yolk vesicles with the extravesicular cytoplasm seen between the zona radiata and the yolk globules is termed the cortical alveoli. As growth and maturation of the oocyte continues, the yolk globules also increase in number finally filling the whole of the oocyte leaving a very thin layer of cortical alveoli. Thus in *L. thermalis* the yolk is of the non-massed type and the formation of the yolk is similar to that reported by Konopacka (1935) in *Cyprinus* and *Gobio*, Narin (1937) in *Saccobranchus*, and Mas (1952) in *Perca*.

4.2. *Germinal vesicle*

The nucleus of the oocyte which after maturation is termed the germinal vesicle is the most vital organelle. In the chromatin nucleolus stage, the nucleus constitutes the major portion of the oocyte. The chromatin matter presents the form of a lightly staining reticulated mass. In this network few scattered chromatin particles are also seen in addition to a single deep staining nucleolus. As the oocyte grows, the reticulated appearance of the nucleus gradually diminishes and it becomes filled with evenly staining nucleoplasm possessing a single nucleolus. When the oocyte reaches the perinucleolus stage, the nucleus assumes a spherical homogenous mass in the centre with a thin nuclear membrane. A number of nucleoli are seen arranged in the periphery of the nucleus inner to the nuclear membrane.

According to Chaudhary (1952) and Mas (1952), in the origin of the nucleoli, the single nucleolus seen in the early phase divides into numerous fragments and each fragment becomes a nucleolus. Yamamoto (1956) and Bara (1960) are of the view that these nucleoli are formed from the chromatin particles present in the reticulam. No division of the nucleolus was visible in the present study, but the chromatin particles were seen fusing together resulting in the formation of these nucleoli. The "lamp-brush" chromosomes characteristic of teleostean and amphibian oocytes appear in the nucleoplasm in the perinucleolus stage of *L. thermalis* oocytes. In the primary yolk stage, these chromosomes disappear from the nucleoplasm.

The nuclear membrane is very thin and surrounds the nucleus. In the early stages, this layer is even, but in the perinucleolar stage, this membrane becomes wavy in many parts. In the preceding growth stages as the germinal vesicle shifts to the animal pole, this membrane becomes inconspicuous and the germinal vesicle looses its spherical shape. The nucleoli decrease in number and in the ripe egg stage they become completely absent.

4.3. *Formation of the egg membrane*

Regarding the formation of the zona radiata (term used for egg membrane) different views exist. Waldayer (1870) thought that egg membrane is a product of the follicular epithelium. Cunningham (1898) and Padmanabhan (1955) have shown that the zona radiata is produced by the thin layer of cortical layer of protoplasm. In the present case a thin cortical layer of cytoplasm is seen in the periphery of the oocyte just beneath the follicular layer and this thickens gradually and forms a deep-staining homogeneous layer around the oocyte. Minute radial canals can be seen in this layer under high magnification. The follicular layer plays no part in the formation of the zona radiata.

4.4. *Formation of the micropyle*

The micropyle owes its origin to the follicular cells (Eigenmann 1890; Yamamoto 1963; Aravindan and Padmanabhan 1972). In *L. thermalis* a single follicle cell in the region of the animal pole gets differentiated into a large cell which helps in the formation of the micropyle as in *Macropodus cupanus* (Padmanabhan 1955) and *Etroplus suratensis* (Rita and Padmanabhan 1976) unlike in forms like *Leucius* (Hoffmann 1881) and *Stigmatogobius javanicus* (Aravindan and Padmanabhan 1972) where more cells are involved.

4.5. *Yolk nucleus*

The presence of a true yolk nucleus in the oocytes of fishes has been reported among others by Rai (1967), Bhargava and Saxena (1971), Sobhana and Nair (1977). However in *L. thermalis* the yolk nucleus was not visible in any stage of oocyte maturation.

4.6. *Resorption of unspawned oocytes*

The process of resorption of unspawned oocytes is somewhat similar to that described in the case of *Mystus seenghala* by Dixit (1956).

4.7. *Source of a new crop of oocytes*

In *L. thermalis* the germinal epithelium is the main source for a new crop of oocytes as in *Gobius giuris* (Sreeramulu and Rajalakshmi 1968) and in *Tilapia mossambica* (Aravindan and Padmanabhan 1972) though occasionally ruptured follicular cells also contribute to their formation.

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