

3-бөлім
ГИСТОЛОГИЯ

Раздел 3
ГИСТОЛОГИЯ

Section 3
HISTOLOGY

**Sarmoldayeva G.R.^{1,2*}, Shalgimbayeva S.M.², Omarova Zh.S.²,
Jumakhanova G.B.², Kairat B.K.², Dautbayeva K.A.²**

¹Kazakh scientific research institute of fishery, Kazakhstan, Almaty

²al-Farabi Kazakh National University, Kazakhstan, Almaty,

*e-mail: gafiza_94@mail.ru

THE HISTOMORPHOLOGIC RESEARCH ON REPRODUCTIVE SYSTEM OF CARP (*CYPRINUS CARPIO* L.) FEMALES IN LAKE BALKHASH

Lake Balkhash is an internal reservoir which has a great number of fishes in comparison with other reservoirs in Kazakhstan, and it contains 20 % of yearlong fishing rate at all in our country. Last years, the water in the lake has declined until 40,0 cm. Thus led to the reduction in the flow of the river, the atmospheric distortion in the pool and the depletion of glaciers at the mountains. Due to the aggravation of ecological factors there might be noticed different changes in the sex cells. Thus the comparative ecological – morph physiological study of industrial important fishes is actual problem today. The carp belongs to the species of *Cyprinus carpio* (Linnaeus, 1758). The cyprinids are widely spread in inner water reservoirs and most of them are perfect objects for fish farming. On the other hand, the cyprinids always attract the scientists attention by their broad range of morphological and ecological changes, and by broad adaptability at the area edge. As a result of histomorphologic research there was defined the resorption of oocyte`s yolks on the vitellogenesis phase, the released follicles, bunch of connecting tissue and atresia, also the continuous process of a vacuolation, the bunch of yolks in egg cell, and the developing of zona radiate and chorion layers in the outside of oocyte. According to the results of histological research on high-important industrial carp females, one might say that today`s ecological condition of Lake Balkhash is permanent.

Key words: lake Balkhash, *Cyprinus carpio* L., the reproductive system of females, histomorphologic research.

Сармолдаева Г.Р.^{1,2*}, Шалгимбаева С.М.², Омарова Ж.С.²,
Джумаханова Г.Б.², Қайрат Б.Қ.², Дәуітбаева К.Ә.²

¹Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.

²Қазақ балық шаруашылығы ғылыми зерттеу институты, Қазақстан, Алматы қ.

*e-mail: gafiza_94@mail.ru

Балқаш көліндегі аналық сазан (*Cyprinus carpio* L.) балықтарының репродуктивті жүйесіне жүргізілген гистоморфологиялық зерттеулер

Балқаш көлі Қазақстандағы ішкі су қоймалары арасында ең көп балық қорына ие, жалпы ауланымы еліміздегі жылдық балық ауланымының шамамен 20 %-ын құрайды. Соңғы жылдары көлдің су деңгейі шамамен 40,0 см-ге төмендеді. Бұл өзен ағындарының қысқаруына, бассейндегі атмосфералық шөгінділердің азаюына және биік таулы мұздар қорының сарқылуына алып келді. Экологиялық факторлардың ұшығуына байланысты жыныс клеткаларының дамуында түрлі деңгейдегі өзгерістер байқалуы мүмкін. Сондықтан да кәсіптік маңызы бар балық түрлерінің көбеюін салыстырмалы эколого-морфологиялық тұрғыдан жан-жақты зерттеу қазіргі таңда өзекті мәселелердің бірі болып саналады. Сазан *Cyprinus carpio* (Linnaeus, 1758) тұқы тұқымдасына жататын балық. Тұқылар – еліміздің ішкі суқоймаларында кең таралған және олардың көпшілігі тоған шаруашылықтары үшін қолайлы объект болып саналады. Екінші жағынан, сүйекті балықтардың арасында тұқылар өздерінің кең диапазонды морфологиялық және экологиялық өзгергіштігімен және кең ареал шегінде жоғары бейімдеушілік мүмкіндіктерімен

үнемі зерттеушілер назарын аударып жатады. Гистоморфологиялық зерттеулер нәтижесінде вителлогенез фазасындағы ооциттер саруызының резорбциясы, босаған фолликулдар, дәнекер ұлпалы түйіндердің шоғыры және атрезия, сонымен қатар қалыпты вакуолизация процесі, жұмыртқа клеткасында саруыз түйіршіктерінің жиналуы және ооциттің сыртқы қабықтарының zona radiata және хорион қабаттарының қалыптасуы анықталды. Жоғары кәсіптік маңызы бар сазан аналықтарының көбею жүйесін гистологиялық зерттеудің нәтижелеріне сүйене отырып Балқаш көлінің қазіргі экологиялық жағдайы қалыпты деп айтуға болады.

Түйін сөздер: Балқаш көлі, *Syrpinus carpio* L., аналық репродуктивті жүйе, гистоморфологиялық зерттеу.

Сармолдаева Г.Р.^{1,2*}, Шалгимбаева С.М.², Омарова Ж.С.²,
Джумаханова Г.Б.², Кайрат Б.К.², Даутбаева К.А.²

¹Казахский научно-исследовательский институт рыбного хозяйства, Казахстан, г. Алматы

²Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы

*e-mail: gafiza_94@mail.ru

Гистоморфологическое исследование репродуктивной системы самок сазана (*Syrpinus carpio* L.) озера Балхаш

Озеро Балхаш – внутренний водоем, в котором водится большое количество рыб по сравнению с другими водоемами Казахстана: 20 % всей рыбы, вылавливаемой в нашей стране. В последние годы уровень воды в озере снизился до 40,0 см, что привело к замедлению течения озера, искажению атмосферы в бассейне и истощению ледников в горах. Обострение экологических факторов отражается на морфологии половых клеток местной рыбы. Сравнительное эколого-физиологическое изучение строения важных промышленных рыб является актуальным направлением на сегодняшний день. Карповые получили широкое распространение во внутренних водоемах, и большинство из них является идеальным объектом для рыбного хозяйства. С другой стороны, карповые всегда привлекают внимание ученых из-за широкого спектра морфологических и экологических изменений и высокую адаптивность к внешним условиям. В результате гистоморфологического исследования были определены: резорбция ооцитов в желтке на фазе вителлогенеза, освобожденные фолликулы, пучок соединительной ткани и атрезия, а также непрерывный процесс вакуолизации, скопление желтка в яйцеклетке, развитие слоев zona radiata и хориона снаружи яйцеклетки. Анализ параметров самки караса показал устойчивое экологическое состояние озера Балхаш.

Ключевые слова: озеро Балхаш, *Syrpinus carpio* L., репродуктивная система самок, гистоморфологическое исследование.

Introduction

Lake Balkhash is located in the drought area of Asia, and in the south – east of Kazakhstan. It takes the third place by its area after the Caspian and Aral seas. The water minerality in the west and east parts of the lake are different, and the parts are connected by thin passage. The water is stale in the west part and salty in the east. Lake Balkhash covers the regions of Almaty, Karagandy, Zhambyl and East Kazakhstan. There are 20 species of fish in the lake, and 6 of them are the local fishes (Ili, Balkhash ottoman, Balkhash perch, etc.), and other species came from aside (carp, ide, ship, pike perch, Aral barbell) are irreplaceable objects in fish farming. The carps consist 70% of our country's catching (Kudekova T.K., 2002: 301).

The pollution of inner reservoirs by industrial flow waters does harm to many country's fish farming. The industrial poisonous things fall into the reservoir and harm the fish environment in all cycle:

from nucleus caviar till the adult. Those toxics destroy the fields and spawning places, complicate the fish's migration, decrease the regularity, cause injuries and spoil the trade quality (Lukyanenko V.I., 1967: 202; Arukwe A., 2000: 160).

In recent years household actions of the people influence the environment and it grows every day, and leads to various negative changes existing in the nature. Consequences of such changes are great, and give long-term effect, in most cases to irreversibility. Changes in the habitat of fish are intimately bound to action of a person. Therefore, some fishes living under the influence of anthropogenic factors of Lake Balkhash demand the deep professionally significant research. Changes of fishes in manifold-ing of generation influence on their steady associations and a kinetics. Expansion of system of generation Mani folding fishes on the basis of development process which are the gametogenesis (oo- and spermatogenesis) and a uterus of sexual cycles (Shikhshabekov M.M., 2003: 72).

It indicates connection between a surrounding medium and an organism, and plays a crucial role in efficiency of fish, and as migration influences to the structure of oscillation. In most cases of fish migration in natural reservoirs, features and conditions of efficiency, a condition of dwelling of juveniles are estimated only by its usage, there was not attached importance on function of reproductive system and character. The gametogenesis, including an oogenesis, is an important milestone in development of an individual organism of fish. The nutrients defining accumulation and formation of fund after morphogenetic information develop in this period of an organism (Sharova Y.N., 2003:72; Chmilevskiy D.A., 2003: 377).

Toxins which are the wide range of the pollutants arriving on reservoirs promote process a gametogenesis in living organisms, and also lead to decrease in reproductive potential of individuals and violation of activity and appearance inability of generation. Idiosyncrasy of this type of pathology is not the gonad, according to numerous researches; it meets in various systematic groups of fishes (Lukyanenko V.I., 1990: 26; Wyanski D.M., 2010: 205; Shikhshabekov M.M., 2004: 105; Barkhalov R.M., 2005: 202).

Specifics of process of sexual manifolding a gametogenesis and annual cycles are considered more important link in the course of close connection of separate types. Due to the aggravation of ecological factors some changes in development of sex cells at various levels are observed: nonuniform development of cages, frustration can be observed in dynamics of annual phases development. Therefore ecomorphophysiological comprehensive investigations of professionally significant species of fish is one of actual problems now. A research objective is a gistomorfology research on reproductive system of carp females in Lake Balkhash, with evaluating its current state.

Materials and methods of research

The materials for histological research were gathered in summer of 2015 during the expedition of Kazakh Scientific Research Institute of Fishery (KazSRIF). There were taken 30 female carps for this experiment from stations in the West part of Lake Balkhash, which is named Ulken (Almaty region, Zhambyl district) and in the East, which is Karzhaubai (Almaty region, Karatal district) (table 1).

Table 1 – The main morphological indexes of examined carp females

Station	Q, g	q, g	l, mm	Age
Ulken	675±1440	580±1005	403±315	4-6
Karzhaubai	200±1000	100±820	285±400	3-5

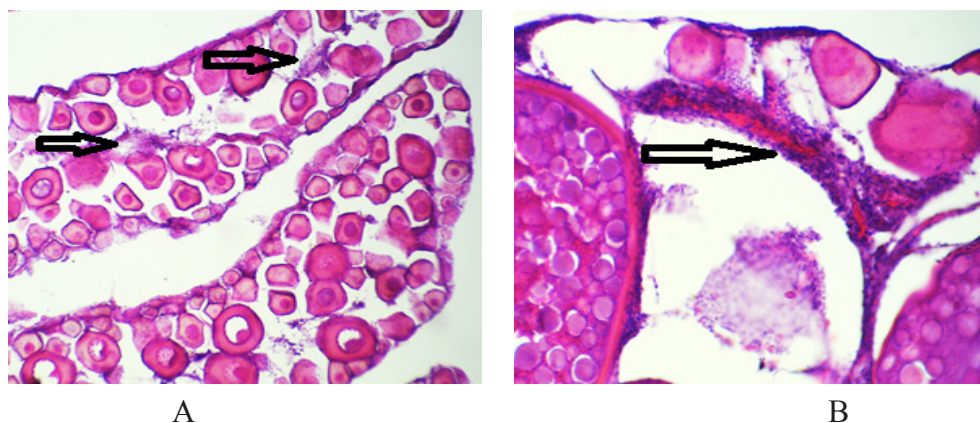
The morphobiological analysis of examined fishes processed by standard methods (Pravdin I.F., 1966: 105). The statistical analysis was carried out with use of the Microsoft Office Excel 2013 program. Organs of reproductive fishes in 4% formalin solution were fixed. Histomorphologic researches were conducted in Ikhtiopatologiya laboratory of KazSRIF. In preparation of tissue specimens the techniques presented by specialists of the All-Russian Research Institute of fishery and oceanology were followed (Mikodina E.V., 2009: 96). The microscope Leica DFC320 (Germany) was used for receiving micrographs from medicines.

Results and discussion

The Ulken station. Age and sex composition of the studied carp females were near at the age of 4-5 year. Parent gonads have the period of ripeness at the VI-VI and III-II stages. As a result of histomorphological research there was defined the resorption of oocyte's yolks on the vitellogenesis phase, the released follicles, bunch of connecting tissue and atresia, also the continuous process of a vacuolation, the bunch of yolks in egg cell (figure 1).

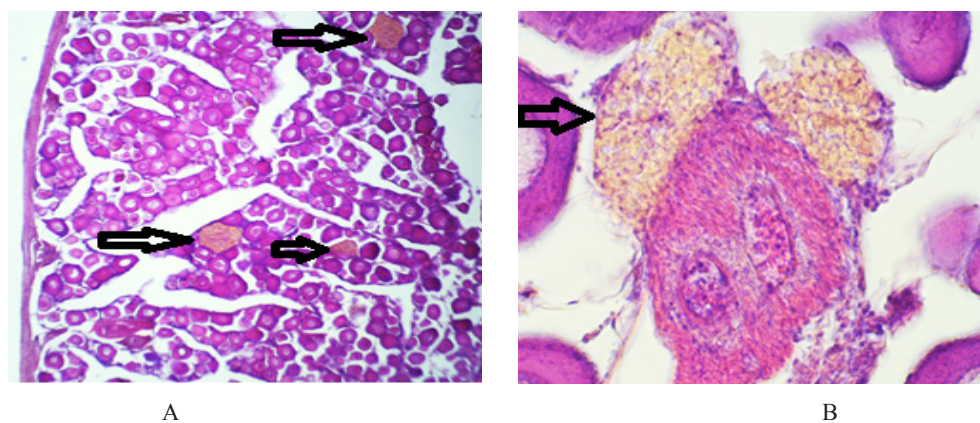
Atresial bodies in the VI-II stage were often found in the 5th age female. in a figure 2 A it is accurately shown that among oocytes of follicles at a one-stage there are atresial bodies. it is possible to tell that it is last year's yolks of an oocyte, in connection with preservation of Atresial bodies in a gonad for 1-1,5. These atresial bodies will eventually collapse and be destroyed (figure 2).

The Resorption of separate oocytes at a stage 2 (D_2) – 3 (D_3) vitellogenesis was found after histological research on female carp organs in the western part of Lake Balkhash. Trophoblastic growth resorption of an oocyte was formed from division of vitelline envelope and from decomposition of nutrients in an oocyte, and the splitting of process cortical alveolar resorption of oocytes in a phase of histologic D_2 and D_3 phases are given below (figure 3).



The released follicles of connecting tissue. A – 100× enlarge, B – 200× enlarge.
The Hematoxylin-eosine dye.

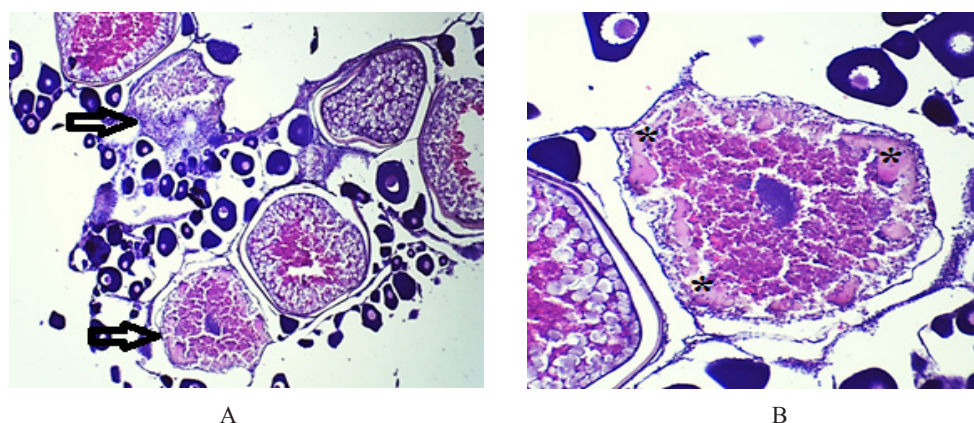
Figure 1 – The carp (*Cyprinus carpio* L.) oocyte section



The atresial bodies. A – 40× enlarge, B – 400× enlarge.the Hematoxylin eosine dye.

Figure 2 – The carp (*Cyprinus carpio* L.) oocyte section.

The VI-II phase of maturing of genitals of females. One-stage follicle phase.



The resorption of oocytes. (*) – the splitting of cortical alveolar.
A – 40× enlarge, B – 100× enlarge. The Hematoxylin-eosine dye.

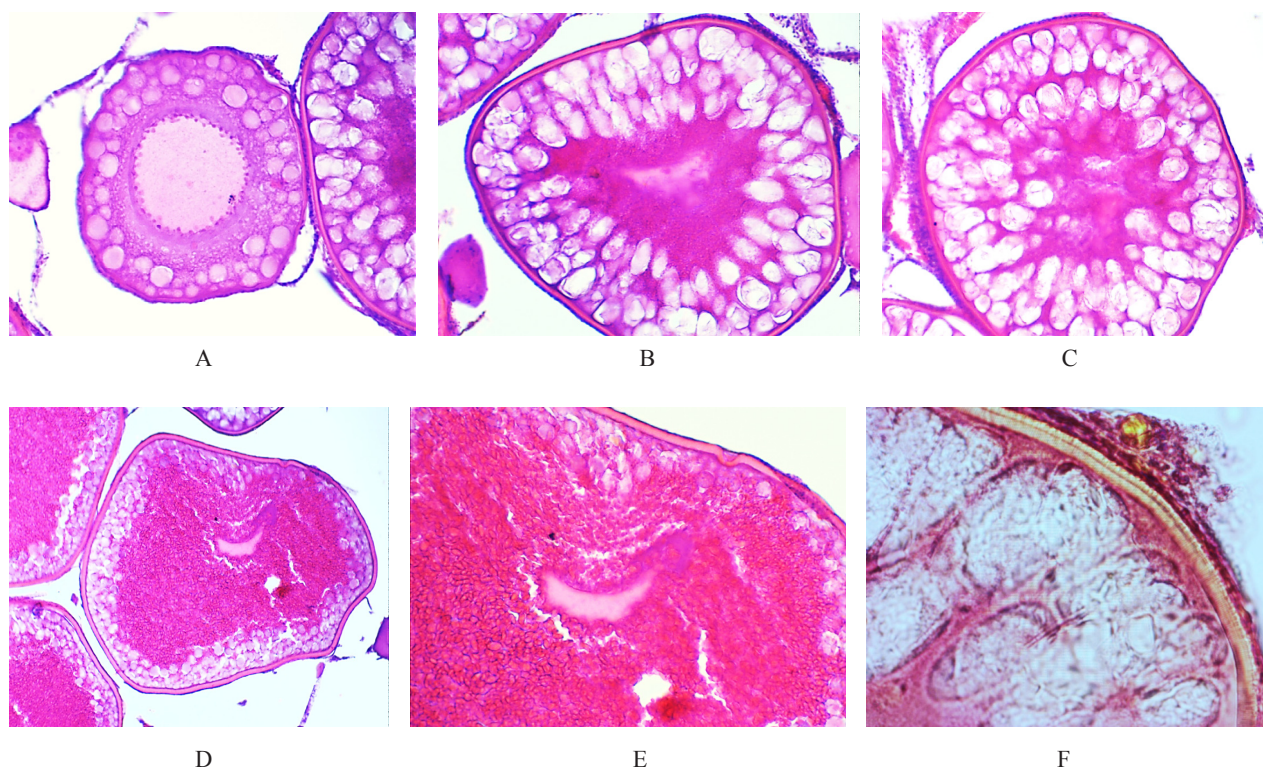
Figure 3 – The histological section of carp's (*Cyprinus carpio* L.) oocyte.
Trophoblastic growth resorption of an oocyte.

Besides, as a result of a research it is revealed oocytes in the period of a vitellogenesis. The sexual products of fishes collected in the station Ulken during Trophoblastic growth it was characterized as follows. On early phases of "D₁" of the oocytes located in a zone of the periphery form serial vacuoles. Core volume round, quantity of yadroid 7-8, it is obvious. on the phase "D₂" a part of an oocyte is filled with 2-3 rows of a vacuole, with a diameter of sex cells at 110-200 microns. Besides, increase in quantity of vacuoles, their diameter of 8-13 microns is registered (an average value on 12,7 microns). the vitelline envelope reached 2-4 microns thick (figure 4, A).

After that vacuoles appear in the inner part of cytoplasm and own completely a zone to its part until the core. It is the "D₃" phase. Vacuoles can reach diameter of 6-16 microns. Vacuoles are located evenly in cytoplasm. Diameter of oocytes is 170-280 microns. The quantity of vacuoles in a circle

of edge of an oocyte is about 40 (figure 4, B). At the phase "E₁" an envelope of a yolk is gradually condensed. Between internal and external vacuole the yolk begins to develop. A diameter of external vacuole remains without changes (figure 4, C). The yolk fills the interior of an oocyte, and also vacuoles outside of an oocyte fill empty interspace. It is the phase "E₂". Diameter of oocytes reaches 450-490 microns.

On the phase "E₃" the yolk fills all parts of an oocyte. The diameter of an oocyte reaches 740-760 microns. With the end of process of collecting a yolk, cage of eggs reach definite criteria (figure 4, E). On the anomaly part of an oocyte the micro Pyle appears. When the oocyte reaches a definite form, it is possible to see legibly external bark of an oocyte. In our histologic figures you can clearly notice layers of Zona radiate of an oocyte and a layer chorion. And also the parts of externa and interna found in a beam part (figure 4, F) are well visible.



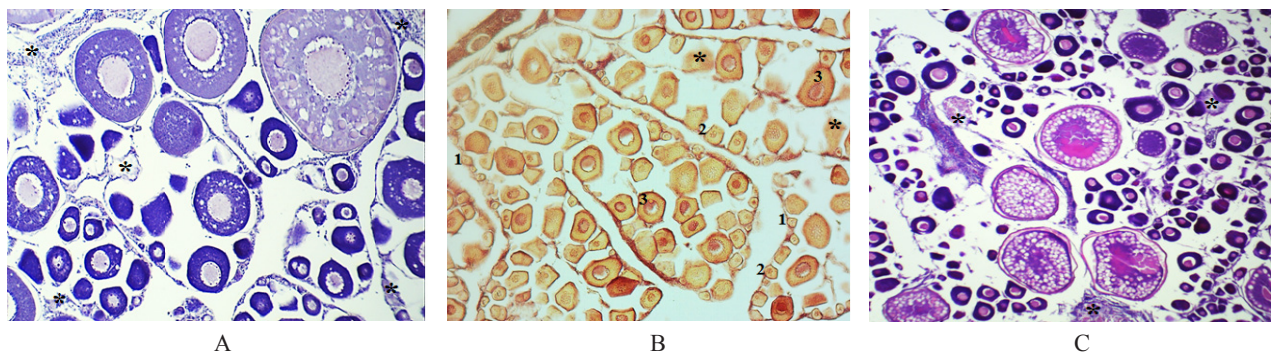
A – The phase «D₂». The two- three lined vacuoles along the periphery. Enlarge 200×. The Hematoxylin-eosine dye.
 B – The phase «D₃». The outer parts of oocytes filled with yolks. Enlarge 200×. The Hematoxylin-eosine dye.
 C – The phase «E₂». The yolks between the middle and extreme vacuoles. Enlarge 200×. The Hematoxylin-eosine dye.
 D – the phase «E₃». The yolk fills all zones, except the alveolus which are cortically located in 3-4 layers. Enlarge 100×. The Hematoxylin-eosine dye.
 E – The definite volume of oocyte. The replacement of core to the anomaly poles. The formation of funnel shaped Micro Pyle. Enlarge 200×. The Hematoxylin eosine dye.
 F – the oocyte crust. Enlarge 400×. The Masson dye.

Figure 4 – The construction of *Cyprinus carpio* L. during the different phases of trophoblastic growth

The Karzhaubai station. The age list of carp females that were investigated in the east part of Lake Balkhash was 3-5 years. As a result of histologic research, there were found the clusters of connecting tissues, a resorption during protoplasmatic body growth, Trophoblastic growth and Vitellogenesis resorption of oocytes. After the analysis at the VI-III stage of maturing, the released follicles resorption which related only for sex cells was found. (figure 5, A) The released follicles resorption met in all fishes at this stage. It is normal physiological process. Over time happens a degeneration to these follicles. the degradation of released follicles is carried out as follows: the calves clenches after an ovulation and its interior is filled with follicular cages of an

epithelium, connecting tissue is deformed and closed by system of capillaries.

During the histologic research of females oosids there was revealed the oogoniya during protoplasmic body growth and oocytes at the phase of a one-stage follicle (figure 5, B). The period of protoplasmic body growth of maturing egg cells is characteristic to this individual and to the II stage of maturing. During the histologic research of oosids it is registered a resorption of the Pre-vitilic oocytes, and also a resorption of oocytes in the period of a vitellogenesis (figure 5, C). A resorption of oocytes is characteristic to the phases D_1 - D_3 of Trophoblastic growth. The VI-III stages of maturing are characteristic to such females.



A – the VI-III phase of maturing : (*) – the released follicles. Enlarge 200×.
 B – protoplasmic body growth: (*) – resorption of the Pre-vitilic oocytes. 1 – oogoniums; 2 – oocytes of juvenal phase;
 3 oocytes at the one-stage follicle phase ; enlarge 40×. C – oocytes at the one-stage follicle phase:
 (*) – the resorption of the Vitellogenin oocytes. Enlarge 100×. The Hematoxylin-eosine dye.

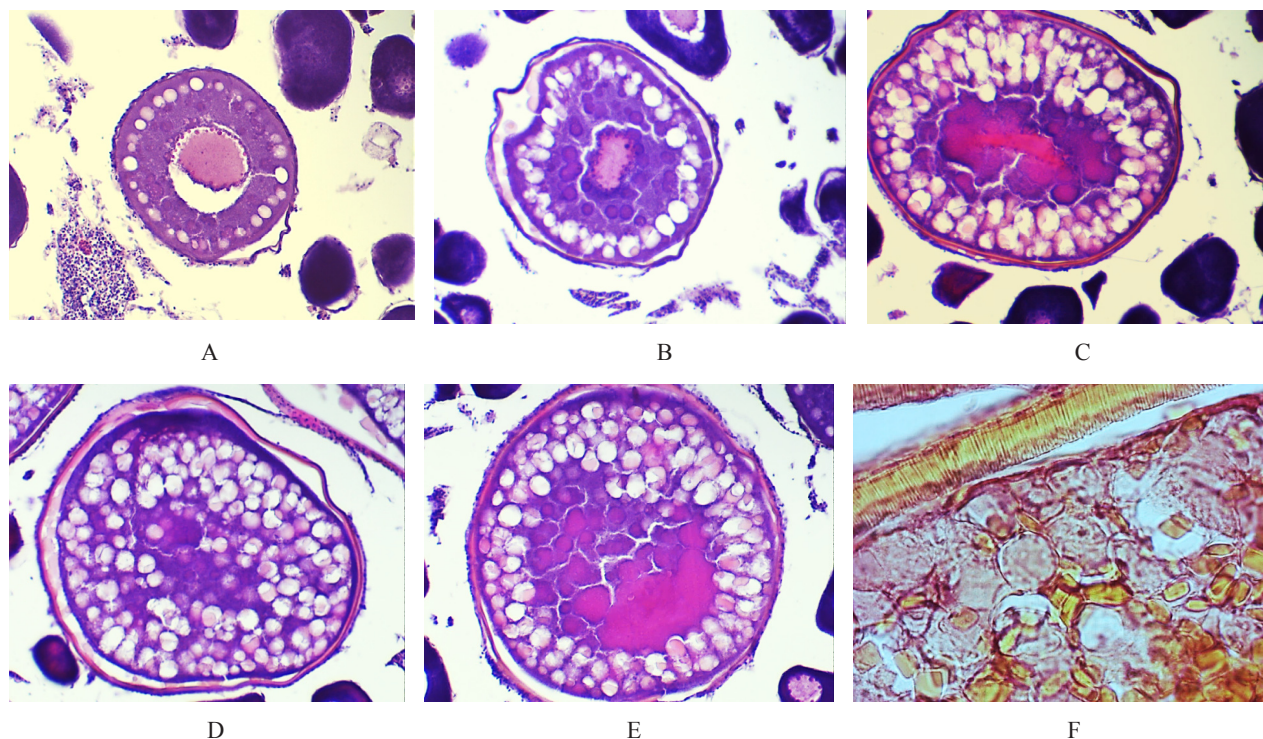
Figure 5 – Histologic figure of *Cyprinus carpio* L oocytes.

Cells of parental egg of carp females in Trophoblastic growth in Karzhaubay station is described as following. the first vacuoles in an oocyte of a carp were formed in the periphery, their quantity reached from 26 to 32. The diameter of the Vacuole of an oocyte, located in one number (their diameter of 3-10 microns) of 75-110 microns and it is the “ D_1 ” phase. Vacuoles are located sequentially. an envelope of oocytes made 3,4 microns (figure 6, A). on the phase “ D_2 ” the vacuole of an oocyte made 2-3 rows. An increase in diameter of vacuoles (4-15 microns) is revealed several times. The diameter of an oocyte was made by 100-130 microns, the envelope of oocytes is 4 microns (figure 6, A).

After the vacuole oocytes fill all cytoplasm, it passes to the phase “ D_3 ”, and the diameter reaches 130-150 microns. The vacuole reaches the diameter from 6 to 17 microns . Shallow vacuoles developed

separately from large vacuoles, that evolved around the oocyte core. And in Average and extreme zones the yolks begin to develop (figure 6, C).

In the phase « E_1 » of a yolk becomes much. diameter of the Oocyte increased a little, these are 130-160 microns (figure 6, D). On the phase “ E_2 ” the yolk fills a half of an oocyte and a vacuole came nearer in cortical zone and their diameter decreases, (on average by 9 microns). The diameter of oocytes is 150-170 microns (figure 6, E). “ E_3 ” phase starts when the oocyte is completely filled with a yolk, and diameter of cortical vacuoles in 2-3 rows remains invariable. the diameter reaches the maximal size and oocytes will be ready for an ovulation. On the phase “ E_1 ” the cover of a yolk oocyte and shell connecting tissue are completely formed. On our micrographs received from histologic specimens it is possible to see legibly beam bark of an oocyte (figure 6, F) .



A – The «D₁» phase. 26-32 vacuoles in one row periphery. Enlarge 200×. The Hematoxylin-eosine dye. B – The «D₂» phase. 4-5 vacuoles fill the peripheral part of the oocyte. Enlarge 200×. The Hematoxylin-eosine dye. C – The «D₃» phase. The peripheral part of the oocyte is filled with vacuoles and it takes the area until the nucleus. Enlarge 200×. The Hematoxylin-eosine dye. D – The «E₁» phase. The yolk fills the vacuoles which are in the middle and peripheral parts of the oocyte. Enlarge 200×. The Hematoxylin-eosine dye. E – The «E₂» phase. The yolk is gathered between the middle and peripheral vacuoles. Enlarge 200×. The Hematoxylin-eosine dye. F – The «E₃» phase. The yolk fills all the part, except the cortical crust where the 3-4 row alveolars are located the oocyte crust. Enlarge 1000×. The Masson dye.

Figure 6 – The parent eggs cells structure of *Cyprinus carpio* L during the different stages of Trophoblastic growth

Conclusion

The result of the histomorphologic research on reproductive system of Carp females (*Cyprinus carpio* L.) in Lake Balkhash is as following:

There were not revealed dangerous Anomalies in a reproductive system of carp females in the station Ulken. As a result of histomorphological research there was defined the resorption of oocyte's yolks on the vitellogenesis phase, the released follicles, bunch of connecting tissue and atresia, also the continuous process of a vacuolation, the bunch of yolks in egg cell.

There was not revealed dangerous pathologies in a reproductive system of carp females in the station Karzhaubai. The stages of D₁, D₂, D₃, E₁, E₂, E₃ and maturing with yolk granules were normal.

However, the resorption of oocytes in the period of a vitellogenesis, the infringement of outer chorion layers, and atresia were defined.

It can be given a good mark on today's state of reproductive system of Carp females in Lake Balkhash. We can make the concept that the microstructural changes are the Normal adaptive physiological state.

Histologic researches of gonads of fishes of Lake Balkhash at the tissue level in the modern ecological conditions proved that the periods and stages of development of an oocyte are developing constantly.

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