STUDIES ON THE ENDOCRINE GLANDS OF THE SALMONOID FISH,
AYU, *PLECOGLOSSUS ALTIVELIS* TEMMINCK ET SCHLEGEL
IV. THE FATE OF THE UNSPAWNED EGGS AND THE NEW CROPS
OF OOCYTES IN THE SPENT OVARY

(With 15 Text-figures)

Yoshiharu HONMA*

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In a previous paper I reported that all the so-called Otu-nen Ayu which have survived after spawning and are found in rivers are female, and that no new oocytes as younger oocytes produced by partial reabsorption of reserve-fund eggs are found in the spent ovary (HONMA, 1959 b). These facts have been regarded as points of difference between the land-locked Ayu which spawn two or three times during their life-span since SUZUKI (1939) has confirmed the existence of young oocytes in this form. More recently, I have been puzzled to find young oocytes in the spent ovary of some of the so-called Otu-nen Ayu collected from the same places as those previously described. Re-examination of my original material has not revealed oocytes, so an account of these new specimens is of interest.

In fish which spawn several times over the course of several years, it is considered that the corpora lutea originating from both post-ovulation and pre-ovulation (=atretic follicles) are the source of hormone production (BRETSCHNEIDER and DE WIT, 1947; HOAR, 1955; STOLK, 1951, 1957; TATEISHI, 1956 etc.). I have therefore re-examined the histological picture of the course of regression of unspawned eggs in the spent ovary of the Ayu.

Materials and Methods

All twenty-one spent fish, consisting of the ordinary Ayu and Otu-nen Ayu, used in this study have been described in previous papers (HONMA, 1959 a, b; 1960). The material was mostly fixed in BOUIN's solution, embedded in paraffin, cut serially at 8 μ , and stained with DELAFIELD's haematoxylin-eosin, HEIDENHAIN's haematoxylin-light green and Azan triple stain.

Observations

The belly of adult fish just before spawning swells remarkably, owing to the

* Dept. Biol., Fac. Sci., Niigata Univ., Niigata, Japan. (本間義治, 新潟大学理学部生物学教室)

presence of a great number of mature eggs. After spawning, the body rapidly becomes emaciated. Four specimens among the fish examined and caught on November 20th, 1958, have a number of abortive eggs in the spent ovary. It is interesting that all the Otu-nen Ayu collected after January have ovaries consisting of two components. The greater part of the body cavity is occupied by the ovarian sheath containing the unspawned ovarian eggs, while in the vicinity of the cloaca there is another small block of eggs derived from the regressing left ovary. A similar block is found in the right side, and both protrude outside the abdominal cavity when the belly is slit open.

Most of the unspawned eggs are immature and deposited in the ovary as abortive eggs. All of them are undergoing degeneration. There are a small number of eggs in the yolk vesicle stage and a large number in the yolk globule or later stages. The unspawned eggs may be distinguished from normal ones by the absence of nuclei in the former. However, the unspawned eggs have a perfect oolemma with distinct radial striations surrounding the fine granular yolk ball, and many vacuoles are recognized in the egg cavity. The granulosa cells (=cells of the follicular epithelium) are present in the various conditions: very high columnar cells with elliptical nuclei in their basal ends; cells of moderate height separate from each other; and cells with indistinct outline containing ovoid nuclei. The periphery of the granulosa is surrounded by a thin theca with capillaries. These atretic features correspond to those of the *a*-phase described by Dutch workers (Figs. 1 and 2).

Ovulation scars are scattered among the unspawned eggs, and two types of granulosa can be recognized. One is shaped like a pinna, since a point of rupture still remains. The other is ellipsoid since the rupture has united. In either case the broad empty follicle cavity (=follicular hole) remains in the central part of the scar; the outline of the granulosa cells is indistinct; the shape and size of the nuclei are irregular; and the cells are in a disorderly arrangement (Figs. 1 and 2). The granulosa is surrounded by a thick theca constructed chiefly of smooth muscle fibres which make contact with the ovarian lamellae. Between the granulosa and theca there are fibres of connective tissue.

In the specimens taken in February the follicular hole becomes reduced and the granulosa reaches a state of hypertrophy. These high columnar cells have their nuclei located towards the follicular hole. On the other hand, the ovarian lamellae surrounding the vestiges of ovulation and the atretic follicles become thick gradually. It is characteristic of the lamellae that large irregular yellow pigment granules are scattered within it (Fig. 3).

We shall now consider the process of oolysis in the atretic follicles prior to ovulation. First, the yolk globules become united with each other, form a large mass, and pass gradually into a state of liquefaction. Vacuoles are found in the yolk mass, but the majority of them are situated just below the oolemma. These features are

still characteristic of the *a*-phase.

The granulosa cells become hypertrophied with large, pale, ovoid nuclei in their central region. A part of the yolk which lies near oolemma becomes completely liquefied, and some of the granulosa cells cross the oolemma and invade the region of liquid. These granulosa cells appear to be involved in phagocytosis. In some sections, yolk granules, varying in size and likely to have been gathered by phagocytosis, are scattered through the granulosa cells. It seems probable that these particles of yolk are derived from the reunion of yolk ingested by the granulosa cells. Sometimes, vacuoles are found in the granulosa. The degree of contact between the granulosa and oolemma is variable: the two components may remain in contact or be quite separate, the space between them being filled with liquefied yolk. These features are comparable to those of the *b*-phase (Figs. 4 to 7).

As a result of the absorption of the contents of the eggs, the following features of atretic follicles are found. (1) Follicles with scanty, thin liquefied yolk in their cavities, and surrounded by a hypertrophic granulosa. (2) Empty follicles surrounded by degenerated granulosa; the follicle having the so-called signet-ring appearance, although the oolemma still exists. However, discrimination between the vestiges of ovulation and atretic follicles can be made without difficulty. More immature ovarian eggs regress without hypertrophy of the granulosa, only a homogeneous yolk remaining.

Beyond this stage, atretic follicles of spherical contour are seldom encountered. The spent ovary of fish which have survived beyond February is characterised by the presence only of a highly convoluted oolemma, without egg contents or granulosa. However, a small quantity of ingested egg contents and invaded granulosa cells may be found in the reduced cavity. It is suggested that this sequence show the course of degeneration and absorption of the content of the eggs. As a result of regression of ovarian eggs the ovary itself becomes reduced to a thin band. These corpora lutea are comparable to those of the *c*-phase (Fig. 8).

As mentioned above the rate and mode of regression of ovarian eggs in a single ovary are not uniform. Furthermore, another type of regression should be mentioned: here the granulosa is indistinct, the remnant of the oolemma is discontinuous and convoluted, and some granulosa cells occupy the ovum cavity. These cells are in a state of hypertrophy indicating the production of some substance, in the regression, and in a spherical mass (=so-called cell pearl). Mature corpora lutea with yellow pigment granules comparable to the *d*-phase of Dutch workers have not been found although the regressive ovarian egg has begun the formation of an atretic corpus luteum which has some sinusoids within its ovum cavity (Figs. 9 and 10).

As was described in the preceeding paper, young oocytes could not be recognized in the spent ovary of Otu-nen Ayu caught as late as February 11th, 1958, and the same was true of 1959. However, in the spent ovaries of four fish out of five obtained on April 10th, 1959, some young oocytes were encountered. These were situated in the

hypertrophic lamellar membrane after ovulation. Young oocytes may be seen with naked eye, since many of them are located near the ovarian sheath. Others are scattered among the atretic follicles. In each case, however, four to six young oocytes can be counted in a section of a single cyst. Occasionally, cysts communicate with each other and form a large mass (Figs. 11 and 12).

The most developed young oocytes have reached the stage of having peripheral nucleoli, and their homogeneous ooplasm with ovoid contour surrounds the periphery of the cycloid nuclei. The mean diameter of the oocytes is $60\ \mu$ (mx. $80\ \mu$) in the longer axis, $50\ \mu$ in the shorter, and the diameter of nucleus is ca. $35\ \mu$, rarely reaching $42\ \mu$.

In the somewhat younger oocytes the nuclei are rather more elliptical, and are surrounded by coarse ooplasm. More younger oocytes, in the state of chromatin nucleoli, have an indistinct outline and scanty ooplasm. There may be ten or more of these in a single ovary. The nuclei of these oocytes are round in shape, ca. $14\ \mu$ in diameter, and the chromatin is concentrated in a clearly recognizable mass. Some of the nuclei are packed close together. There are a few clusters of oocytes with nuclei, ca. $7\ \mu$ in diameter; the chromatin substance of which is disposed in the peripheral region of the karyoplasm. It seems probable that these are derived from nests of oogonia, although the oogonia have not yet been demonstrated clearly. In addition, a few oocytes which had reached the peripheral nucleolus stage were detected in the spent ovary of a fish collected on May 21st, 1958 (Figs. 13 to 15).

The young oocytes are surrounded by the lamellar membrane which contains scattered, yellow pigment granules and melanin.

Considerations

The process of regression in the unspawned eggs of the Ayu coincides well with that of other species of fish described by previous investigators. The oolemma with distinct radial striations maintains its affinity for dyes, and exists for a long time as a thick folded membrane. The course of collapse and ingestion of yolk by the active granulosa (*a*, *b*, and *c*-phase) is similar to that described by other workers (BRETSCHNEIDER and DE WIT, 1947, *Rhodeus*; STOLK, 1951, *Lebistes*, 1957, *Dermogenys*; HOAR, 1955, *Carassius*; and BEACH, 1959, *Carassius* etc.). In the ovary of the Ayu, however, the ultimate corpus luteum (= *d*-phase) containing yellow pigment granules in the ovum cavity has not been observed, and only indications of the process of formation of corpora lutea (= *a*, *b*, and *c*-phase) have been demonstrated. HOAR (1955) considered that hypertrophy of the granulosa is a constant feature of the preovulation corpus luteum of teleosts and the source of the ovarian hormones. STOLK (1951, 1957) states that the histological structure of the corpus luteum of *Dermogenys* corresponds closely with that of *Lebistes*, and that luteal cycles during the gestation are similar

although these two species of viviparous fish belong to systematically very remote families. It seems, therefore, that the corpora lutea accomplish an important task during gestation. On the other hand, BEACH (1959) is of the opinion that the granulosa of *Carassius* merely produces enzymes which digest the yolk and prepare it for absorption into the blood, since there is no histological evidence that these cells produce hormones. The granulosa of the Ayu also plays a role in the destruction, transfer and ingestion of yolk, but there is no histological indication of hormonal secretion. HISAW and HISAW (1959) suggest that in elasmobranch fishes this activity of the granulosa represents the original function of luteal bodies and that endocrine function, as seen for example, in mammals, is a later adaptation, although the corpora lutea are formed by atresia of yolk-laden follicles and subsequent to ovulation. STENGER (1959) found many atretic follicles in the spent ovary of *Mugil*, but no structure comparable with those of mammals and other species of fish. It seems necessary to clarify the chemical nature and origin of the yellow pigment granules scattered in the lamellar membrane of the spent ovary of Ayu, and the author proposes to attempt this in the near future.

Reports dealing with the formation and maturation of ovarian eggs, the process of ovulation, and the regression of unspawned eggs in the spent ovary of some species of fish have been published by several Japanese workers, such as YAMAMOTO, K. (1954, 1956, *Liopsetta*), TATEISHI (1956, *Carassius*) and YAMAMOTO, T. S. (1955 a, b, *Oryzias*, *Oncorhynchus*, *Clupea*, and *Lampetra*). SUZUKI (1939), HONMA (1959 b) and SUKO (1960) reported on the spent ovary of the Ayu, *Plecoglossus*. The results of these investigations it has been suggested that the eggs in a single ovary of some salmon and salmonoid fish which die after their first spawning are peculiar in that all the eggs develop to almost same stage, and that extraordinary younger oocytes are not encountered in the spent ovary. However, the present investigation has demonstrated that the ordinary spent post-spawning fish (so-called Otu-nen Ayu) have young oocytes which are in a stage comparable to those of the young Ayu and the land-locked Otu-nen Ayu. WEISEL (1947) reported that the oocytes are present in the spent ovary of the sockeye, *Oncorhynchus nerka*. ROBERTSON and WEXLER (1960) did not find any cells similar to oogonia in the king salmon, *O. tshawytscha*, although a few immature ova of normal appearance were present after ovulation. The young oocytes found in the spent ovary of Ayu are neither immature unspawned eggs (so-called reserve fund eggs) nor younger eggs reorganized owing to partial reabsorption of ooplasm as in *Liopsetta* (YAMAMOTO, K., 1954). Therefore, it seems probable that the new oocytes of the Ayu are derived from the residual oogonia, although nests of oogonia like those in the spent ovary of the mullet, *Mugil cephalus* (STENGER, 1959) have not been distinctly recognized.

It is difficult to discover whether or not these new oocytes continue their vitellogenesis and maturation, and develop into a fully ripe condition. Not many new

oocytes can be counted in a section (from two or three only to several hundreds), and even if the fish could survive the winter it seems likely that they would die before the following June in the natural environment (KOBAYASHI, 1938). Moreover, it is still questionable whether these eggs are ejected after maturation. Thus, we cannot say with certainty whether or not the spent fish can deposit two sets of eggs. The author is now planning an examination of the spent ovary of the naturally land-locked Otu-nen Ayu, and attempting to collect specimens from various districts of Japan proper, since the materials used for the present account have been limited to the rivers of Niigata Prefecture, on the coast of the Sea of Japan.

Summary

The spent ovaries of Otu-nen Ayu that have survived in the rivers after spawning have been examined cyto-histologically.

The problems concerned are:

1. Morphological changes in the ovulation scar in the course of time.
2. The process of regression of unspawned eggs, and particularly, the collapse and ingestion of yolk attacked by the active granulosa or follicular epithelium, the persistence of the oolemma, and the development of atretic follicles.
3. The appearance and descent of young new oocytes found mainly in the hypertrophic lamellar membrane.

Explanation of figures

- Fig. 1.** Atretic oocytes corresponding to the primary yolk stage and ovulation scars. $\times 50$.
Fig. 2. *a*-phase atretic oocytes and an empty follicles. $\times 75$.
Fig. 3. Ovarian lamellae including large irregular yellow pigment granules. $\times 150$.
Fig. 4. A part of a *b*-phase atretic follicle showing active granulosa and oolemma. Some of granulosa cells are invading the region of liquefied yolk. $\times 150$.
Fig. 5. *a*-, and *b*-phase atretic follicles. $\times 150$.
Fig. 6. A *b*-phase atretic follicle showing liquefied yolk. $\times 50$.
Fig. 7. A *b*-phase atretic follicle and some young oocytes in the peripheral nucleolus stage. $\times 50$.
Fig. 8. *c*-phase atretic follicles showing convolution of oolemma. $\times 50$.
Fig. 9. A *c*-phase atretic follicle with ovum cavity and young oocytes in peripheral nucleolus stage. $\times 50$.
Fig. 10. A *c*-phase atretic follicle indicating invasion of granulosa cells and remnants of oolemma. $\times 50$.
Fig. 11. A mass of young oocytes corresponding to peripheral nucleolus stage and a *c*-phase atretic follicle. $\times 50$.
Fig. 12. A mass of young oocytes located near the ovarian sheath. $\times 50$.
Fig. 13. Scattering oocytes found in the ovarian sheath. $\times 50$.
Fig. 14. Cysts of younger oocytes with scanty ooplasm. $\times 50$.
Fig. 15. Nests of more younger oocytes corresponding to chromatin nucleolus stage and three oocytes in peripheral nucleolus stage. $\times 200$.

In the ovary of Ayu the corpora lutea are formed from granulosa both of post-ovulation (=vestige of ovulation) and of pre-ovulation eggs (=atresia of yolk-laden follicles), but production of yellow pigment granules in the ova cavities has not been recognized. It seems likely that the granulosa of pre-ovulation corpora lutea merely play a part in the destruction, transfer, and ingestion of yolk, since there is no clear indication of hormonal secretion.

The young ovarian eggs in the spent ovary seems to be new oocytes originating from the residual oogonia, but not the immature reserve-fund eggs nor younger ones reorganized by partial absorption of ooplasm. Whether or not these young oocytes can develop to mature functional eggs is undetermined.

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