

***SEASONAL HISTOLOGICAL CHANGES IN THE OVARIES OF
MUGIL SEHELI FROM SUEZ BAY***

By

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ABSTRACT

The sequence of oocyte maturation in Mugil seheli from the Suez Bay is histologically divided into nine stages, these histological identified stages are related to seven morphological stages. The histology of the ovaries of Mugil seheli is examined to give a picture of the reproductive cycle of this species. Seven developmental stages are described in details. Oocyte's maturation was divided into nine stages; chromatin, early perinucleolus, late perinucleolus, yolk vesicle, primary yolk, secondary yolk, tertiary yolk, mature and rip stage. Oogenic development in Mugil seheli is belonging to asynchronous type. In this type the spawning season was found to be long and extending from November to March.

INTRODUCTION

The study of oogenesis in fishes has attracted the attention of many investigators (El-Agamy et al., 1983; 1987, Abdin, 1986, clay 1989). The maturity stages, in teleost fishes were described histologically by many authors (Rastogi, 1968; Latif and Saady, 1973; El-Agamy et al., 1983; 1987; El-Gharabawy and Abdel-Aziz, 1988; Zaki and El-Gharabawy, 1991 and Asem 1992). They were mainly interested in the morphological and histological changes which took place in the course of oocyte growth. The gonads of bony fishes have clear cyclic changes, on the basis of which the sexual cycle is characterized.

Mugil seheli, which belongs to the family Mugilidae, is an important commercial fish in Suez Bay. It supports a good capture fishery and is used extensively as stock in farms. In spite of its considerable economic importance, to date there has been no such study undertaken on their gonads.

The present work investigates the ovarian cycle and spawning season of this important fish.

MATERIALS AND METHODS

Living specimens of *Mugil seheli* were collected from Suez Bay during the period, May/1991 and April/1992. Total length, total weight and the date of capture were recorded. The abdominal cavity was opened and the ovaries were removed, fixed in Bouin's fixing fluid, dehydrated, cleared and embedded in paraffin wax. Sections 5-8 um thick were stained with Ierich haematoxyline, Heidenhain iron haematoxylin (eosin was used as counter stain) and Milligant trichrome stain. To determine GSI (gonadosomatic index), gonad weight was recorded as a percentage of the gutted body weight.

RESULTS

Macroscopic structure and maturity stages

The maturity stages designated in this study are based on macroscopic appearance of the ovaries and the microscopic structure of the ova, according to Bara (1960) and Yamamoto and Yamazaki (1961).

The ovary of *Mugil seheli* consists of two lobes of almost equal size, lying below the alimentary canal along the body cavity. The maturity of gonads can be classified into seven stages according to the shape, colour and size of the ovaries. These stages are;

Stage I (Immature virgin): Ovaries are thin and thread like, translucent and colourless. Sexes usually can not be distinguished.

Stage II (Developing virgin and recovering spent): More rounded, translucent and colourless, eggs not evident to the naked eye.

Stage III (Maturing): Thickening, opaque and pale yellowish. Eggs small but not visible to the naked eye.

Stage IV (Mature): The ovary fills most of body cavity, opaque and yellow in colour, eggs large.

Stage V (Ripe): Ovaries distended and fill the body cavity, yellow colour. Eggs large and extruded by pressure on abdominal wall.

Stage VI (spawning): Ovaries show somewhat shrinkage due to discharge of a considerable amount of eggs.

Stage VII (Spent): Ovaries are thin and flaccid, congested, sometimes contain large opaque-yellow residual eggs.

The classification of oocyte maturation stages

The active sequence of events that growing oocytes in this fish passes through before getting transformed into an ovum can be conveniently arranged in the following nine stages and is based on the general scheme used by Yamamoto and Yamazaki (1961), with some modification, for the gold fish (Carassius auratus). These stages are;

I- Chromatin nucleolus stage:

Very minute oocytes, sparsely distributed in the ovary. They are found embedded in the ovigerous lamella and have a diameter below 13 μm and their nucleus has diameter of 11 μm . The cytoplasm is seen as a narrow, weakly basophilic zone around the basophilic nucleus. One or two nucleoli can be found in the nucleus. A thin follicle layer is seen to surround the oocytes (Fig. 1).

II- Early perinucleolus stage

The size of oocytes in this stage ranges from 15 to 36 μm in diameter and are rounded to polyhedral in shape. The cytoplasm is highly basophilic, staining a deep colour with haematoxylin. The nucleus is still relatively large, having a diameter ranging from 9 to 24 μm and lightly staining. In section it contains an average of 8 basophilic nucleoli arranged more or less peripherally. These are of various size and usually round in form, (Fig. 2).

III- Late perinucleolus stage

The diameter of oocytes in this stage ranges from 50 to 120 μm and are usually more rounded than those in the preceding phase. The cytoplasm is at first still basophilic and homogenous in appearance. The nucleus increases in size, have diameter that ranges from 30 to 73 μm . The nucleoli increase in number and become

more peripheral in position. In the early phase of this stage yolk nucleus lies close to the nuclear membrane and then moves to the periphery of the cytoplasm with the growth of oocytes. The oocytes are surrounded with a very thin follicular layer. At the end of this stage the yolk nucleus disappears, (Fig. 3 and 4).

IV- Yolk vesicle stage

Most of the oocytes in this stage range from 140 to 160 μm in diameter. The nucleus is spherical in form and ranges from 60 to 73 μm in diameter. The yolk vesicles and vacuoles are formed in the cytoplasm. Zona radiata is also recognizable between the cytoplasm and the follicular layer, it measures about 6 μm , (Fig. 5). By the appearance of yolk vesicles the outer region of the cytoplasm becomes more darkly stained than that lying inwards or round the nucleus (Fig. 5).

V- Primary yolk stage

At this stage the yolk globules begin to appear (Fig. 6). The formation of the globules proceeds by the inner part of the cytoplasm and leaves small area of yolk free cytoplasm near the nucleus. Oocytes in this stage measure about 270 μm in diameter. Nucleus in this stage is some-what round and measures about 70 μm and the nucleoli are distributed in the periphery of the nucleus. The nuclear membrane assumes an irregular outline of several nucleoli and seen pushed into its granulations. The yolk globules which are eosinophilic and stained black with heidenhain iron haematoxylin, was about 11 μm . Also the vacuoles increase in size and measure about 11 μm . Zona radiata becomes thick, measuring about 9 μm , (Fig. 7).

VI- Secondary yolk stage

Oocytes in this stage measure from 300 to 360 μm in diameter. Yolk globules seem to be accumulated very rapidly in the inner part of the cytoplasm & measures about 13 μm in diameter. The nucleus at the beginning of the stage becomes oval in shape due to the increase of yolk globules and the fat vacuoles which increase in size to be about 18 μm . At the end of this stage the nucleus becomes irregular in shape and take place for the beginning of migration to the animal pole. Zona radiata increase in thickness and measures about 11 μm , (Fig.8).

VII- Tertiary yolk stage

Oocytes at this stage generally measure about 400 μm . The characteristic feature of this stage is the migration of the nucleus to the animal pole. The nucleoli are distributed inside the nucleus. The yolk globules increase in diameter. Zona radiata increase in thickness to 14 μm . The fat vacuoles increase in size and measure about 18.5 μm in diameter, (Fig. 9).

VIII- Mature egg stage

Oocytes at this stage measure about 450 μm . The nucleus can not be seen in the cytoplasmic mass. The yolk globules tend to coalesce. Fat vacuoles (oil droplets) increase in size and ranged from 36 to 86 μm in diameter and tend to coalesce. Zona radiata increase in thickness to about 20 μm , (Fig. 10).

IX- Ripe egg stage

Oocyte in this stage measure about 553 μm . In this stage the ripe oocytes start to be ovulated, which is usually recognized as the final step in oocyte maturation. The contents of the oocyte is similar to that in the preceding stage with small changes, such as the oil droplets (fat vacuoles) coalesce more than the preceding stage to form the oil drop which is the characteristic feature for the pelagic eggs. Zona radiata increased in thickness to about 30 μm , (Fig. 11).

Some atretic oocytes may be found in the ovaries amongst the normal oocytes at all stages of development. These atretic oocytes are usually derived from those between the yolk vesicle and tertiary yolk stage and are characterized in sections by their deformed shapes and the breakdown of their cytological structure, (Fig. 12).

Monthly changes in the ovary during annual reproductive cycle

In May and June, The females of *M. seheli* were in the recovery stage. Others were found in immature stage. Sections of the ovaries show that, the body wall of the ovary (tunica albuginea) is very thick and sometimes wrinkled. The ovigerous lamella did not fill the ovary lumen and contained oocytes in the chromatin stage (oogonia), early perinucleolus and very few numbers of late perinucleolus oocytes. All these types of oocytes were found along the ovigerous lamellae, and the center of ovary was empty. The average GSI is about 0.26.

In July, the ovary exhibited the characters of the preceding period, but in some sections the ovigerous lamella expanded and filled the ovary. Inside the sac the oocytes, chromatin, early perinucleolus and late perinucleolus filled the sac and increased in diameter. Gonadosomatic index is about 0.3, (Table 1).

In August and September, the body wall of the ovary decreased in thickness. The early and late perinucleolus oocytes filled the ovary and the diameter of the oocytes increased. The yolk nucleus appeared in the start of this period and disappeared at the end. Gonadosomatic index ranged from 0.46 - 0.58, (Table 1).

In October, the ovaries of some fishes exhibited the same characters of the preceding period, but in the other fishes the vacuolized and yolk vesicle oocytes appeared. The GSI was increased from 0.58 to 1.09 in average, (Table 1).

In November the spawning season started, the sections of ovaries displayed different stages of developmental oocytes. Some of them contained primary, secondary and tertiary yolk stage in addition to a few number of early perinucleolus oocytes. Very few fishes which was in the spawning stage, their ovaries contained a considerable quantity of ripening eggs, as well as a relatively large number of yolk vesicle and primary yolk stage, in addition to empty follicles. The average GSI was very high (9.42), (Table 1).

In December, January and February, the ovaries displayed different stages of development. Every month, some of them was in the ripe stage and contained a fair number of ripening eggs, while others which already have discharged a considerable quantity of ripening eggs, contained yolk vesicle & primary yolk oocyte, as well as empty follicles and small immature oocytes. The average GSI decreased from 8.36 to 6.23 (Table 1).

Table (1): Monthly variations of GSI for females Muqil seheli collected from Suez Bay (1991/1992).

Month	No.of fish	Maximum	Minimum	Average	± S.D.
May 1991.	20	0.35	0.10	0.259	0.07
Jun.	20	0.40	0.06	0.260	0.11
Jul.	31	0.66	0.10	0.330	0.12
Aug.	33	0.67	0.32	0.460	0.08
Sep.	25	0.86	0.43	0.580	00.11
Oct.	26	6.80	0.08	1.090	1.20
Nov.	45	26.89	1.58	9.420	5.46
Dec.	47	18.95	2.33	8.360	5.25
Jan. 1992.	15	15.47	1.00	0.960	5.41
Feb.	30	15.33	0.62	6.230	4.86
Mar.	17	15.84	0.26	4.050	5.05
Apr.	17	0.76	0.20	0.420	0.17

In March and April most of the ovaries were in a spent condition. GSI decreased to 0.42. Some of the sections contained yolk laden oocytes, these were oocytes residual which would not be spawned due to unfavorable conditions. These would undergo degeneration and resorption. Empty follicle, atretic oocytes and small oocytes were found.

DISCUSSION

The course of development of the egg is divided into stages, phases or periods in order to differentiate the gradual changes in its conditions or peculiarities (Forberg, 1982; Kaneko and Hanyu, 1985, Zaki and El-Gharabawy 1991 and Asem, 1992). In the present study the classification of egg is based on the general scheme used by Yamamoto and Yamazaki (1961), with some modification. These modifications are: the chromatin stage in the present study is similar to the pre-maturation period of Zaki *et al.*, (1986), immature stage of Hickling (1930), the premeiotic phase of Rastogi (1963) and the synapsis period of Latif and Saady (1973). These stages are; (I) chromatin stage, (II) early perinucleolus stage, (III) late perinucleolus stage, (IV) yolk vesicle stage, (V) primary yolk stage, (VI) secondary yolk stage (VII) tertiary yolk stage, (VIII) mature stage and (IX) ripe stage.

In the present study, oogonia were found throughout the annual cycle of the ovary of Mugil seheli, this means that they act as a reserve for more oogonia to the next season. The previous conclusion is supported by the presence of some oogonia during the resting phase of the ovary. This agrees with Hann (1927) in Coltus barrdi, Belsar (1962) in Ophiocephalus punctatus. Moreover, Yamamoto (1956) in Liopsitto obscura believed that, the new crop of oogonia is produced by follicular epithelial cells of the spent follicles.

At the beginning of the late perinucleolus stage, closely contiguous to the nucleus a small organelle appeared, to which the names archoplasm, centrosphere, crop vitelline, Balbiani bodies or yolk nucleus. As the growth of oocyte advances and by the end of late perinucleolus stage, the yolk nucleus migrate to the peripheral region of the oocyte where it disappeared.

It is worth to mention that, in Mugil seheli the yolk deposition appeared after the yolk nucleus was disintegrated. At the end of late perinucleolus stage follicular epithelium was formed.

In the yolk vesicle stage the oocyte surrounded by two layers, the inner zona radiata and the outer follicular epithelium. This stage followed by primary, secondary and tertiary yolk stage respectively. In these stages the yolk globules deposit, so

the nucleus was compressed and became irregular in shape. At the end of this stage, the nucleus migrate to the animal pole. Also the fat vacuoles appear which is the characteristic feature of pelagic eggs.

Autoradiographic studies by Korfsmeier (1966) with the zebra fish demonstrated the transfer of protein from the blood to form yolk. Therefore, most yolk proteins appear to be synthesized outside the oocyte (heterosynthetic). By using the electron microscope Yamamoto and Onozata (1965) observed that, the yolk protein originated intra ovarian, i.e. autosynthetic. Whereas, Norrevang (1968) and Anderson (1974) mentioned that the yolk protein in teleostes appears to consists of both autosynthetic and heterosynthetic types.

Moreover, Balinsky (1970) showed that the nucleus becomes irregular and the flattened nucleoli are in close contact with the cytoplasm. This probably reflect the RNA transportation from the nucleoli into the cytoplasm where it is to be used in the building of yolk.

After the period of yolk deposition, maturation and ripening of oocyte occur very rapidly. In these stages after the migration of nucleus, the yolk globules coalesced together into a mass. Also the nucleus membrane can not be seen. The fatty droplets coalesced to form the oil globules which is found in the ripe eggs.

Marza (1938) placed the rhythm of maturation of the oocytes in three categories: a- Total synchrony, in which all the oocytes of the ovary developed synchronously, as in Oncorhynchus mason (Yamamoto *et al.*, 1959). Partially synchrony, when group of oocytes can be distinguished, indicating that spawning takes place once, a year, with a short and definite season (Guraya *et al.*, 1975) a synchrony, in which several batches of oocytes at different stages are present indicating that the spawning season is long, with several spawnings as in Punilius sophore (Dixit and Agrawala 1974).

In the present study Mugil seheli appears to be belong to the third category (asynchrony) and their spawning season was found to be long period extending from November to March. The spawning pattern in Mugil seheli appears to be of the fractional spawning type through their spawning period. Therefore, the individual Mugil seheli may spawn more than once along the spawning period.

After the end of spawning, some atretic oocytes in the spent ovary was found in addition to small oocytes which constituted the stock for the following year. The wall of the spent ovary becomes thick. Polder (1961) claimed that, resorption of atretic oocytes in the herring was brought about by degeneration of the membrane followed by active phagocytosis.

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LIST OF FIGURES

- Figure 1: Transverse section of the ovary of Mugil seheli showing the chromatin stage cell (A) .IH (X 250).
- Figure 2: Transverse section of chromatin stage cell (A) and early perinucleolus stage cells at different sizes (B). IH (X 25).
- Figure 3: Transverse section of the ovary of Mugil seheli showing yolk nucleus (A), Beginning of late perinucleolus stage cells (B). EH (X 100).
- Figure 4: Transverse section through the end of late perinucleolus stage cells (B), chromatin cells (A), follicular epithelium. Milligant trichrome (X 100).
- Figure 5: Transverse section of the ovary of Mugil seheli showing the yolk vesicle stage cell. Nucleus (N), Nucleolus (Nu), Follicular Epithelium (FE), vacuoles (V), yolk vesicles (YV) and zona radiata (ZR). IH (X 100).
- Figure 6: Transverse section of the ovary showing the beginning of yolk globules deposition. Follicular Epithelium (FE), Nucleus (N), Nucleolus (NU), yolk globules (YG), yolk vesicles (YV) and zona radiata (ZR). IH (X 50).
- Figure 7: Transverse section through the primary yolk stage cells. Follicular Epithelium (FE), fat vacuoles (Fva), Nucleus (N), Nucleolus (Nu), yolk globules (YG), yolk vesicles (YV), zona radiata (ZR). Eirlich eosin haematoxylin (X 50).
- Figure 8: T.S. of the ovary showing the secondary yolk stage cell. EH (X 50).
- Figure 9: T.S. of the ovary showing the tertiary yolk stage cell. EH (X 50).
- Figure 10: T.S. of the ovary showing the mature oocyte stage. Fat vacuoles (Fva), Nucleolus (NU). Zona radiata (ZR). EH (X 50).
- Figure 11: Transverse section of ripe oocyte. Fat vacuoles (FVa), Zona radiata (ZR). EH (X 50).
- Figure 12: The ovary at the end of spawning season. Atretic oocyte (A), Empty follicle (B), Resting oocyte (C), artery of ovary (D), vein of ovary (E), Blood cell (F). EH (X 50).





