

A HISTOLOGICAL STUDY OF THE OVULAR ATRESIA IN THE CATFISH, *MYSTUS SEENGHALA* (SYKES)

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(With 2 Plates)

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I—INTRODUCTION

Brambell (1956) observed that “the follicular atresia is common and may be regarded as a process in the ovaries of vertebrates at all ages” The resorption of eggs appears to be a common occurrence in the growing ovaries of vertebrates (Hisaw & Albert, 1947). Earlier reports on the degeneration of the ovarian ova of teleosts are of His (1873), Brock (1878), Emery (1880), Owsianikov (1885), Barfurth (1886) (cited by Wallace, 1903) and Cunningham (1897). The works of Cunningham (1897) and Wallace (1903) give comprehensive reviews of the earlier studies on the subject. Turner (1933, 1938), Matthews (1938), Frazer (1940), Mendoza (1943), Bretschneider and de Wit (1947), D’Ancona (1950) and Stolk (1950, 1951) are some of the recent authors who have dealt with the subject. Many investigations on the ovarian ova of teleosts lack references to the atresia of oocytes, while some workers have made passing remarks on this phenomenon (James, 1946 ; Sathyanesan, 1959). The extensive studies of Bretschneider and de Wit (1947) reveal that in *Rhodeus amarus*, homologous structures function as endocrine organs. Basing on its function, the above authors termed this structure as “pre-ovulation corpus luteum” Dixit’s (1956) observations on the atretic oocyte of *Mystus seenghala* are rather fragmentary.

In this paper an attempt is made to study in detail the histological changes occurring in the atretic oocyte of *Mystus seenghala* during its resorption.

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II—MATERIAL AND METHODS

For this study specimens were collected from the river Ganges every month for one complete year. Fishes were killed by severing the head, and portions of the anterior, middle and posterior regions of the ovary were fixed in appropriate fixatives immediately. Bouin's picro-formol, Allan's modification of Bouin's picro-formol, Zenker-formol and Pereny's fluid were used as fixatives. Paraffin sections were cut at 4, 6 and 8 μ thick and stained in Delafield's Haematoxylin, Heidenhain's iron Haematoxylin and Erlich's Haematoxylin; Eosin and Orange-G were the counterstains employed.

III—OBSERVATIONS

The ovary of *M. seenghala* shows an all round enlargement during the pre-spawning phase. In the larger oocytes yolk deposition is in progress, and in many cases the deposition is even complete. In the spawning phase the ovary is turgid and completely distended. During these phases a number of oocytes abort and are in different stages of resorption. Degeneration of the immature oocytes is not so common as that of the matured and maturing ones. The terms "degenerating or atretic oocyte" (Matthews, 1938; Mendoza, 1943) and "pre-ovulation corpus luteum" (Bretschneider and de Wit, 1947) are used by workers to describe homologous structures.

The ripe oocyte of *M. seenghala* has a prominent vitelline membrane. The follicular layer which overlies it is made up of a single row of columnar cells. A thin vascular theca corresponding to the follicular membrane in the oocyte of *Pleuronectes* (Cunningham, 1890) covers the follicular layer (Pl. 1, Fig. 2a). The vitelline membrane and the follicular layer of *M. seenghala* correspond to the oolemma and the granulosa layer described in the oocyte of *Rhodeus amarus* (Bretschneider and de Wit, 1947); the thin theca may be homologous with the theca interna, while the basement membrane and the theca externa of *Rhodeus amarus* is not clearly differentiated in *M. seenghala*.

In *M. seenghala*, during its resorption, the atretic oocytes present different patterns. Though the end product of atresia is the same, the pattern of the process by which it is accomplished varies.

In the abortive oocytes initially the nucleus disappears and the follicular layer shows indications of hypertrophy. Liquefaction of the contents of oocyte is evident towards the periphery (Pl. 1, Fig. 1), even when the vitelline membrane persists and the follicle cells retain their definition (Pl. 1, Fig. 2). The vitelline membrane is deflected off from the follicular layer (Pl. 1, Fig. 2) and may be thrown into folds. During the next stage prominent cytoplasmic granules are seen in the follicle cells. On further hypertrophy, these cells lose their definition and the entire follicular layer becomes a syncytium (Pl. 1, Fig. 3). At a later stage vacuoles of varying size and shape appear in this syncytium (Pl. 1,

Fig. 4). Some of these vacuoles contain homogeneous globular bodies (Pl. 1, Fig. 5). Such bodies are recorded in the hypertrophying post-ovulatory follicle of *Fundulus heteroclitus* (Matthews, 1938) and in the hypertrophying follicle of the atretic oocyte of *Zoarcetes* (Wallace, 1903). The above authors considered these globular bodies to be of fatty or oily consistency, which dissolve during the preparation of the slides. As the process of resorption is in progress the cavity of the oocyte (oocoel) is narrowed down by the approximation of the hypertrophying follicular layer which is thrown into folds (Pl. 1, Fig. 6). Blood cells are always present among the infiltrating follicular element ; but they abound in the folds of the follicular layer (Pl. 1, Fig. 6). Probably they arise from the vascular membranous theca which covers the follicular layer. When the oocoel is almost obliterated the follicular layer becomes more compact and the vacuoles gradually dwindle (Pl. 1, Fig. 6a). In the subsequent stage the entire mass becomes more compact and presents a yellowish hue. When this structure becomes more condensed a close examination reveals that it is made up of irregular yellow granular mass and round or oval cells (Pl. 2, Fig. 3) having yellow pigments in them. In some case the non-cellular pigment mass abounds. Gradually the intracellular pigment disappears. These cells lose their regular form, become variously shaped, migrate along strands of connective tissue (Pl. 2, Fig. 2) and finally are lost among the stromal elements. The entire process appears to take about two to four months to accomplish. In the final stage of the histogenesis of the "pre-ovulation corpus luteum" in *Rhodeus amarus* Bretschneider and de Wit (1947) record necrosis and migration of the cells as two different ways to decrease the number of cells from this structure. In *M. seenghala* the yellow pigment mass may be the remnant of necrosis. And the migrating cells described, have close similarity to that reported in *Rhodeus amarus*.

In some oocytes after the disappearance of the nucleus the follicular cells hypertrophy and show distinct numerical increase. They lose their definition and the entire follicular layer becomes a syncytium. Vacuoles and globular bodies as reported in the preceding case are not evident (Pl. 1, Figs. 7 and 8). The granulation of the cytoplasm of the follicular syncytium and the liquefaction of the yolk contents of the oocyte are not extensive. In some cases, in the region of the theca, capillaries are prominently seen (Pl. 1, Fig. 7). The proliferation of follicular cells is not uniform all around. In the areas of active proliferation these cells form regular or irregular groups. Some of these groups of cells have a regular shape resembling the so-called "cell pearls" (Pl. 2, Fig. 4) described in the "pre-ovulation corpus luteum" of *Rhodeus amarus* (Bretschneider and de Wit, 1947) and *Lebistes reticulatus* (Stolk, 1951). Dixit (1956) observed that "the invasion of the follicular cells is more pronounced at the corners than at other points" The present writer does not recognise any region in the atretic oocyte which can be termed as corner with any justification. Also, the pronounced invasion of the follicular elements at certain points as noticed by Dixit (1956), is not a general feature, since in some cases it is seen that the follicular invasion is more or less uniformly spread over (Pl. 1, Fig. 8). Irrespective of the initial pattern of arrangement of these proliferating follicle cells, they steadily invade the ovular contents. This centripetal

migration of the follicular cells results in the resorption of the ovular contents, and its replacement by the moving mass of cells (Pl. 2, Fig. 1). This strongly recalls the structure of corpus luteum atreticum. Bullough (1951) defines the corpus luteum atreticum as a solid body similar to the normal corpus luteum, which is formed from the ovarian follicle when an egg is resorbed. The fate of this cellular mass is similar to that described earlier.

In some cases of atresia the follicular hypertrophy is less pronounced than that of the preceding two types. However, the follicular layer becomes a syncytium which is generally devoid of vacuoles (Pl. 2, Figs. 5 and 6). Granulation of their cytoplasm is moderate. Liquefaction of the ovular contents is generally not prominent. It is interesting to note that the vitelline membrane is retained almost entire even when the major part of the ovular contents is resorbed. The follicular components together with the vitelline membrane are thrown into several irregular digitate folds, leaving only a narrow strip of obliterating oocoel inside (Pl. 2, Fig. 6).

IV—DISCUSSION

The follicular components of the teleostean oocyte is found to vary in size and appearance depending on the stage of maturity and the species of the fish. Generally the vitelline membrane is covered by the follicular layer, which is enveloped by the thecal elements (Cunningham, 1890, 1897; Wallace, 1903; Bailey, 1933; Hann, 1927; Matthews, 1938; Mendoza, 1943; James, 1946; Bretschneider and de Wit, 1947; Chaudhry, 1956; Sathyanesan, 1959). But in *Pomoxis* (Cooper, 1952) the only follicular covering is formed by a capsule-like layer of loose reticular fibres. The terms zona radiata (James, 1946; Chaudhry, 1956) granulosa layer (Bretschneider and de Wit, 1947) and connective tissue covering or follicular membrane (Cunningham, 1890, 1897) are used by authors to denote the vitelline membrane, follicular layer and the theca respectively.

Several workers have studied the structure and changes occurring in the teleostean post-ovulatory follicles. But studies on the structure, function and fate of the atretic oocytes of teleosts are few. Cunningham (1897), and others observed the process of resorption of dead eggs to be similar to that of the empty follicles. Wallace (1903) and Bretschneider and de Wit (1947) have reported that these two processes are different.

Opinions differ as to the part played by the different components of the follicle in the resorption of the atretic oocyte. Cunningham (1897) reported that the cells from the connective tissue covering of the follicle proliferate and invade the contents of the oocyte to effect resorption. According to Wallace (1903) the cells of the follicular layer are responsible for the resorption. During the first stage in the formation of the preovulation corpus luteum, Bretschneider and de Wit (1947) observed that the granules of the granulosa act as a disintegrating ferment to dissolve the oolemma. The absorption of the ovular contents by the granulosa cells takes place through resorption of liquefied ovular substances and phagocytosis of solid yolk elements. During the second stage they reported penetration of the thecal cells into the follicular space by breaking through the granulosa layer. Thus it appears that

in *Rhodeus amarus* the theca cells also have some part to play in the formation of this structure. In *Heterandria formosa* (Frazer, 1940) and *Neotoca bilineata* (Mendoza, 1943) the follicular layer takes part in the resorption of the atretic oocyte. Hoar (1955) observed that the function of the follicular derivatives is by no means clear.

The role of blood cells in the resorption process appears to vary. Ruge (cited by Wallace, 1903) records a simultaneous incursion of blood and follicular cells into the degenerating egg and he regards both kinds of elements as taking active part in the breaking down of the egg. Wallace (1903) noted in *Zoarces* the appearance of leucocytes at a late stage in the resorption process. He attributed them, the probable function of conveying the product of degeneration (the yellow non-adipose granules) into the lymph or blood channel. Mendoza (1943) believes that the slight lymphocyte infiltration usually occurring during the process may be instrumental in aiding the process of disintegration. Barfurth (1886, cited by Wallace, 1903) considers that the leucocytes play only a subordinate part in the degeneration of the eggs in the trout. Bretschneider and de Wit infer that the hormone of the lutein-cells (transformed granulosa cells) is directly secreted into the blood vessels lying close to them.

In *M. seenghala* the cells of the follicular layer hypertrophy, proliferate and penetrate into the substance of the oocyte, The blood cell seen among the hypertrophying follicle may probably belong to the theca. These blood cells persist throughout the entire process of resorption. As in the case of goldfish (Hoar, 1955), marked vascularisation and hypertrophy of the theca is not seen in this species. However, in some cases prominent capillaries are present overlying the theca. The so-called "cell pearls" of the *Rhodeus amarus* is formed by the invading theca and granulosa cells having a part of the oolemma remnant inside. In *M. seenghala* the "cell pearl-like" bodies are formed by the excessive proliferation of follicular cells at certain areas. These bodies may be sometimes regular, but more often they are irregular in shape.

The process of resorption of atretic oocyte in *M. seenghala* exhibits wide variations. In the first type described elsewhere in this paper, the hypertrophy of the follicular layer is seen. Liquefaction of the ovular contents is profound. The follicular syncytium has granules, vacuoles and globular bodies. In the second type, numerical increase in the follicular cells is noted. The follicular syncytium is devoid of vacuoles and globules. Granulation is sparse and liquefaction is much less in comparison to the former. In the third type the hypertrophy of the follicular layer is less pronounced than that of the preceding cases. The liquefaction of the ovular contents is meagre. The vitelline membrane is almost intact even when the entire content is resorbed. Unlike the "pre-ovulation corpus luteum" of *Rhodeus amarus*, the considerable variations involved in the process of resorption in *M. seenghala* render it difficult to arrange them into continuous series of interrelated stages.

Dixit (1956) reports that atresia in *M. seenghala* appears to take place in "two general ways" "In one case the cytoplasm loses its normal yolk-laden structure, vacuoles make their appearance and the whole mass appears to be somewhat a vacuolated mass of debris. In

the second type the follicle cells lose their normal cellular appearance, proliferate and invade the enclosed yolk-laden cytoplasm from the sides", resulting in their gradual disappearance. The study of the present writer on the same species reveals that, what the "two general ways" or types of atresia mentioned by Dixit (1956) may be two stages in a particular type of resorption.

Variations in the process of resorption of the atretic oocyte are suggested by several workers. Cunningham (1897) has recorded the presence of aborted eggs in a state of resorption in the spent and immature ovaries of teleosts. He observed that their appearance differs very much according to the stage at which their development has been arrested. Mendoza (1943) states that in *Neotoca bilineata* "the degeneration picture most definitely is not one of complete breakdown of all or most cells at once. In a few isolated cases an abnormally heavy lymphocyte infiltration may occur and in others follicle may precede the egg in degeneration" Further he adds that regardless of the method of atresia the end result is the removal of the degenerating mass of cells from the stroma of the ovary. Hoar (1955) writes that in goldfish he did not observe marked vascularisation and hypertrophy of the theca described in the second stage of corpora lutea formation in *Rhodeus amarus* (Bretschneider and de Wit, 1947). Wallace (1903) reports that he is unable to find some intermediate stages in the fatty degeneration of the atretic oocyte of *Zoarces*. Speaking of follicular atresia in general Brambell (1956) states that atresia exhibits much variation not only in different species but also in follicles of different size.

The findings of the above authors and the present writer suggest the absence of a regular sequence of stages in the resorption of the abortive oocytes. It is probable that, depending on the growth stage at which atresia sets in and the rate of proliferation and hypertrophy of the follicular cells, stages in the process of resorption may be added or inhibited or telescoped.

Bretschneider and de Wit (1947) found that the "pre-ovulation corpus luteum" secretes a hormone which is responsible for the development of the ovipositor in *Rhodeus amarus*. It would be interesting to investigate the endocrinological implications involved in the case of *Trigla hirundo* (Cunningham, 1897) and the trout (Barfurth, 1886; cited by Wallace, 1903) where the whole batch of ripe ova meant for spawning are resorbed *in situ*.

More recently in summing up the present position of this problem in fishes (Hoar, 1957) states, "several writers have noted the degeneration and atresia of developing eggs and have speculated as to the significance and cause of this atresia. Others have emphasised the hypertrophy of the follicular cells, the apparently healthy condition of the resulting structure and the possible endocrinological significance. There is not yet sufficient information to establish the physiology of the follicular layers and their derivatives" Bursting atresia occurring in the ovary of monotremes, birds and reptiles is considered to be a method by which heavily yolked eggs are eliminated when atresia sets in (Garde 1930, Davis 1942, Dominic 1960, Bragdon 1952). Instances of comparable burst atretic follicles are reported in *Mystus seenghala* (Sathyanesan 1960). These findings support

the inference of Beach (1959) that the formation of "corpus luteum" like structure in the resorptive oocyte is apparently associated with the removal of the yolk rather than secretion of hormone. However it is probable that these may perform both the functions.

V—SUMMARY

1. The presence of atretic oocytes in the maturing and mature ovary of *M. seenghala* is not uncommon as reported by Dixit (1956).
2. The presence of variations in the process of resorption of the atretic oocytes is evident.
3. It may be difficult to arrange all the stages in the resorption process recorded in this species into a continuous series.
4. It is likely that these variations may depend on the growth stage of the oocyte at which atresia sets in and the varying activity of the follicular component during resorption.
5. Histological observations reveal that the follicular cells have a major role in removing the yolk. It is likely that this structure may also be responsible for the production of hormone.

VI—REFERENCES

- BAILEY, J. 1933. Ovarian cycle in the viviparous teleost *Xiphophorus helleri*.—*Biol. Bull. Woods Hole, Woods Hole, Mass.* **64**, pp. 206-225.
- BEACH, A. W. 1959. Seasonal changes in the cytology of the ovary and of the pituitary gland of the goldfish.—*Canadian J. Zool.* Ottawa **37**, pp. 616-625.
- BRAGDON, D. E. 1952. Corpus luteum formation and follicular atresia in the common garter snake, *Thamnophis sirtalis*.—*J. Morph.*, Philadelphia, **91**, pp. 413-437.
- BRAMBELL, F. W. R. 1956. Ovarian changes. 'Marshall's Physiology of Reproduction' **1**, pp. 397-542.—London, (Longmans, Green & Co.).
- BRETSCHNEIDER, L. H. and DUYVENE DE WIT, J. J. 1947. Sexual Endocrinology of Non-mammalian *Vertebrates*.—Amsterdam (Elsevier).
- BULLOUGH, W. S. 1951. *Vertebrate sexual cycles*.—London (Methuen & Co., Ltd.).
- CHAUDHRY, H. S. 1956. The origin and structure of the zona pellucida in the ovarian eggs of teleosts.—*Zellforsch.* Berlin, Wien. **43**, pp. 478-485.
- COOPER, L. J. 1952. A histological study of the reproductive organs of crappies (*Pomoxis nigro-maculatus* and *Pomoxis annularis*).—*Trans. Amer. micr. Soc.*, Lancaster, Pa., **71**, pp. 393-405.
- CUNNINGHAM, J. T. 1890. *A treatise on common sole*. Plymouth.
- CUNNINGHAM, J. T. 1897. On the history of the ovary and ovarian ova in certain marine fishes.—*Quart. J. micr. Sci.*, London, **40**, pp. 101-163.

- D'ANCONA, U. 1950. Follicoli atresici e corpi lutei nei Teleostei.—*Arch. ital. Anat. Embriol.*, Firenze, **54**, pp. 413-419.
- DIXIT, R. K. 1956. Atretic oocytes in the ovaries of *Mystus seenghala* (Sykes) and *Wallago attu* (Bloch).—*J. Zool. Soc. India*, Calcutta, **8**, pp. 91-94.
- DAVIS, D. E. 1942. The bursting of Avian follicles at the beginning of atresia.—*Anat. Rec.*, Philadelphia, **82**, pp. 153-165.
- DOMINIC, C. J. 1960. An unique type of follicular atresia in the ovary of birds.—*Sci. & cult.*, **25**, pp. 653-654.
- GARDE, M. L. 1930. The ovary of *Ornithorhynchus*, with special reference to follicular atresia.—*J. Anat.*, Cambridge, pp. **64**, 422-453.
- HANN, H. W. 1927. The history of the germ cells of *Cottus bairdii*.—*J. Morph.*, Philadelphia, **26**, pp. 427-498.
- HISAW, F. L. and ALBERT, A. 1947. Observations on the reproduction of the spiny dog fish *Squalus acanthias*.—*Biol. Bull., Woods Hole*, Woods Hole, Mass., **92**, pp. 187-199.
- HOAR, W. S. 1955. Reproduction in teleost fishes.—*Mem. Soc. Endocrinology*, Cambridge, No. 4, pp. 5-24.
- HOAR, W. S. 1957. Gonads and reproduction. The physiology of fishes. pp. 287-321.—New York, (Academic Press Inc.).
- JAMES, M. F. 1946. Histology and gonadal changes in blue gill *Lepomis macrochirus* and the large mouth bass *Huro salmoides*.—*J. Morph.*, Philadelphia, **79**, pp. 63-89.
- MATTHEWS, S. A. 1938. The seasonal cycle in the gonads of *Fundulus*.—*Biol. Bull. Wood's Hole*, Wood's Hole, Mass., **75**, pp. 66-74.
- MENDOZA, G. 1943. The reproductive cycle of the viviparous teleost *Neotoca bilineata*, a member of the family Goodeidae. IV The germinal tissue.—*Ibid.*, **84**, pp. 87-97.
- SATHYANESAN, A. G. 1959. Seasonal cycle in the gonads of *Cirrhina reba* (Hm.).—*Indian J. Veter. Sci.*, Delhi, **29**, pp. 27-34.
- SATHYANESAN, A. G. 1960. On the occurrence of burst atretic follicles in the teleost *Mystus seenghala* (Sykes).—*Naturwissenschaften*, Berlin, **13**, p. 310.
- STOLK, A. 1950. Histo-endocrinologische analyse van de graviditeits verschijnselen bij de cyprinodontide *Lebistes reticulatus*. Thesis. Utrecht.
- STOLK, A. 1951. Histo-endocrinological analysis of gestation phenomena in the cyprinodont *Lebistes reticulatus*. II. The corpus luteum cycle during pregnancy.—*Proc. Kon. Ned. Akad. v. Wetensch.*, Amsterdam, **54**, pp. 558-565.
- TURNER, C. L. 1933. Viviparity superimposed upon ovoviviparity in the Goodeidae, a family of cyprinodont fishes.—*J. Morph.*, Philadelphia, **55**, pp. 207-251.
- TURNER, C. L. 1938. Adaptations for viviparity in embryos and ovary of *Anableps anableps*.—*Ibid.*, **62**, pp. 323-349.