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Source of a new crop of oocytes in *Tilapia mossambica*

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EXTRAIT: **Origine des poussées ovocytaires chez *Tilapia mossambica*.** L'ovaire de *Tilapia mossambica* a été étudié dans le but de préciser l'origine des poussées ovocytaires. Après la ponte l'ovaire n'est pas vide pour autant, et il y subsiste des ovocytes à tous les stades du développement. Les replis de l'épithélium germinatif et les tractus épithéliaux ramifiés contiennent des flots de cellules qui produisent des ovogonies primaires, lesquelles se transforment en ovocytes et en cellules folliculaires. Il ne semble pas qu'une nouvelle poussée ovocytaire puisse se faire à partir des résidus des follicules des ovocytes atrétiques. Seuls les ovules mûrs non évacués par la ponte subissent une atrésie et se résorbent; les ovocytes immatures ou en voie de croissance ne dégénèrent pas. L'asynchronisme de *T. mossambica* se traduit par la présence simultanée, dans l'ovaire, d'ovocytes à tous les stades de croissance et de maturation.

INTRODUCTION

The source and origin of a new crop of oocytes in teleosts has been studied by a number of investigators, and different opinions have been put forward by them. CALDERWOOD (1892) assumes that the new crop of oocytes arises by a process of nuclear coalescence in a nest of ovarian epithelial cells. According to WHEELER (1924) and YAMAMOTO (1956), new oocytes arise from cells of the ruptured follicles of the preceding crop. HONMA (1961), YAMAMOTO & SHIRAI (1962) consider that the residual oogonia, available in a spent ovary, give rise to a fresh crop of oocytes. MENDOZA (1943), working on the viviparous teleost *Neotoca bilineata*, came to the conclusion that epithelial cells of the ovigerous fold are the seat of oocyte formation. BULLOUGH (1942) and YAMAMOTO (1962) state that the germinal epithelial cells give rise by proliferation to fresh oocytes. SREERAMULU & RAJALAKSHMI (1965) are also of the opinion that the enlarged germinal epithelium proliferates the primary oogonia. Recently, DUTT & GOVINDAN (1968) have argued that in *Anabas scandens* new crops of oocytes arise from the germinal epithelium. In addition, some oogonial cells are differentiated occasionally from the remnants of the follicle cells of the pre-ovulatory corpus luteum.

In the present investigation an attempt has been made to elucidate the source of fresh crops of oocytes in *Tilapia mossambica* (PETERS), a cichlid fish which has been introduced into freshwater bodies of India from Thailand.

MATERIAL

Tilapia mossambica (PETERS) is a tropical fish which has remarkably acclimatized itself freshwater ponds and reservoirs of Kerala. The fish breeds throughout the year both under natural conditions and also in the laboratory. Even though it is a perennial spawner it has been noticed that during the rainy months there is an appreciable increase in the frequency of spawning. For the present study fish were collected from local tanks and fresh pieces of the ovary were fixed in BOUIN's fluid. Care was taken to select immature, maturing, fully-mature, post spawned and reorganising ovaries so as to get a complete picture of the growth, formation, liberation and subsequent origin of oocytes. Sections of 8 μm in thickness were stained with HEIDENHAIN's iron haematoxylin and counter stained with eosin.

RESULTS

The ovary of *Tilapia mossambica* is an unpaired structure but bilobed in its anterior part with a midventral groove which demarcates the organ into left and right halves. It is attached to the dorsal body wall by the mesovarium. The wall of the ovary is composed of three layers. The outermost layer is the peritoneum, which is transparent and thin and is so intimately apposed to the basal layers that sometimes it becomes difficult to recognise its identity. Beneath the peritoneal layer is a fairly thick layer of mesenchymal connective tissue known as tunica albuginea, made up of muscle fibres with a copious supply of blood vessels. Inner to the tunica albuginea lies the germinal epithelium composed of ellipsoidal cells with meagre cytoplasm and comparatively large and deeply staining spherical nuclei. The germinal epithelium in some regions is found to have lost its contact with the tunica albuginea and abuts into the ovocoel in the form of lamellae. The lamellae are not of the same size and shape during the different stages of ovarian development. The oocytes are found to take their origin from clusters of cells within the ovigerous lamellae. In addition to this, the germinal epithelium proliferates cells which arrange themselves into irregular strands ramifying into the ovocoel (Fig. 1a). The cells from these strands also give rise to oogonial cells as well as follicle cells.

For a complete picture of the origin of the new crop of oocytes in the ovary an essential prerequisite is a knowledge of the various changes occurring in the ovary.

In a fully mature *Tilapia mossambica* the germinal epithelium is comparatively thin and the ripening oocytes get so compactly packed that the ovocoel and the ovigerous lamellae are obliterated. Since the fish is a perennial breeder, in a ripe ovary oocytes in various stages of growth can be seen and these oocytes can be grouped into three categories namely (1) those which are ready to be spawned, (2) those which are in the growth stages, and (3) those in the differentiating stages (Fig. 1b). The ripe yolk-laden oocytes are those which are ready to be spawned; oocytes which have entered the growth stages and are in the active state of vitellus formation are those which are preparing for the subsequent spawn; while the undifferentiated stages represent the primary oogonial cells and follicle cells.

During one spawning most of the ripe oocytes are extruded and the originally distended ovary shrinks in size, but does not become thin and ribbon-like as reported in fishes in which all the ova are spawned at a time (HILDEBRAND & CABLE 1930). In such an instance, after one spawning is over, the ovary which has become flabby,

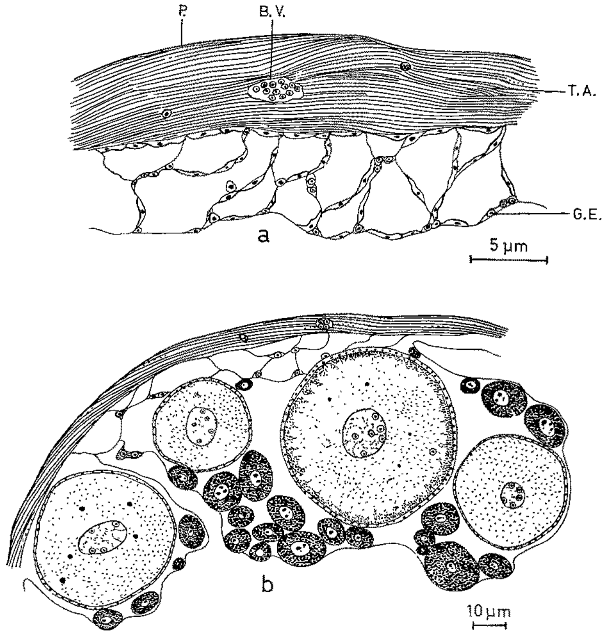

Fig. 1: *a* Section of ovary showing the ovarian wall. (P. teritoneum; T.A. tunica albuginea; G.E. germinal epithelium; B.V. blood vessels) – *b* Section of ovary showing oocytes of different growth stages

transparent and ribbon-like may rightly be termed a "spent ovary". In the case of *Tilapia mossambica*, a spent condition of the ovary as described above has not been noticed, since after a brood has been extruded, a sufficient number of ripening oocytes for the next spawn, which may happen within two weeks, occupy the ovocoel. In addition to the ripening oocytes, a large number of immature oocytes, a few ripe oocytes which have escaped extrusion during the previous spawn, as well as a large number of undifferentiated cells form the bulk of the ovary. The few oocytes which have escaped extrusion soon undergo atresia. The follicular cells hypertrophy and show marked numerical increase (Fig. 2). All the follicular cells lose their characteristics owing to intense hypertrophy, and are transformed into an irregular mass of cells. These cells invade the oocyte, and gradually the entire contents of the latter get resorbed. It is interesting to note that, after one spawning is over, the follicular layer of the mature unspawned ova alone undergoes atresia, and neither the immature oocytes nor the oocytes which have entered the growth stages undergo this degeneration. However, atresia of immature oocytes has been reported in *Gobius giuris* by SREERAMULU & RAJALAKSHMI (1965).

After spawning, the ovarian wall remains quite thick. The germinal epithelium reveals a large number of folds in addition to several strands of cells arising from the germinal epithelium and ramifying within the ovocoel. Within the folds of the epithe-

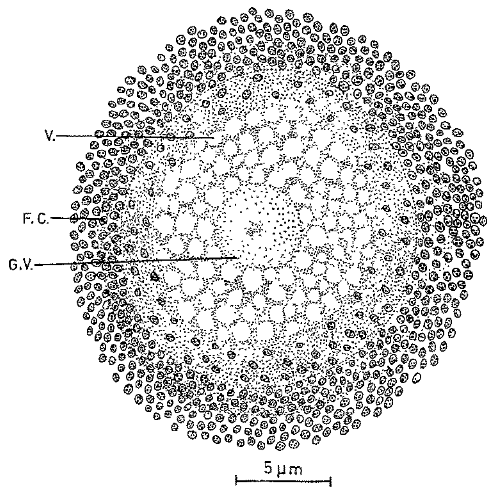

Fig. 2: Section of an unspawned egg showing degeneration (F.C. follicle cells; G.V. germinal vesicle; V. vacuole)

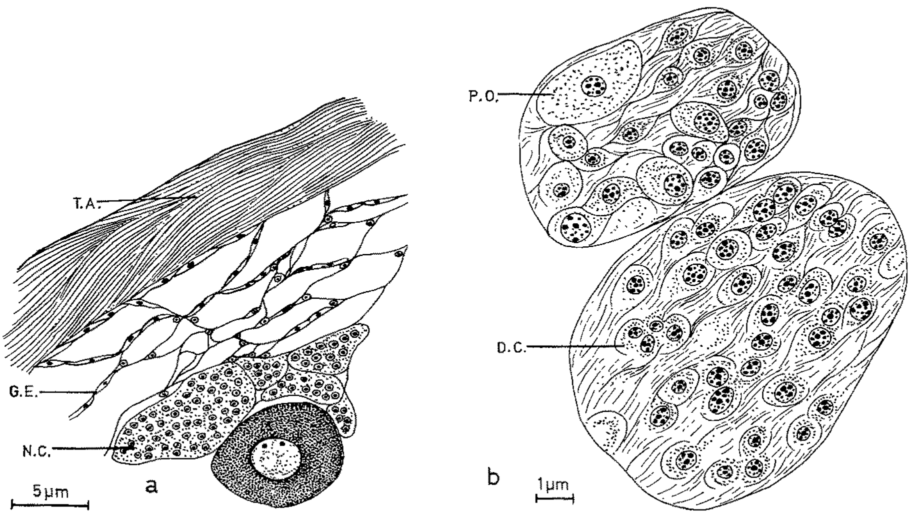

Fig. 3: *a* Section of ovary showing nests of cells (T.A. tunica albuginea; G.E. germinal epithelium; N.C. nest of cells) – *b* Nests of cells containing differentiating primary oogonia (P.O. primary oogonia; D.C. differentiating cells)

lium, nests of cells appear as a result of rapid mitotic activity (Fig. 3a). A few of the cells of the nest become large, enclosing an eccentric nucleus surrounded by a small amount of cytoplasm (Fig. 3b). Each nucleus contains reticulated chromatin studded with dark-stained particles within the nucleoplasm and a large deeply stained nucleolus. These cells form the primary oogonia and soon get pushed into the ovocoel, and by mitotic division give rise to the primary oocytes as well as the follicular cells. Thus, in nests which are found in an ovary from which one brood has been released, differentiating oogonia, follicle cells and undifferentiated primary oogonial cells can be noted.

Consequently, the source of the fresh crop of oocytes in *Tilapia mossambica* is attributable to the nest of cells and the ramifying strands of cells which both owe their origin to the germinal epithelium.

The stages from immature oocytes to mature ova are as follows:

(1) Chromatin nucleolus stage. The oogonia at this stage are irregular in shape and are seen crowded together in the folds of the germinal epithelium. Measuring 19 to 20 μ in diameter, each oogonium contains a nucleus 8 to 9 μ in diameter bearing a strongly basophilic nucleolus and strands of ramifying chromatin (Fig. 4a).

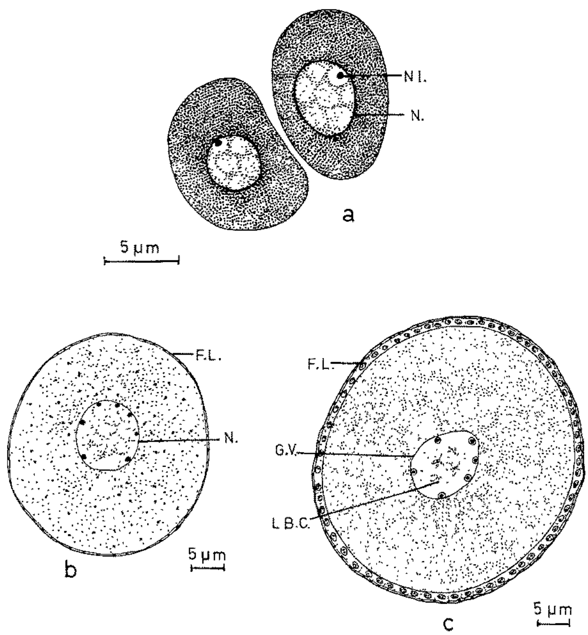

Fig. 4: *a* Early oocytes (Nl. nucleolus; N. nucleus) – *b* Perinucleolus stage (F.L. follicular layer; N. nucleus) – *c* Yolk vesicle stage (F.L. follicular layer; G.V. germinal vesicle; L.B.C. lamp-brush chromosomes)

(2) Perinucleolus stage. The oogonia have transformed into oocytes and are distinguishable by the characteristic peripheral arrangement of a number of nucleoli along the inner side of the nuclear membrane. The chromatin threads increase in number and appear as a close meshed network in the centre of the nucleus. The nucleus of each oocyte from now on is termed germinal vesicle. The oocyte measures about 175 μ in diameter, and a thin follicular layer surrounds it (Fig. 4b).

(3) Y o l k v e s i c l e s t a g e. The appearance of yolk vesicles in the peripheral zone of the cytoplasm is the distinguishing feature of this stage. As the oocyte grows, the vesicles too increase in number and size. The centrally placed germinal vesicle measures around 105 μ in diameter. The position occupied by chromatin threads is now taken up by "lamp-brush" chromosomes. A distinct egg membrane the "zona radiata" makes its appearance at this stage (Fig. 4c).

(4) P r i m a r y y o l k s t a g e. The entire cytoplasm outside the germinal vesicle, apparently filled with yolk vesicles, now contains a coagulated substance, indicating the formation of fatty yolk. "Lamp-brush chromosomes" are not visible at this stage (Fig. 5a).

(5) S e c o n d a r y y o l k s t a g e. Yolk globules appear in the cytoplasm and are moved towards the peripheral region. The germinal vesicle reveals a reduction in size.

(6) T e r t i a r y y o l k s t a g e. Yolk globules increase considerably and a large number of yolk platelets and yolk spherules appear. The nuclear wall shows signs of dissolution and becomes greatly reduced in thickness.

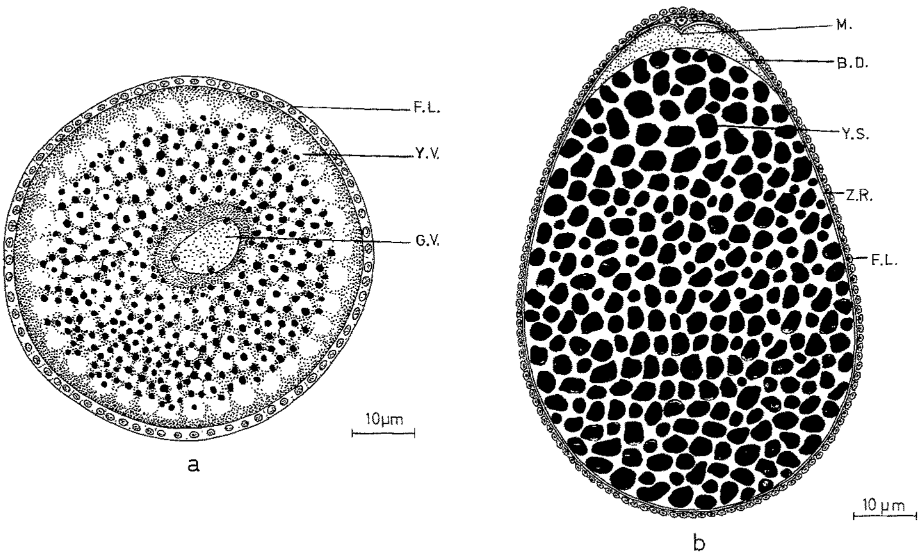

Fig. 5: *a* Primary yolk stage (F.L. follicular layer; Y.V. yolk vesicle; G.V. germinal vesicle) – *b* Ripe egg stage (M. micropyle; Z.R. zona radiata; F.L. follicular layer; Y.S. yolk spherules)

(7) M i g r a t o r y n u c l e u s s t a g e. A small quantity of yolk-free cytoplasm accumulates at one pole of the egg. The germinal vesicle gradually moves towards the periphery. The micropyle appears as a funnel-shaped depression near the yolk-free cytoplasm.

(8) P r e - r i p e n i n g s t a g e. The germinal vesicle reaches the animal pole beneath the micropyle formed in the previous stage.

(9) R i p e - e g g s t a g e. A fully mature ovum measures about 3 mm in its long axis and 2.5 mm in the short axis. It is translucent and yellow (Fig. 5b).

The fully mature ova which are still retained in the ovary after a spawn, undergo atresia as has already been stated.

DISCUSSION

Tilapia mossambica, breeding almost throughout the year, does not show distinctive morphological and histological changes in a post-spawned ovary. On the other hand, in annual breeders like *Gobius giuris* (SREERAMULU & RAJALAKSHMI 1965), the ovarian wall, after spawning is over, undergoes morphological transformations and histological changes preparatory to the next cycle. After one spawning is over in *Tilapia mossambica,* the ovary contains an adequate number of potential oocytes for subsequent spawnings. Thus, in a mature ovary, oöcytes of different categories can be noticed, each category representing oocytes for a specific spawning. A careful scrutiny of the composition of the ovary of a mature fish reveals at least six crops of oocytes which would be released one after another by rapid growth of the oocytes of a crop soon after one crop has been extruded.

Different views are held with regard to the source of a new crop of oocytes in ovaries of teleost fishes. In regard to *Pleuronectes limanda,* WHEELER (1924) states that cells of the follicular layer of the previous crop form the source of new crop of oocytes.

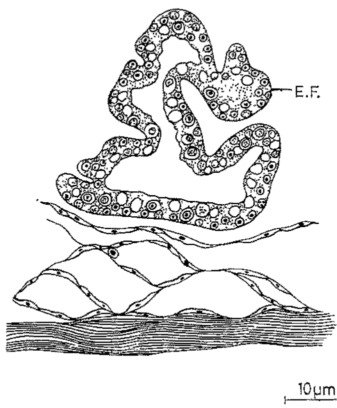

Fig. 6: Section of post-spawned ovary showing a degenerating empty follicle (E.F. empty follicle)

YAMAMOTO (1956) has come to the same conclusion in *Liopsetta obscura.* But in *Tilapia mossambica,* the follicular cells retained within the ovary after the oocytes are extruded undergo degeneration, or some of the cells coming into contact with the growing oocytes function as nutritive cells for these (Fig. 6). In *Gobius giuris,* even the ruptured follicle cells are retained for some time even after extrusion. They eventually disintegrate. The follicle cells neither show any hypertrophy to form a corpus luteum nor divide to form new oocytes (SREERAMULU & RAJALAKSHMI 1965). YAMAMOTO &

YAMAZAKI (1961) found that in the gold fish *Carassius auratus* the ovary is replenished from residual immature oocytes retained within it after spawning. In *Gobius giuris,* the residual immature oocytes form corpora atretica which are eventually resorbed. But in *Tilapia mossambica,* only fully ripe unspawned ova undergo atresia by hypertrophy of follicle cells, and these atretic ova form corpora atretica; eventually, resorption of the degenerating oocyte takes place.

According to HONMA (1961) and YAMAMOTO & SHIRAI (1962), residual oogonia give rise to new a crop of oocytes by their multiplication. DUTT & GOVINDAN (1969) claim that a new crop of oocytes arises in *Anabas scandens* from the germinal epithelium or from the remnants of follicle cells of the "pre-ovulatory corpus luteum".

In conclusion, it may be stated that, in *Tilapia mossambica,* the replenishment of the ovary following spawning is traced to the already available number of oocytes which are present there for 5 or 6 subsequent spawnings. A fresh crop of oocytes arises from the nest of cells of the germinal epithelium as well as from the ramifying strands of epithelium and there is no evidence that the degenerating follicle cells form a source for a new crop of oocytes. This finding agrees with results of BOLLOUGH (1942), YAMAMOTO (1962), SREERAMULU & RAJALAKSHMI (1965) who, in their studies on *Phoxinus laevis, Oryzias latipes* and *Gobius giuris* respectively, have indicated that the source of a new oocyte crop is the germinal epithelium. The occurrence of various groups of oocytes (immature, maturing, and fully ripe ova) in a single ovary at a particular time accounts for "asynchronism" in the ovary of *Tilapia mossambica* as opposed to "group synchronism" in *Gobius giuris* (SREERAMULU & RAJALAKSHMI 1965), or "total synchronism" in *Onchorhyncus masou* (YAMAMOTO et al. 1959).

SUMMARY

1. In the teleost *Tilapia mossambica* (PETERS), new crops of oocytes arise from nests of cells of the germinal epithelium as well as from the epithelial strands ramifying into the ovocoel.
2. There is no evidence to indicate that degenerating follicle cells form a source for a new crop of oocytes.
3. After one spawning is over, the follicular layer of the mature unspawned ova alone undergo atresia.
4. Neither immature oocytes nor oocytes in the growth stages undergo degeneration.
5. The occurrence of immature, maturing, and fully ripe ova in the ovary at a particular time account for asynchronism in *Tilapia mossambica.*

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