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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON OOGENESIS OF COMMON CARP, CYPRINUS CARPIO (L.)

BY

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Key words : Oogenesis, Histochemistry, Gonadosomatic index, *Cyprinus carpio* (Teleostei) .

ABSTRACT

Common carp, Cyprinus carpio has short spawning season in April and May. The gonadosomatic index increase gradually from late August and reach its maximum value in February and March. In April and May the gonadosomatic index decreased due to throw of ripe eggs and some of atretic oocytes. The water temperature during the spawning season ranged between 23 – 25.5°C. The annual cycle of ovary undergoes two successive developmental phases. The primary growth phase includes the immature oocyte characterized by a large nucleus, containing one large nucleolus. Thereafter, the nucleoli increase in number and scatter in the center of nucleus. At the end of this stage, the nucleoli are mostly arranged towards the periphery of nuclear membrane. The secondary growth phase includes vacuolization of cytoplasm, yolk depositions and mature ova. The yolk granules appear around the nucleus, first in the large oocyte, occupy the cytoplasm, with exception the periphery of cytoplasm. The fully mature ova reach its maximum size 970micron in diameter. By using histochemical techniques, there are three types of yolk materials in the oocytes of Cyprinus carpio namely oil droplet, yolk globues and cortical granules. It seems likely that, the oil droplet first appear followed by cortical granules and then yolk granules. The yolk granules are mainly composed of protein, carbohydrate and lipid. However, the vacuoles in the periphery of cytoplasm are composed of carbohydrate, and protein. On the other hand, the granulosa cells in the wall of the ripe ova are mainely composed of carbohydrates and protein. However, the thecal cells are composed of lipid and protein.

INTRODUCTION

Common carp, *Cyprinus carpio* is considered as one of the most economic cultured fish in fresh water fish farms. In Egypt, *Cyprinus carpio* is reared in polyculture fresh water ponds together with grass carp, silver carp, tilapia species and most species of family Mugilidae e.g *Liza ramada* and *Mugil cephalus*. The culture of this fish is closely depended on availability of carp fry that are obtained from hatcharies. Subsequently, the competition between the owners of fish farms for obtaining the common carp fry lead to raising the fry price. Although, this type of fish is successfully cultured in fish farms, but its productivity and spawning in natural fresh water is limited. Therefore, special technique must be used under control to reach the oocytes to final maturation is outmost importance (El Gamal, 2000).

Limited study has been carried out on this type of fish and there is no enough data concerned on the morphology and histology of gonads during the annual cycle. However, numerous studies were carried out on the histology of gonads of many other teleosts comprising, *Schizothorax richardsonii* (Bisht and Joshi, 1975); *Mystus tengara* (Guraya *et al*, 1975); *Synodontis schall* (Rizalla and Yoakim, 1977 a and b); *Limand a limanda* (Htun-Han, 1978); *Fundulus grandis* (Greely *et al*; 1988); *Mugil cephalus* and *Liza ramada* (Mousa, 1994 and 2002), *Solea valgaris* and *Solea aegyptiaca* (Assem,1995), *Dicentrachus labrax* ((Abdo, 1996) and *Sparus aurata* (El Gamal, 1997).

Little attention has been directed to the histochemical changes in connection with gonadal development. In teleosts as in other nonmammalian vertebrates, it has been demonstrated that the female-specific protein (vitellogenin) which is synthesized by the liver in response to -17-β oestradiol, then transported to the ovary (Wallace, 1978). In the oocytes of most animals, yolk is important constituent of teleost oocytes. In teleosts there are three distinct types of yolk material in vitellogenic oocytes namely: oil droplet, yolk vesicles and yolk globules (Nagahama, 1983). The sequence of the appearance of the yolk material varies with species. In the rain bow trout the oil droplet appear soon after the formation of yolk vesicles (Yamamoto *et al*, 1965).

As the oocytes grow, the yolk vesicles increase in both size and number and at maturity they become as cortical alveoli in the periphery of oocyte (Wallace and Selman, 1981). The study on histological and histochemical changes of the ovaries is of outmost importance. Therefore, the present study was planned to investigate cyclic changes occurring in the ovary of common carp, *Cyprinus carpio* in connection with gonadal development and throw light on chemical composition of yolk inclusions of secondary growth phase i.e. vitellogenic oocytes.

MATERIALS AND METHODS

The temperature of water was measured at least three times every week. The monthly minima and maxima of the temperature were averaged to produce a monthly mean of water temperature.

Fish collection:

Cyprinus carpio, was collected alive monthly from fish farms throughout the period extended from (January - December 2002). About 55 females were collected during this period. The total lengths and body weights of collected fish ranged from 15 to 59cm in length and 0.750 to 5.5kg in weight. The ovary was weighed in each fish for nearest 0.01gm, thereafter, the gonadosomatic index is determined as the percentage of the gonad weight to the gutted weight.

Histological and Histochemical techniques:

Small pieces of ovary were fixed in Bouin's fluid for about 48 hours. The fixed ovary was dehydrated through an ascending concentration of ethanol, cleared and embedded in paraplast parafin (m.p. 56-58^{5}C). Transverse sections were cut at 6 to 8 micron in thickness. Section of the ovaries were stained with haematoxylin after Harris, (1900), aqueous solution of eosin was used as counterstain. Some sections were also stained with Mallory triple stain.

The oocyte diameters were measured in section by using caliberated eye-piece micrometer (measuring the maximal and minimal diameter of each oocyt), then the mean of oocyte diameters were calculated.

For histochemical studies, the following procedures were followed :-

1- Sudan black technique for determination of lipid after (Chiffelle an Putt, 1951).

2- The mercury bromophenol blue for determination of total protein after (Bonhag, 1955).

3- The periodic acid Schiff's (PAS) reaction for determination of carbohydrates after (Mc Manus, 1948).

Five stages of gonadal development were recognized according to Zaki *et al.* (1986) and Ashour *et al.* (1990) .

RESULTS

The water temperature during the collection of samples ranged from 28 to 29.5^{5}c in summer and decreased gradually in November to 16.4^{5}c. The lowest of water temperature ranged from 13.0 to 15.5^{5}c in December, January and February. (Fig.1) .

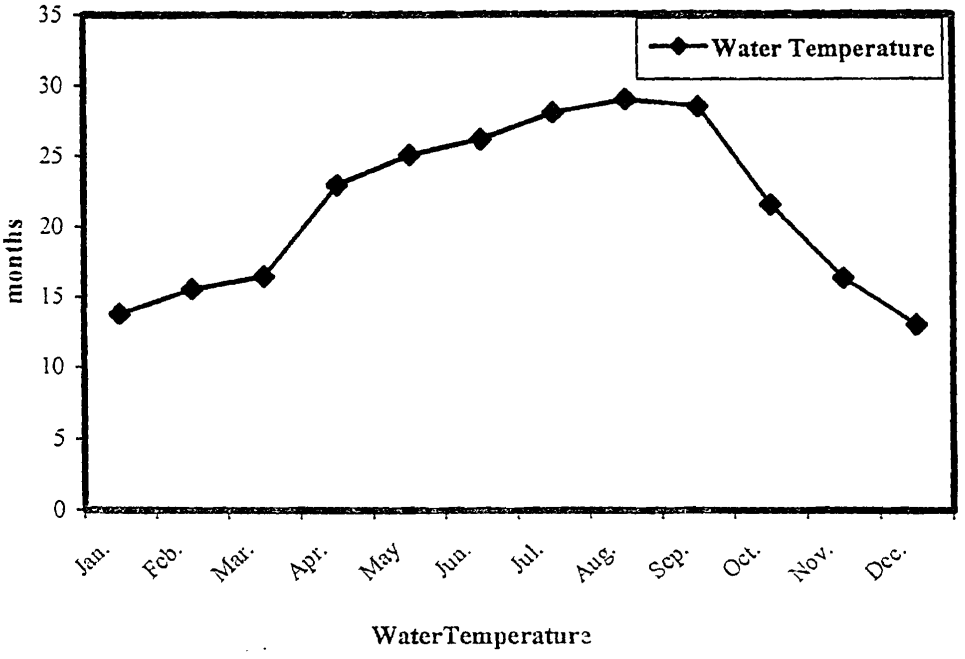


Fig. 1: Monthly fluctuations in water temperature during the sampling period (January - December, 2002).

Cyclic changes in the ovary:

Successive changes occurred in the cytoplasm of oocytes and the nuclei of those cells during the ovarian development. The course of egg development can be divided into five stages. These stages are stage I , immature oocytes (resting stage); stage II , vacuolization of the cytoplasm (preparatory stage); stage III , beginning of yolk deposition (prespawning stage); stage IV , the mature ovum (spawning stage); stage V , egg resorption (postspawning stage).

Histological observations:

Stage I , immature oocytes (resting stage)

This stage appear in the middle of June and continued until late of July in fish ranging from 15 to 25cm in total length. The ovary is transparent and pale rose in colour, occupying about one third of the body cavity. Microscopical examination of the young ovary of *Cyprinus carpio* showed that the oocytes appear small in size. These cells appear in spherical shapes, measuring from 30 to 50 micron in diameter. The nuclei are spherical and occupy most of the cells, measuring about 17-30 micron in diameter. The nucleus is basophilic, containing one single large nucleolus located towards its center . (Fig.2a) . As the young oocytes increase in size (55-85 micron in diameter), more than one of small nucleolei become inside the nucleus. As growth of the oocytes progress , they increase in size and measure about 190 micron in diameter each . The cytoplasm is basophilic and occupy the greater part of the ovum. The nucleus is round in shape and measure about 95 micron in diameter. The nucleoli increase in number and become smaller in size and locate near the periphery of nucleus.(Fig.2b) . The wall of oocyte is thin and composed of follicular epithelial cells.

Stage II , vacuolization of cytoplasm (preparatory stage)

This stage first appears in early of August and ends in late of September, comprising fish over than 25cm in total length. The ovary occupies more than half length of the body cavity and acquires pinkish colour with scattering blood vessels on its external surface. Microscopical examination of sections of ovaries at this stage revealed that the ovary becomes filled with previtellogenic oocytes and some oocytes appear in vacuolated stage. At the beginning, few number of small vesicles appear at the peripheral region of cytoplasm in the oocyte which is now measuring about 195 micron in diameter. These vacuoles have not stained with Harris' haematoxylin (Fig.2b) , but stained light blue after application with Mallory triple stain . The nucleus is more or less round in shape and measure about 97 micron in diameter, having small nucleoli that increase in number and became small in size (Fig.2c) .

As the growth of the oocyte proceeds, the mass of cytoplasm increases and the nucleus becomes irregular in shape. At the end of this stage, these vacuoles increase gradually in size and number and finally the oocyte appears in sieve shaped and measures about 550 micron in diameter. (Fig.2d) . The wall of oocyte is thin and externally composed of follicular epithelial cells and internally thin layer of zona radiata towards the cytoplasm was observed (Fig.3a).

Stage III, beginning of yolk deposition (prespawning stage)

The ovary at this stage become yellow in colour, measuring about more than two thirds of the body cavity. This stage starts by the early of October and extends until the end of March. The oocyte is characterize by increasing in size, with changes in the cytoplasm, nucleus and oocyte membrane. In transverse sections, the ovary is filled with oocytes at different stages of yolk deposition. At the beginning, the nucleus occupies a central position, then its shape become irregular and measures about 95 micron in diameter . At this stage, the oocyte diameter ranged from 570 to 720 micron in diameter. The yolk granules appear as small spheres in the inner part of cytoplasm around the nucleus, leaving the peripheral vacuoles towards the periphery of cytoplasm without having any of yolk depositions. (Fig.3b) . The oocyte membrane measures about 18 micron in thickness and composed of three layers, the outer layer is called thecal, the middle layer is granulosa and the inner layer is striated, zona radiata. (Fig.3c) .

Stage IV : mature ovum (spawning condition ovary)

This stage appear in the middle of April until end of May. Anatomically, the ovary occupies most length of the body cavity and become orange-yellow in colour. At this stage, the ovary is filled with fully ripe oocytes having small disintegrated spheres of yolk depositions (Fig.3d) . The animal pole which could be distinguished by having a small pit (i.e. micropyle) in the wall of oocyte. (Fig.4a & b) . At this stage, the oocyte increases in size and becomes oval in shape, measuring about 980 micron in diameter. The egg membrane is thin and measures about 15 micron in diameter having more striations in zona radiata layer. (Fig.4c) . The ovary at this stage, composes beside the eggs which had reached final maturation, few small eggs with different diameters as well as some oocytes in atretic state.

Stage V , egg resorption (post spawning stage).

This stage appears in late of May and continued for 10 days after the postspawning period. Anatomically, the ovary was reddish, collapse and severely shrunken in size. Sections passing through postspawning ovary showed that the oocytes become in atretic phase in which many of hyaline yolk in oocytes are observed. The granulosa cells are hypertrophied and thecal cells transform into macrophages to invade the oocyte contents. (Fig.4d) .

Histochemical observations:

The histochemical studies on the ovary of *Cyprinus carpio* give an accurate information about the chemical composition of cytoplasm, nucleus and the wall of oocyte. Certain histochemical techniques has been applied for this purpose such as

Sudan black for lipid, bramophenol blue for the total protein and periodic acid – Schiff's reagent for carbohydrate inclusions. Subsequently, the deposition of these stains indicate that the tissue either contains protein, lipid or carbohydrate material. There are three types of yolk materials could be identified in the oocyte of *Cyprinus carpio*, namely oil droplet, yolk globules and cortical granules.

Oil droplets

The yolk vesicles of large ova (i.e. vitellogenic oocyte) appear as empty vacuoles and negatively stained with harris' hematoxylin. The negatively staining may be resulted durng artifact processing in alcohol as in (Fig.2d) . Sudan black B technique is applied for detecting lipid inclusion in cryostat cut sections. The thecal cells and yolk granules as well as a layer underneath zona radiate are positively stained with Sudan black B. However, the vacuoles towards the periphery of cytoplasm and zona radiata are not stained with Sudan black B (Fig.5a & b) .

Yolk globules

The oocytes in the ovary of *Cyprinus carpio* in early developmental stages show an intensive deposition of protein in cytoplasm and nucleoli. However, the chromatin of nucleus exhibited a light protein staining (Fig.5c) .

The oocyte in yolk vesicle stage show a moderate of protein staining in the egg membrane, nucleus and the remnant of cytoplasm. However, the nucleoli show an intense protein staining (Fig.5d). At the end of this stage, the egg membrane and nucleus react strongly with protein staining. However, the remnant of cytoplasm appear as empty vacuoles and stain weekly with bromophenol blue. (Fig.5d) . The deposition of yolk globules are first noticed in oocytes reaching about 570 micron in diameter. Small spheres of yolk deposition are first appear in the inner region of cytoplasm near to the nucleus. The egg membrane, yolk granules and the nucleus show strong reaction with bromophenol blue. However, cortical alveoli in the peripheral region of cytoplasm and the yolk vesicles in the remnant part of cytoplasm show weak reaction with bromophenol blue. (Fig.6a) . With more advancement, the ova become fully mature, measuring about 970 in diameter. The yolk granules acquire an intense protein staining and are scattered throughout the cytoplasm . The peripheral region of cytoplasm which have a weak reaction to bromophenol blue staining (Fig.6b).

Cortical granules

Initially, PAS positive reaction appears as intense rosy dots inside the vesicles scattering along the inner side of peripheral border of cytoplasm of vitellogenic oocytes.

However, the vacuoles located near the nuclear membrane and small oocytes in early developmental stages have not reacted with PAS reaction (Fig.6c) .

With progression in growth of oocytes (yolk deposition), yolk granules around the nucleus, the egg membrane particularly, granulosa layer and the yolk vesicles near the peripheral border of cytoplasm are reacted strongly with PAS staining, However, the nucleus is reacted negatively for PAS staining. This mean that this region is empty from any polysaccharides inclusions (Fig.6d and 7a) . As maturation in oocytes proceeds, the polysaccharides increase in number until each yolk granule became reacted positively with PAS staining and become containing polysaccharide inclusions (Fig.7b). The egg membrane, particularly granulosa layer reacted strongly with PAS staining, however the outer layer of egg membrane, theca layer, and zona radiata in the inner layer stained weakly with PAS staining. (Fig.7c) .

Gonadosomatic index (GSI):

The gonadosomatic index of females of *cyprinus carpio* at different stages of maturation Table (1) and Fig.(8) divided the annual cycle of egg development into five stages:

Stage I, (immature oocytes) started at the period from the middle of June and ended at the late of July. At this stage the mean of gonadosomatic index was 1.107 ± 0.245.

Stage II , vacuolization of cytoplasm (preparatory stage).

This stage extended from early of August to end of September. The gonadosomatic index increased and reached to 4.052 ± 0.852.

Stage III, (prespawning stage).

This stage began in early of October until end of March, the gonadosomatic index reached its maximum value 8.633 ± 1.489 .

Stage IV, (spawning condition ovary).

The spawning stage started from the middle of April until late of May. The gonadosomatic index decreased to 5.912 ± 0.928 due to explosion of some fully ripe oocytes and other oocytes appear in atretic phase.

Stage V, (postspawning stage).

This stage began from late of May and extended about 15 days after postspawning. The gonadosomatic index reached its minimum value 0.630 ± 0.197.

Table 1: Mean of Gonodosomatic index (GSI ± SD) of female common carp, *Cyprinus carpio* at different stages of maturation.

Stages	No. of Fish	Gonodosomatic index		
		Maximum	minimum	Mean ± SD
I	7	1.30	0.81	1.107 ± 0.245
II	6	5.52	3.10	4.052 ± 0.852
III	9	10.53	6.31	8.633 ± 1.489
IV	8	7.50	4.52	5.912 ± 0.928
V	5	0.89	0.39	0.630 ± 0.197

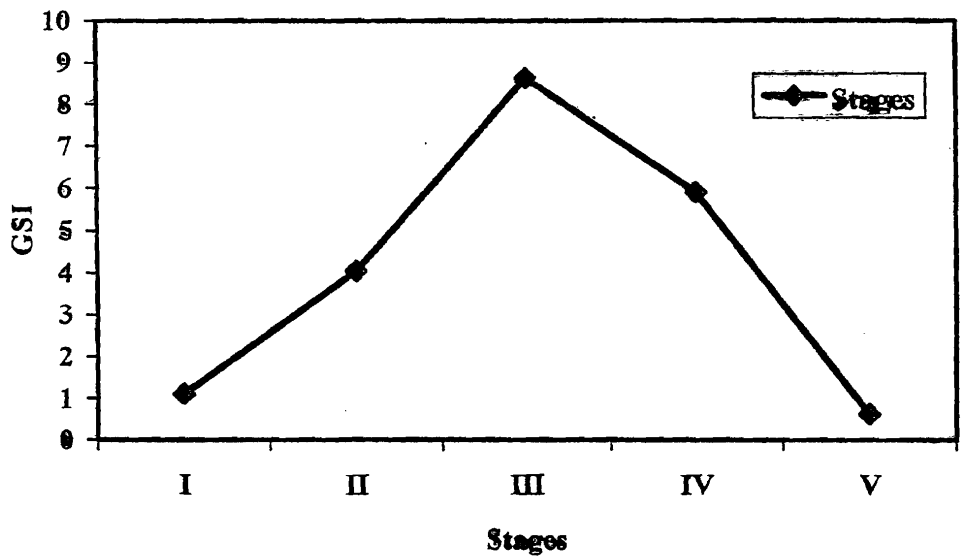


Fig. 8 : The relation between the gonadosomatic index (GSI) of female common carp, Cyprinus carpio at different stages of maturaion.

DISCUSSION

The pattern of fluctuations in the ovaries *Cyprinus carpio* are characterized to each developmental phase and reflected the level of the annual cycle in the ovary . It seems likely that, the variations in photoperiod and fluctuations in water temperature during the annual cycle are two major environmental cues that mediate reproduction activities in *Cyprinus carpio*. The results gathered from the present study have ensured that the water temperature during the fish collection in winter season ranged from 13 – 15.5^5 C, thereafter it increased to reach 23-25^{5}C in spring season . During this period, the ovarian development clearly indicated that the gonadosomatic index increased gradually from August until February – March and started to decrease in April and May. These findings are in accordance with those reported by Yaron and Zermonsky (1986) on *Cyprinus carpio*. In this respect, El Gamal (2000) reported that the gonadosomatic index of *Cyprinus carpio* reached its maximum value in prespawning season (February – March), thereafter it decreased in April and May (spawning season). Khallaf *et al* .(1986) attributed the changes in the gonadosomatic index related to variation of daily photoperiod and water temperature. As in other teleosts, the increase of gonadosomatic index in the ovary of *Cyprinus carpio* during the prespawning season mainly was due to the deposition of yolk material inside the mature eggs as indicated by many authors (Larson, 1974; and El Gamal, 1997).

Cyprinus carpio under the present study is related to the spring spawners in which the water temperature and daily photoperiod started to increase. In this respect, Ashour *et al* (1990) divided fishes into two main groups according to the relation between the ripening of their sexual cells and the water temperature. The first group are summer spawners and the second group are winter spawners. To follow up the ovarian development, several criteria have been used to identify the different stages of oogenesis in *Cyprinus carpio* according to changes in oocytes i.e. composing yolk granules, size of oocytes, nucleus and distribution of nucleoli. Five stages were recognized during the process of oogenesis in the ovary of *Cyprinus carpio* namely: stage I, immature oocyte (resting stage); stage II, Vacuolization of the cytoplasm (preparatory stage); stage III beginning of yolk deposition (prespawning stage), stage IV, the maturation of oocytes (spawning stage) and stage V, egg resorption (postpawning stage). Similar stages were previously described by (Zaki *et al*. 1986 and Ashour *et al*. 1990) in many of other fish species .

It is well known that the growth of oocytes takes place at two developmental phases namely: the primary growth phase and secondary growth phase (i.e. vitellogenic oocyte). The primary growth phase in *Cyprinus carpio* under the present study includes only an immature oocyte which can be divided into three subdivision or phases. Initially, the early young oocyte characterized by having a large nucleus containing one large nucleolus. The early stage of young oocyte of *Cyprinus carpio* is similar to prematuration period of Zaki *et al* . (1986), Synapsis – period of Latif and Saady

(1973), immaturation period of Assem (1992 and 1995) and chromatin – nucleolus stage of El Gamal (1997).

The late immature oocyte stage of the present study characterized by increasing oocyte in size and the nucleoli mostly located towards the nuclear memberane. The late immature stage of the present study is similar to protoplasmic growth of Latif and Saady (1973)and the perinucleolus stage of Mousa (1994 and 2002) and El Gamal (1997).

The second growth phase (i.e. vitellogenic oocyte) of *Cyprinus carpio* includes vacuolization of cytoplasm and yolk deposition. In some other teleosts, the vitellogenic stages were divided into two phases vacuolization and yolk deposition as described by Zaki *et al.* (1986) and Ashour *et al.* (1990). However, in some other fishes, there were four stages: vesicle stages, primary yolk granules stage, secondary yolk granules stage, and tertiary yolk granules stage as described by (Khoo, 1979; Mousa, 1994and 2002 and El Gamal 1997) on the ovary of many other fishes. In *Cyprinus carpio*, the beginning of vacuolization of the cytoplasm appeared in oocytes measuring about 195 micron in diameter. The vacuolization of cytoplasm of ova of *Cyprinus carpio* is similar to maturation stage described by (Yamamoto, 1956 and Yamamoto and Yoshiok, 1964) and vacuolization stage described by (Zaki *et al.* 1986 and Ashour *et al.* 1990).

In the present study, the beginning of yolk deposition first appeared in oocytes measuring about 570micron in diameter in proximity to the nucleus, then scattered in the whole cytoplasm, leaving the vacuoles in the peripheral region of cytoplasm. On the contrary of many other fishes, the yolk depositions first appeared in the peripheral cytoplasm, thereafter scattered towards the center of oocyte as those described by (Zaki *et al.* 1986; Zaki and El Gharabawy 1991; El Gamal, 1997 and Mousa, 2002). In fully ripe ova of *Cyprinus carpio*, small depression in the ovarian wall (i.e. micropyle) could be easily distinguished. The presence of a small opening in the ovarian wall of carp is the place of entrance of the head and trunk of sperm for fertilization . The egg membrane in other teleosts is contained a small opening which is the micropyle through the sperms gain access to enclosed egg (Laale, 1980).

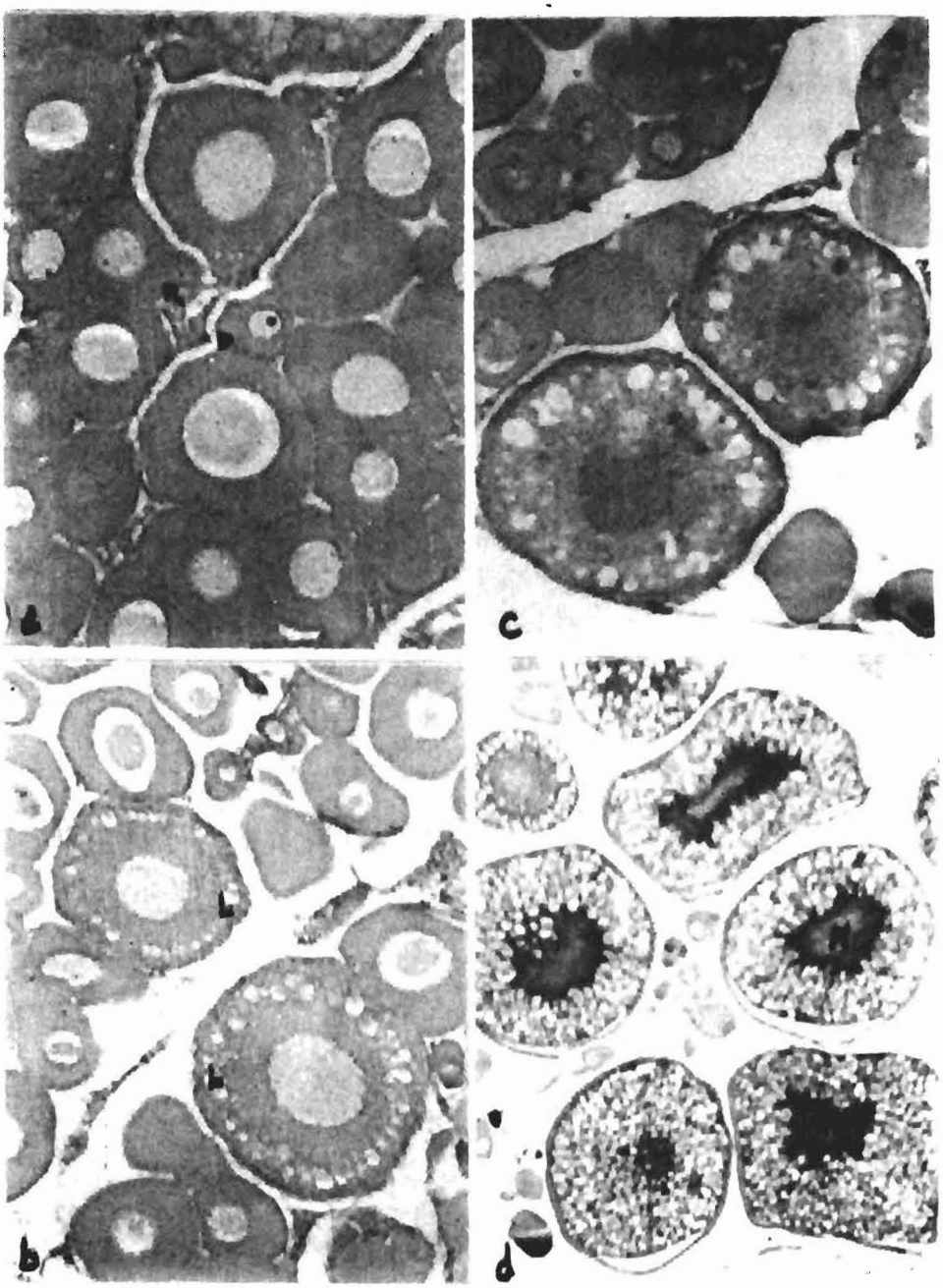
After the oocytes of *C. carpio* reached their final maturation (970micron in diameter), they are spawned. Those which do not succeed to be spawned are reabsorbed and become atretic . The oocyte became amoeboid in shape, in which granulosa layer hypertrophied and yolk granules disintegrated into small granules. Finally, the thecal cells attacked the yolk granules and the follicles appear as sieve and can be easily removed. In this respect, Khoo (1975) gave detailed description of histological changes of follicular atresia of gold fish and found that after complete reabsorption of all remnant oocytes by hypertrophied granulosa cells, it collapse into atrium to form cellular mass. Various authors attributed the follicular atresia to environmental stress (Ball, 1960 and Kamel, 1990).

In teleost as in other nonmammalian vertebrates, it has been demonstrated that a female specific protein (vitellogenin), is synthesized by liver in response to 17-ß estradiol (Wallace, 1978). Most of the yolk protein inside the ova of *Cyprinus carpio* under the present study appeared to be synthesized outside the oocyte (heterosynthetic) and a lesser amount of protein is intraovarian in origin (autosynthetic). Similar results were suggested on the base of electron microscopic study (Yamamoto and Onozato, 1965).

The first appearance of the yolk vesicles (endogenous yolk) in the ova of *C. carpio* appeared in the periphery of ooplasm, thereafter, spread towards the center of oocyte until the whole cytoplasm became vacuolated. The sequence of endogenous and exogenous yolk have been observed by many authors (Ulrich, 1969; Droller and Roth, 1966; Anderson, 1968 and Khoo, 1979). During the endogenous yolk formation in oocytes of *C. carpio* (i.e. vacuolated oocyte), zona radiata is poorly developed, but towards the end of this stage, granulosa and thecal cells have become developed. In this respect, Zaki (1989) suggested the egg membrane of *Solea solea* including zona radiata may be path way for transporing the particles to the interior region of oocytes. The endogenous yolk in oocyte of *C. carpio* has been described as vacuolization (i.e. yolk vesicles). Similar description of endogenous yolk was described with applied histochemical techniques as yolk vesicles, cortical alveoli, vacuoles, yolk globules and yolk spheres (Khoo, 1979). Histochemically, there are three types of yolk material which could be indentified in the oocyte of *C. carpio* namely oil droplet, yolk globules and cortical granules. Two types of yolk inclusions, yolk vesicles and yolk granules were described by (Malone and Hiseka, 1963 and Yamamoto, 1956). However in some other fishes there are three types designated as yolk vesicles, yolk globules and lipid globules Guarya, (1965) The composition of yolk vesicles differ from yolk granules in number of aspects: the yolk vesicles in oocyte of *C. carpio* is mainly composed of polysaccharides However, the yolk granules are mainly composed of protein , polysaccharides and lipids. Khoo (1979) revealed that the yolk granules contain lipids (neutral fat and phospholipids) and protein but no polysaccharides. On the other hand the granulosa cells in the wall of the ripe ova are mainly composed of carbohydrate and protein. However, the thecal cells may be composed of lipid and protein.

CONCLUSION

Cyprinus carpio has short spawning season in April and May. During this period, the gonadosomatic index reached its maximum value and the water temperature ranged from 23 – 25.5^{5}C. The young oocytes are mainly composed of protein, However the large size of oocyte, the yolk granules contain protein, carbohydrate and lipid materials. Depending on these data, we suggest that supplemental food to ripe fish must be supplied with carbohydrate , protein and lipid to improve the quality and quantity of eggs and to accelerate the egg maturation.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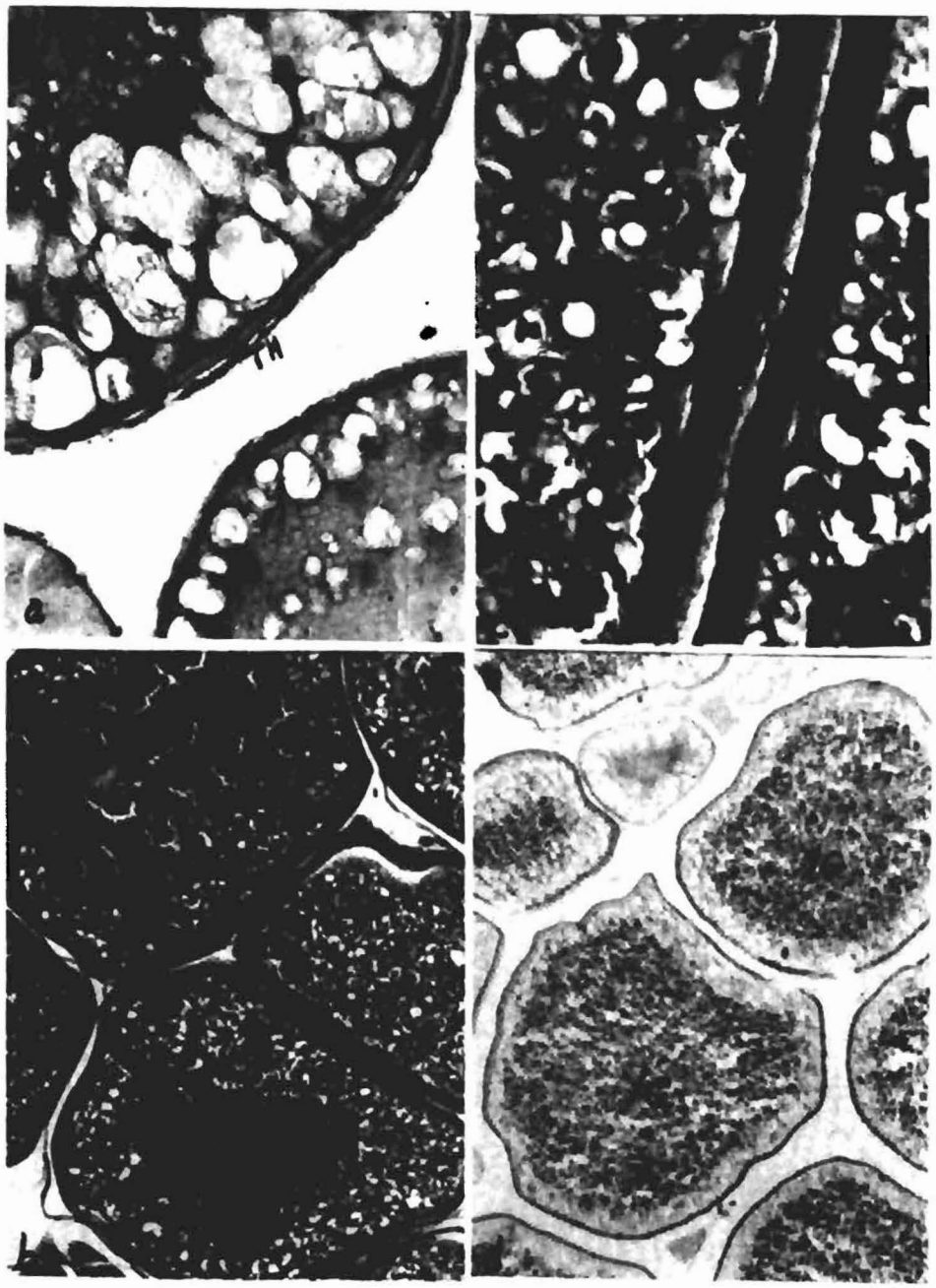

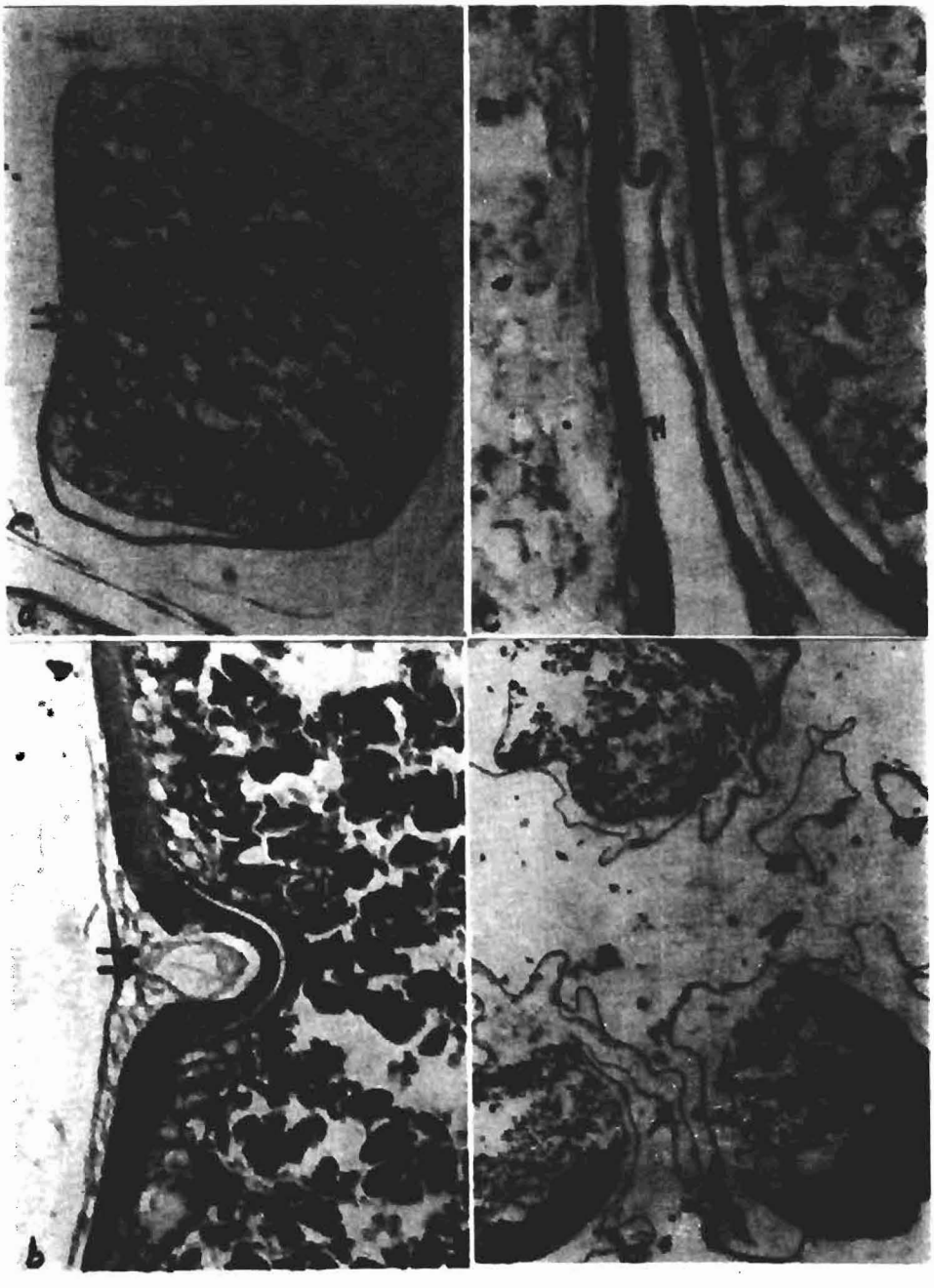

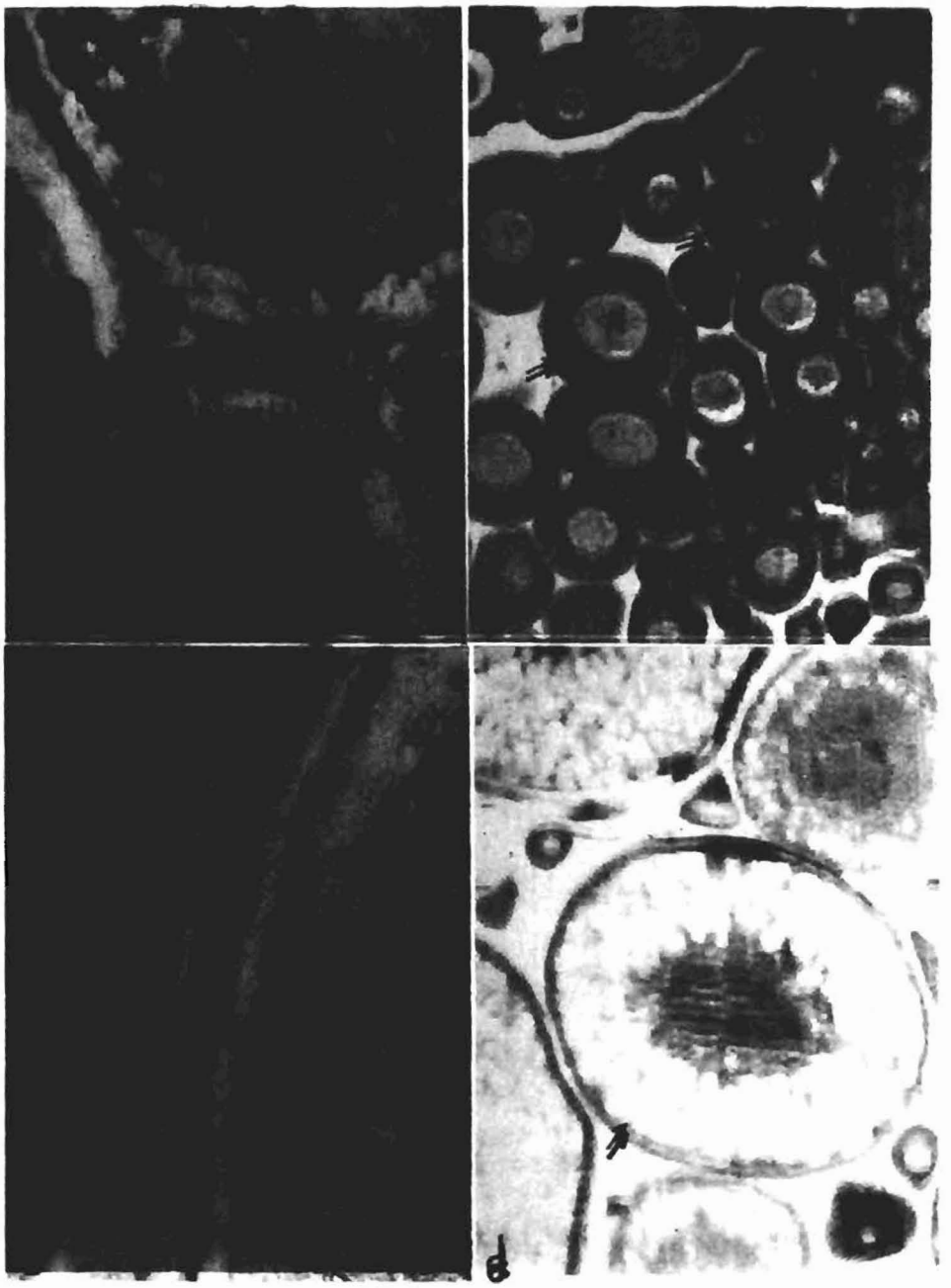

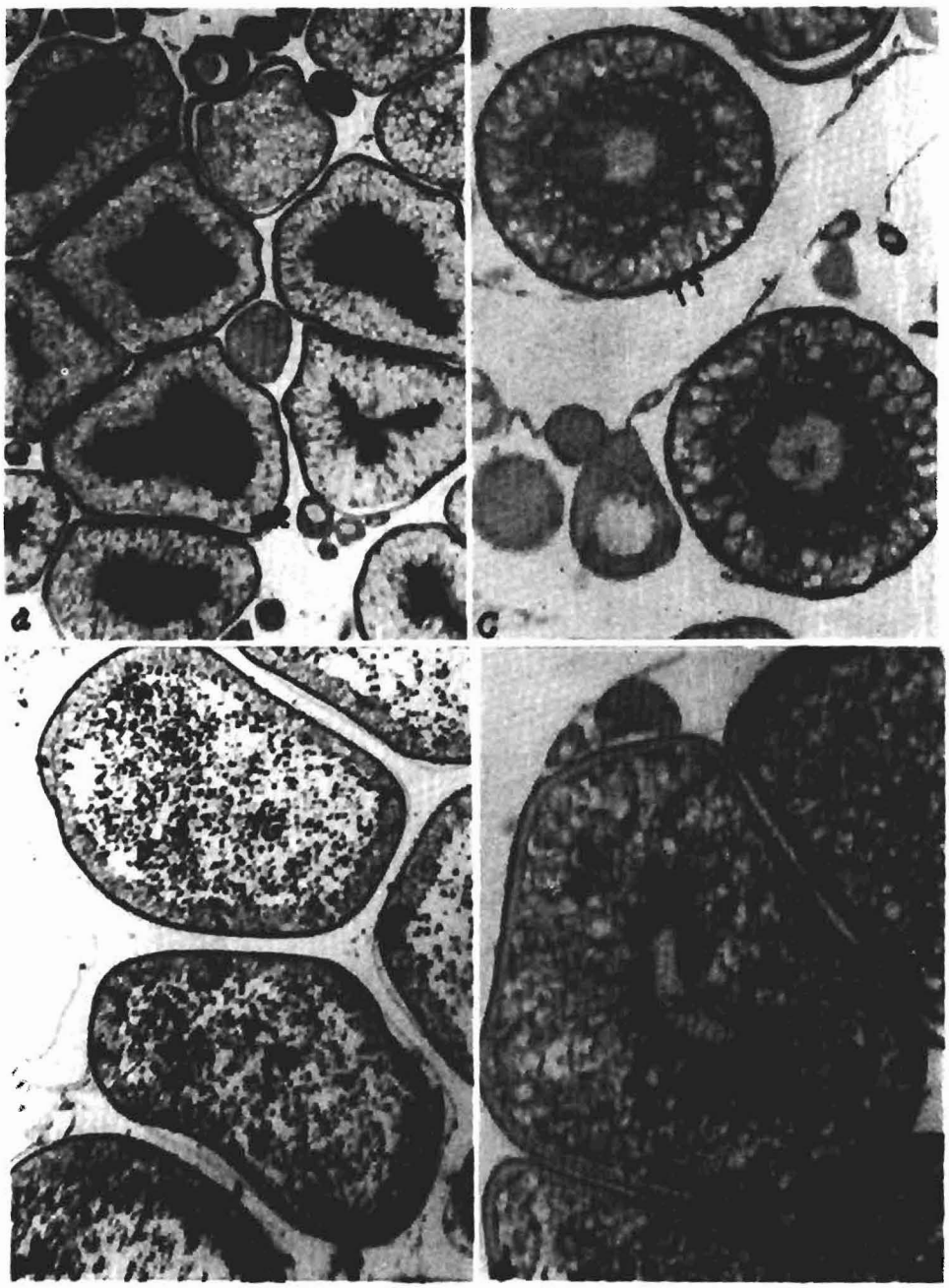

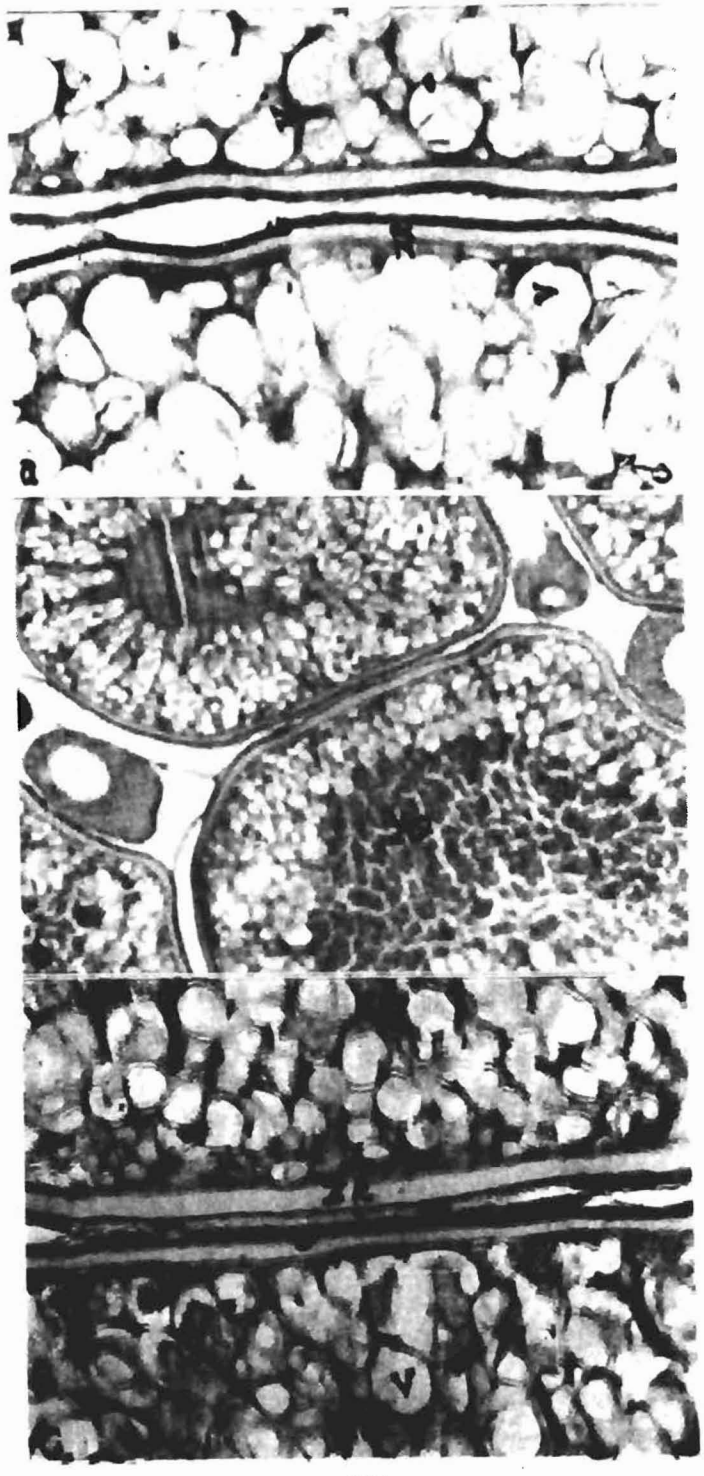

EXPLANATION OF FIGURES

Fig . (2) : Histological appearance of oocyte stage in *C. carpio* at early primary oocyte stage to the end of yolk vesicle stage. Sections a , b and d were stained with Harris's haematoxylin and eosin, section (C) was stained with Mallory triple stain. (a) Early previtellogenic ovary showing, chromatin nucleolus (arrow) and perinucleolus oocytes (arrow head). X 200 (b) the beginning of yolk vesicles of oocytes exhibiting lipid droplet (L) in the periphery of cytoplasm. X 200. (c) similar description as in (b), but, the yolk vesicles appeared as blue colour after stained with Mallory triple stain. X 250. (d) the vesicles in oocytes scatter in cytoplasm and the oocyte appeared as sieve, with exception the area around the nucleus (N) . X 150.

Fig. (3) : Histological appearance of oocyte stages in ovary of c. carpio at the end of yolk vesicles stage to beginning of yolk depositions. Section (a) was stained with Harris,s haematoxylin and eosin, sections b, c and d were stained with Mallory triple stain. (a) Magnified portion in the oocyte wall of yolk vesicle stage showing, thecal cells (TH), granulose (G) and zona radiate (ZR). X500. (b) mid – vitellogenic ovary showing appearance of yolk granules as yellow colour around the nucleus (N) and lipid droplet (L) appeared as blue colour. X100 (c) Magnified portion of (b) showing the follicular wall of vitellogenic oocyte, thecal cells (TH), granulose (G) and zona radiate (ZR). X500. (d) Late vitellogenic oocyte (prespawning ovary), showing tertiary yolk oocytes impregnated with yolk granules (YG). X100

Fig. (4) : Histological appearance of oocytes in ovary of *C. carpio* from tertiary yolk oocyte stage impregnated with yolk globules and atretic oocyte stage. Sections (a), (b) and (c) were stained with Mallory triple stain, section (d) was stained with Harris's haematoxylin and eosin. (a) part of section in the wall of ovum, showing a small pit (i-e. micropyle) (arrow) and yolk granules impregnation (YG). X100. (b) Magnified portion of follicular wall showing micropyle (arrows) . X500. (c) part of section in the follicular wall of tertiary vitellogenic oocyte showing thecal cells (TH), granulose (G) and zona radiate (ZR) stained with yellow colour. X500. (d). part of section in the atretic oocytes showing the wall of ova were disintegrated (arrows), some oocytes were completely removed (arrow heads). X100.

Fig (5) Histochemical appearance of oocytes development in the ovary of *C. carpio.* Sections from (a) to (b) were stained with Sudan black B for lipid, sections from (c). and (d) were stained with bromophenol blue for portions. (a) the yolk granules (YG) were stained positively with Sudan black B. X300 . (b) part of section in the follicular wall of tertiary oocyte, showing the thecal cells were stained positively with Sudan black B (arrow), however zona radiate was not stained with Sudan black B (arrow head). X500. (c) part of section in the early stage of oocyte development, showing an intense protein staining in cytoplasm and nucleus reacted strongly with bromophenol blue staining (arrows). X200. (d) part of section at the end of yolk vesicle stage, showing the egg memrance and nucleus reacted strongly with bromophenol blue staining (arrows) . X200.

Fig (6) : Histochemical appearance of oocyte development in the ovary of *C. carpio.* Sections (a) and (b) were stained with bromophenol blue for protein. Sections from (c) to (d) were stained with PAS for carbohydrate and counterstained with Harris's haematoxylin. (a) part of section in mid-vitellogenic oocytes containing yolk granules reacted strongly with bromophenol blue, also nucleoli and zona radiate. X50. (b). section in tertiary yolk oocytes, the yolk granules (YG) and zona radiate reacted strongly with bromophenol blue the vacuoles (V) underneath zona radiate (ZR) are moderately stained. X50 (C) PAS positive reaction of yolk vesicle stage, showing granulose (G) was stained positively with PAS, however, zona radiate (ZR) showed a weak staining with PAS reaction X200. (d) part of section in the late of yolk vesicle stages , showing granulosa (G) was stained positively with PAS. However, zona radiata (ZR) showed moderate staing with PAS. X300 .

Fig (7) : Histochemical appearance of middle and late vitallogenic oocytes in the ovary of *C. carpio,* Stained with PAS for carbohydrate from section a to c, (a) PAS positive reaction in the wall of middle vitellogenic oocytes showing, granulose (G) reacted strongly with PAS, zona radiate (ZR) gave a weak reaction with PAS reaction. X500. (b) PAS positive reaction in late of vitellogenic oocytes were reacted strongly in the yolk granules (YG), granulose (G) and between the vacuoles of peripheral cytoplasm . X300. (C) Magnified portion of (b) showing granulose (G) and Vacuoles (V) in the peripheral region of cytoplasm were reacted strongly with PAS, zona radiate (ZR) gave a weak reaction with PAS. X500 .

REFERENCES

Abdo, M.A.A. (1996) : Reproductive biology and induced spawning of *Dicentrachus labrax*.Ph.D.Thesis, Faculty of science, Alexandria University, Alexandria, Egypt.

Anderson, E. (1968) : Cortical aveoli formation and vitellogenesis during oocyte differentiation in the pipe fish, *Syngnathus fuscus* and the killifish, *Fundulus heteroclitus*. J. Morphol.125, 23 – 60.

Ashour, M.B; Zaher, M.M. and Rida, S. (1990) : Ecological studies on female reproductive cycle of some fishes of the River Nile at Beni Suef area III. Hisrological studies of the seasonal cycle of the ovary of Chrysichthyes auratus. J. Egypt. Ger. Soc. Zool., 2 : 287 – 297.

Assem, S. (1992) : Reproductive biology and physiology of one species of family sparidae in Mediterranean Sea. M.Sc. Thesis, Faculty of science, Alexandria University, Alexandria, Egypt.

Assem, S. (1995) : Reproductive physiology and induced spawning of Selea species.Ph. D. Thesis, Faculty of science, Alexandria University, Alexandria, Egypt.

Ball. J.N.(1960) Reproduction in female bony fishes. Symp. Zool. Soc. (London) 1: 105 – 135.

Bisht, J.S. and Joshi, M.L. (1975) : Seasonal histological changes in the ovaries of mountain stream teloeost, *Schizothorax richardsonii* (Gray and Hard). Acta Anta., 93(4): 512 – 525.

Bohag, P.F. (1955) : Histochemical studies of the ovarian nurse tissues and oocytes of the milk – weed bug, *Oncopeltus* faciatus (Dallas).

1- Cytology, nuclei acids and carbohydrates. J.Morphol., 96(3) : 381 – 439.

Chiffelle, T.L. and Putt, F.A. (1951). Propylene and ethylene glycol as solvents for Sudan and Sudan black B. Stain Techn. 26 : 51 – 56.

Droller, M.J. and Roth, T.F. (1966) : An electron microscope study of yolk formation during oogenesis in *Lebistes reticulata* guppy. J. Cell. Biol – 28: 209 – 232.

El Gamal, A.A. (1997) : Biological studies on the reproduction of the gilthead bream, *Sparus aurata*, reared in fish farms. Ph.D. Thesis of Science, Mansoura University, Mansoura, Egypt.

El Gamal, A.A. (2000) : Induced spawning of cultivated common carp, *Cyprinus carpio* in fish farms. Bull. Nat. Inst. Oceanoger. and fish., A.R.E., Vol.(26) : 211 – 221.

Greeley, M.S; Macgregor, R. and Marion, K.R. (1988) : Changes in the ovary of the Gulf killifish, *Fundulus grandis* (Baried and Girard) during seasonal and Semilunar spawning cycles. J.fish Biol., 33: 97– 107.

Guraya, S.S. (1965): A comparative histochemical study of fish (*Channa maruleus*) and amphibian (Bufo stomaticus) oogenesis. Z. Zellforsch. Mikrosk. Anat. 65, 665 – 700.

Guraya, S.S.; Kaur, R. and Saxena, P.K. (1975) : Morphology of ovariam changes during the reproductive cycle of the fish, *Mystus tengara* (Ham). Act.Anat., 91: 222 – 260.

Harris, H.F. (1900) : On the rapid conversion of hematoxylin into hematin in staining reaction. J.Appl. Mikcrose., 3 : 777 -780.

Htun, Han, M. (1978) : The reproductive biology of the dab, *Limanda limanda* (L.) in the North Sea : Seasonal changes in the ovary.J. Fish. Biol., 13 : 351 – 359.

Kamel, S.A. (1990) : Study of atresie in the ovary of the Nile Bolti, *Oreochromis niloticus* during its annual reproductive cycle. Proc. Zool.Soc. Egypt, 18 : 1 – 10.

Khallaf, E. A.; Latif, A.F.A. and Alnenaei, A.A. (1986) : Reproduction of *Tilapia nilotica* and *T. zilli* in a Nile canal and its interaction with the environment. Delta. J.Sci., 10(2) : 724 – 747.

Khoo, K.H. (1975) : The corpus luteum of gold fish (Carassius auratus) and its function. Can. J. Zool. 53: 1306 – 1323.

Khoo, K.H. (1979) :The histochemistry and endocrine control of vitellogenesis in goldfish ovary. Can. J. zool., 57 : 617 – 626.

Laale, H.W. (1980): The periviteline space and egg envelopes of bony fishes: A review. Copeia pp. 210 – 226.

Larson, G.L. (1974) : Liver weight of brook trout in a high mountain lake in Washington state. Prog. Fish Cult., 35: 234 – 236.

Latif, A.F.A. and Saady, R.E. (1973) : Oogenesis in the Nile Bolti, *Tilapia nilotica*. Bull. Inst. Ocean. And Fish., Egypt, 3: 183 – 202.

Malone, T.E. and Hisoaka, K.K. (1963) : A histological study of formation of protoplasmic components in developing oocytes of the Zerbrafish, *Brachydanio rerio*. T. Morphol. 112: 61 – 76.

McManus, J.F.A (1948) : Histological and histochemical uses of Periodic acid stain. Technol., 23: 99 – 108.

Mousa, M.A. (1994) : Biological studies on the reproduction of mullet *(Mugil cephalus* L.) in Egypt. Ph.D. Thesis Ain Shams University. pp 278.

Mousa, M.A. (2002) : Immunocytochemical and histochemical study on oogensis in thin – Lipped grey mullet, *Liza ramada* (Risso).J. Egypt.Ger. Soc. Zool., 39c : 549 – 567.

Nagahama, Y. (1983) : The functional morphology of teleost gonads. In: W.S. Hoar. D.J. Randall and E.M. Donaldson (Editors). Fish Physiology. Vol IX(A). Academic Press. New York. Ny. London pp. 233 – 276.

Rizkalla, W. and Yoakim, E.G. (1977a). Studies on the ovary of the Nile Catfish, *Synodontis schall* (Bloch – Schneider). II. Histochemistry and cytology. Proc. Zool. Soc., 5: 229 – 237.

Rizkalla, W. and Yoakim, E.G. (1977b): Studies on the ovary of the Nile Catfish, *Synodontis schall* (Bloch – Schneider). III Pre-ovulatory and post-ovulatory corpora lutea. Proc. Zool. Soc., 5: 239 – 244.

Ulrich, E. (1969): Etude des ultrastructures au cours de l'ovogenese d'un poisson teleosetean, Le danio, Brachydanio rerio J. Microsc. (Paris) 8, 447 – 448.

Wallace, R.A. (1978) : Oocyte growth in nonmammalian vertebrates. In the vertebrate ovary (R.E. Jones, ed.) pp. 469 – 502., New York; Plenum press.

Wallace, R.A. and Selman, K. (1981) : Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool., 21: 325 – 343.

Yamamoto, K. (1956) : Studies on the formation of Fish eggs. 1. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura.* J.Fac. Sci. Hokkaido Univ., Ser., 12: 362 – 373.

Yamamoto, K. and Yoshioka, H. (1964). Rhythm of development in the oocyte of the *Oryzias latipes*, Bull. Fac. Fish. Hokkaido University, 15 (1) : 5 – 19.

Yamamoto, K. and Onozato, H. (1965): Electron microscope study on the growing oocytes of the gold fish during the first growth phase. Mem. Fac. Fish Hokkaido Univ, 13: 79 – 106.

Yamamoto, K. ; Oota, I.; Takano, K.; Ishikawa, T. (1965) : Studies on the maturing process of rainbow trout, *Salmo gairdneri,* irideus. 1- Maturation of the ovary of a one year old fish. Bull. Jpn. Soc. Sci. Fish., 31: 123 – 132.

Yaron, Z. and Levavi-Zermonsky, B. (1986) : Fluctuations in gonadotropin and ovarian steroid during the annual cycle and spawning of the common carp. Fish physiology and Biochemistry Vol. 2 (1-4) : 75-86.

Zaki, M.I.; Dowidar, M.N. and Abdalla, A. (1986) : Reroductive biology of *Clarias gariepinus* (Syn. Lazera) Burchell (Claridae) in lake Manzallah, Egypt. I. Structure of the ovaries. Folia Morphologica, XXXIV (3) : 301 – 306.

Zaki, M.I. (1989): Gametogenesis and sexual cycles of solea (*Solea solea)* in Lake Qarun (Egupt). Problem Ichthiology, 29 (4): 582 – 588.

Zaki, M.I. and El – Gharabawy, M.M. (1991). Histological characters of ovaries of *Mugil capito*, (Egypt) . J.Appl. Sci., 6 (6) : 13 – 23.