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ORIGIN OF THE OOCYTES IN THE ADULT GUPPY,  
*LEBISTES RETICULATUS*

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Although many investigations on the origin of the oocyte have been performed on the adult of viviparous fish, the results reported are variable and give scope enough for controversy.

The conclusions obtained by many workers may be summarized as follows; the oocytes in the adult fish are derived from the germinal epithelium (Wallace, 1904; Essenberg, 1923; Mizue, 1964), from both pre-existing oogonia and the epithelium (Wolf, 1931; Mendoza, 1943), or from the proliferations of pre-existing oogonia (Turner, 1938).

In order to clarify these conclusions, the present study was undertaken, using the guppy, *Lebistes reticulatus*.

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**Material and Methods**

The guppy used in the present study were adult multiparous females. All were reared in the laboratory at 22-28°C and illuminated by artificial light in addition to the natural daylight. The fish were killed at various periods after the last parturition. The ovaries were fixed with Bouin's, Allen-Bouin's, Zenker's, and Regaud's fluids. Serial sections of 7-10 micra were prepared by the usual paraffin method and stained with Heidenhain's iron haematoxylin-light green or Delafield's haematoxylin-eosin.

**Results**

The ovary of the adult *Lebistes* takes a single, oval form, and it is enveloped by a thin peritoneal membrane and lies in the post-dorsal portion of the peritoneal cavity. This organ consists of the ovarian cavity and the ovarian stroma in which many oocytes and embryos in various stages of development are situated.

The ovarian cavity extends ventrally from post-dorsally, branching irregularly in the ovary, and it continues caudally with a single short gonoduct. The wall bordering on the ovarian cavity is lined with a single epithelial layer and sub-

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epithelial connective tissue. As the oocytes increase in size, these two epithelia invaginate into the stroma and form a so-called funnel-like depression or a delle. The delle offers an intimate connection between the ovarian cavity and the oocyte in follicle, and plays an important role at the time of fertilization and extrusion of the embryo, as already reported by Purser (1938) and Mendoza (1943). The general histology of the ovary is similar to that described in other poeciliid fish such as *Xiphophorus helleri* (Bailey, 1933) and *Heterandria formosa* (Fraser & Renton, 1940).

Usually oogonia and young oocytes are found in the wall as small nests. These nests are enclosed with a connective tissue sheath which is connected directly to the subepithelial connective tissue fibers (Fig. 1), and are often situated near the delle of the more developed oocytes (Fig. 2). These facts suggest that the growth of one oocyte among the nest results in the invagination of two surface layers and the formation of the delle, thus the nest composed of the remaining young germ cells takes a juxtaposition to the delle (Fig. 3). Occasionally the nest exists at some distance from the surface of the ovarian wall, but no isolated nest is found in the deep portion of the ovarian stroma. Ordinarily one adult ovary holds as many as a few tens of these nests. Each nest contains young germ cells whose development varies from the oogonia up to the chromatin-nucleolus stage, a few to tens in number.

The resting oogonia, 8 to 12 micra in diameter, have a large spherical nucleus and a very thin sheath of cytoplasm (Fig. 11). The nucleus, ranging from 6 to 8.5 micra, occupies the greater part of the cell, and it contains a single or several chromatin-nucleoli and the linin network scattered throughout it. These resting oogonia are observed in all specimens sacrificed during the gestation period.

Although the number was small, at most several in one ovary, the dividing oogonia (Figs. 5 & 6) were found in the specimens killed on the day of parturition (3 individ.), 3rd, 6th, 7th, 14th, 15th, and 25th day after the last parturition respectively. It may be presumed, therefore, that the multiplication of oogonia occurs in the mature ovary throughout the gestation period. The oogonia exhibit typical mitosis as shown in Figures 12-16. The diameter of the oogonia reaches about 18-20 micra in the late anaphase or telophase (Figs. 14 & 15).

The oocytes in the synaptic stage are also frequently observed in the nests. They are characterized by the nucleus containing the complicately anastomosed chromatins situated on one side of its cavity (Fig. 7). With the progress of synapsis the chromatin threads become thick and loose, while the chromatin-nucleolus continues to maintain a large distinct shape. Then the chromatin threads spread over the nuclear cavity and besides the single large one, there appear some small

nucleoli (Fig. 8). Hereafter the oocyte increases in volume, accompanying the gradual movement of chromatin-bodies toward the peripheral region of the nucleus (Fig. 17). Further growth of the oocytes has already been mentioned in the previous report (Takano, 1964).

Oogonia and very young oocytes are frequently observed within the epithelial layer itself which fronts the ovarian cavity. In general they exist singly, and rarely show division (Fig. 9). Although they develop more or less *in situ*, no advanced oocyte past the earlier phase of peri-nucleolus stage could be seen (Fig. 4). It is uncertain whether these oocytes may invaginate the stroma at a certain time or degenerate at the time of parturition. The epithelial cells themselves, on the other hand, frequently show division (Fig. 10), and they are smaller in size than the dividing oogonia.

It is interesting to note that numerous dividing oogonia have been observed in the ovary of one old female. This female showed no breeding during nearly three months (corresponding to three gestation periods or more) from the last parturition up to the date of sacrifice. She showed a curvature of the spine, her ovary was markedly shrunken, and a large quantity of fatty mass was stored in her peritoneal cavity. In addition to many degenerating oocytes and four yolk-laden ones, many nests composed of resting or dividing oogonia and young oocytes were found in the ovary (Figs. 11-18).

#### Discussion

Based on the histological studies of the embryonic gonads of *Lebistes*, Dildine (1933, 1936) reported that the embryonic gonads are hermaphrodite; gonads exhibit female characteristics first, and then half become testes after the degeneration of synapctic germ cells. In another half, on the other hand, the germ cells in synapsis which begins to occur from 10 to 15 days before birth, continue to grow and form definitive oocytes. Goodrich *et al.* (1934) who also studied this species, reported that the definitive germ cells are derived from the primordial germ cells and that the evidence of the transformation of the stroma cells into the definitive germ cells is slight.

Thus, in this species, it has been thought that the division of the oogonia ceases early in the life-history (Franchi *et al.*, 1962), without any attempt to ascertain the evidence of the mitotic proliferation of oogonia in the ovary of the multiparous female. From the results obtained in the present study, however, it seems probable that the new oocytes of the adult multiparous *Lebistes* are supplied by the multiplication of pre-existing oogonia.

Wallace (1904) reported that the ova and follicular epithelium have a common origin in the ovarian epithelium in *Zoarces viviparus*. In *Xiphophorus helleri*, Essenberg (1923) observed that all primordial germ cells disintegrate and the definitive ova derive from the peritoneal cells. Wolf (1931), studied the female *Platypoecilus maculatus* as far as sexual maturity, suggested that many definitive germ cells originate in the primordial germ cells, and that some transformation of epithelial cells into sex cells continues until the animal is almost mature but no transformation is observed in mature animals. Recently, on the other hand, Mizue (1964) worked on the marine viviparous teleosts, *Ditrema temmincki*, *Sebastes marmoratus* and *Sebastes inermis*, and pointed out that the oogonia appear from the inner cell layer of the germinal epithelium which is a component of the ovigerous fold of the mature ovary. Mendoza's (1943) observations on *Neotoca bilineata* led him to the conviction that in the adult gonad, some of the germ cells arise from the ovarian epithelium of the ovigerous folds. In the viviparous fish examined, oogonial multiplication through mitosis has not been observed.

On the contrary, Turner (1938) ascertained that the mitotic proliferation of oogonia has taken place in the adult ovary of the *Cymatogaster aggregatus*. According to him, some nests of resting germ cells (or oogonia) are scattered through the ovigerous sheets in the ovary in the early stages of gestation. Soon after the ovary is free of embryos, a period of rapid mitosis occurs in some germ cells of the nests. Such a special active period of oogonial multiplication could not be recognized in *Lebistes*, but multiplication of oogonia continues to occur throughout the gestation period. As distinct evidences of a transformation of epithelial cells into germ cells were not obtained in the present study, it may be adequate, therefore, to conclude that new crops of oocytes in the adult *Lebistes* are supplied mainly from the multiplication of residual oogonia.

On the origin of the oocytes in oviparous fish, this has been reviewed extensively by Franchi *et al.* (1962). Recently, in *Oryzias latipes*, Yamamoto (1962) observed that new crops of oocytes are produced yearly from the germ cells in the ovary just before spawning time. Yamamoto & Shirai (1962) further showed that new crops of oocytes originate in the residual oogonia throughout the year in *Rhodeus ocellatus*. Thus, it may be said that new crops of oocytes in the adult fish are supplied in the same way in both oviparous and viviparous fish. Some differences in the occurrence of oogonia are seen between the above oviparous fish and the guppy. In *Lebistes*, the nests of young germ cells are smaller in size and larger in number than in these oviparous fish. The morphological changes in the oocyte after synapsis resemble those in the two oviparous animals.

The dividing figures of oogonia were also found in the ovary of the old female. The ovary was undoubtedly in a senile and inactive condition. Bullough (1942), based on the experiment of oestrone injection on the female *Phoxinus laevis*, suggested that the anterior pituitary gland stimulates indirectly the nuclear divisions of gametogenesis through the intermediary of sex hormones. Since the oogonial division has been observed in goldfish reared for a long time after hypophysectomy, Yamazaki & Yamamoto (1965) suggested that the pituitary is not concerned directly with the multiplication of oogonia. The present finding that active multiplication of oogonia occurs in the old guppy suggests that the oogonial proliferation may be induced by some different mechanism than that of vitellogenesis or maturation.

#### Summary

In *Lebistes reticulatus* the oogonia and the young germ cells are found in the wall bordering the ovarian cavity. They usually make small nests enclosed with a connective tissue sheath which is continuous with the subepithelial connective tissue, but sometimes they are located singly within the epithelial layer which fronts the ovarian cavity. Throughout the gestation period, the multiplication of oogonia takes place by mitotic division. As no distinct transformation from epithelial cells to germ cells was observed in the present species, it seems highly probable that the oocytes in the adult multiparous female are newly supplied by the multiplication of oogonia.

#### Literature

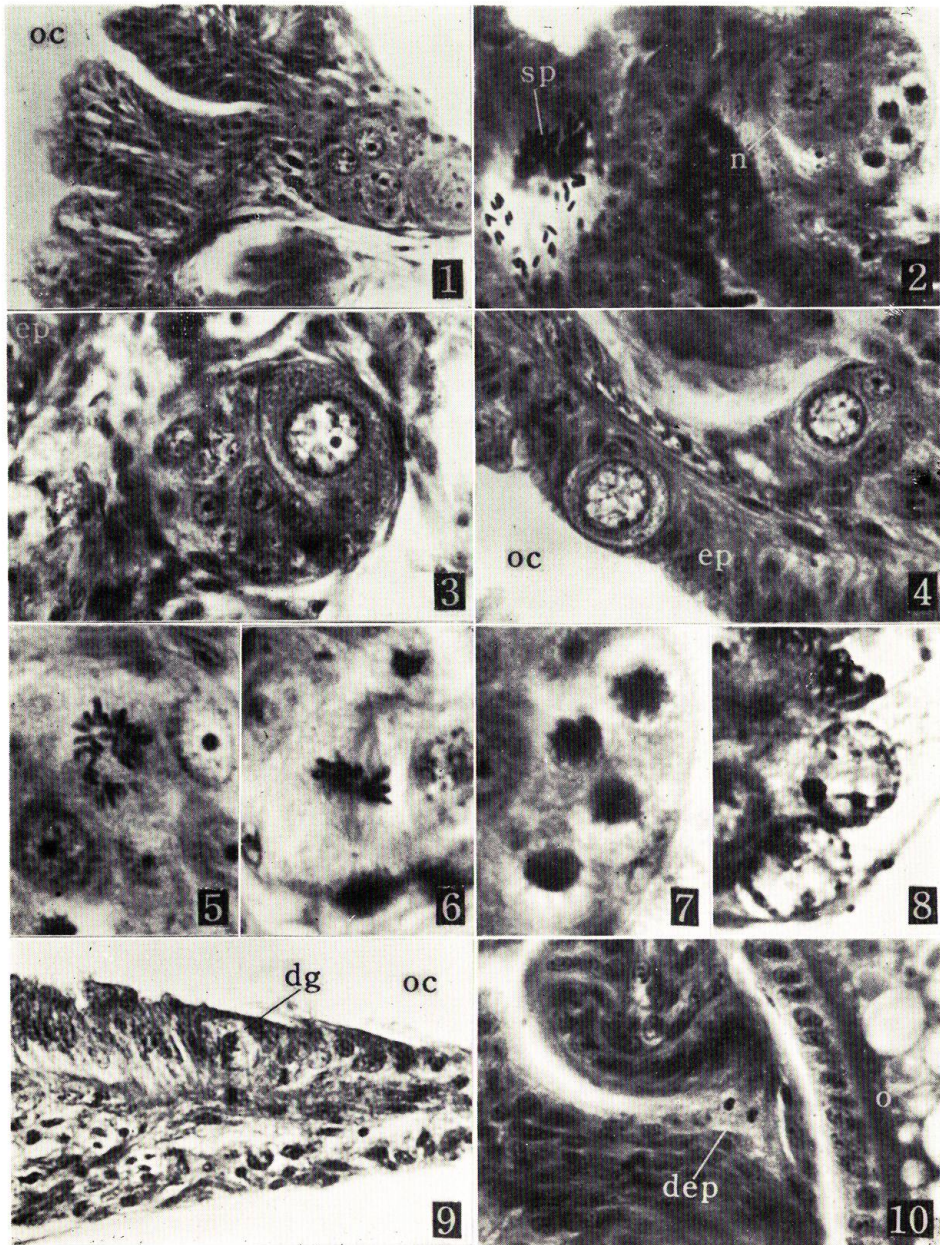
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## Explanation of Plates

## PLATE I

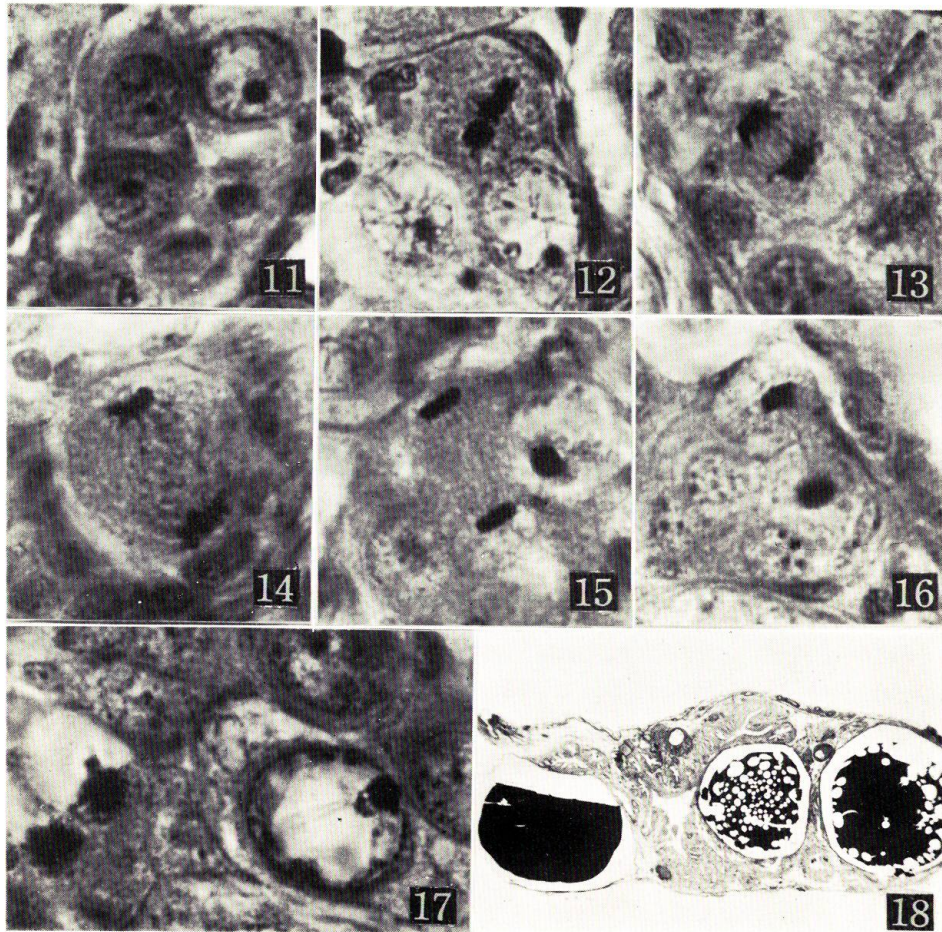
- Fig. 1. Nest of young germ cells located in the surface of the ovarian wall. Regaud's solution and Heidenhain's haematoxylin-light green preparation. *oc*, ovarian cavity.  $\times 280$
- Fig. 2. Nest of young germ cells adjacent to the delle. Allen-Bouin's solution and Heidenhain's haematoxylin-light green preparation. *sp*, sperm in the delle. *n*, nest involved dividing and resting oogonia and synaptic oocytes.  $\times 575$
- Fig. 3. Nest composed of one large and many young germ cells. Regaud's solution and Heidenhain's haematoxylin-light green preparation.  $\times 575$
- Fig. 4. Developing oocyte in the epithelium lining of the ovarian wall. Bouin's solution and Heidenhain's haematoxylin-light green preparation. *ep*, epithelium lining.  $\times 575$
- Figs. 5 and 6. Dividing oogonia in metaphase. Regaud's solution and Heidenhain's haematoxylin-light green preparations.  $\times 1330$
- Fig. 7. Oocytes in synapsis. Regaud's solution and Heidenhain's haematoxylin-light green preparation.  $\times 1330$
- Fig. 8. Oocyte after synapsis. Zenker's solution and Delafield's haematoxylin-eosin preparation.  $\times 1470$
- Fig. 9. Dividing oogonium in the epithelium lining. Zenker's solution and Delafield's haematoxylin-eosin preparation. *dg*, dividing oogonium.  $\times 575$
- Fig. 10. Dividing epithelial cell in the delle. Regaud's solution and Heidenhain's haematoxylin-light green preparation. *dep*, epithelial cell in division. *o*, developing oocyte.  $\times 575$



K. Takano: Origin of oocytes in adult *Lebistes*

## PLATE II

- Fig. 11. Two resting oogonia, from the same specimen as shown in Fig. 18.  $\times 1330$
- Figs. 12, 13, 14, 15, and 16. Progressive figures of oogonial multiplication through division, from the same specimen as in Fig. 18.  $\times 1330$
- Fig. 17. Young oocytes in synapsis and after synapsis, from the same specimen as in Fig. 18.  $\times 1330$
- Fig. 18. Ovary of old female. Bouin's solution and Delafield's haematoxylin-eosin preparation.  $\times 16$



K. Takano: Origin of oocytes in adult *Lebistes*