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# Ultrastructural characterization of Sertoli cells of Salmonidae from Ohrid Lake during the spermatogenetic cycle

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Ultrastructural characteristics of Sertoli cells of Salmonidae from Ohrid Lake during the spermatogenetic cycle have been analysed. Sertoli cells being an integral part of the seminiferous lobules underwent considerable changes, which influenced their cytomorphological features. The degenerative changes of Sertoli cells were manifested by an extreme vacuolisation, mitochondria in degeneration with widened crysts and thickened matrix, disorganised ER, autophagosomes, "myeline-like" structures and lysed cytoplasmic regions. The above mentioned changes were followed by karyopycnisis, complete degeneration and delamination of cells from the wall of the seminiferous lobules, lysis and detritus formations (Sertoli necrotic material) in the lumen of the lobules. The aim of this paper is special research of the ultrastructural characteristics, i.e. the changes on a level with testes which happen in the postspawning period in the two species of *Teleostei* of Ohrid Lake, Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.). The postspawning period is emphasized in *Teleostei* in this relatively short period, when one reproductive cycle finishes and the following has to start, on a level of testicular parenhyma very important histological changes are going on which give special histological identification, i.e. in the postspawning period there is a complete reorganization of the testes.

*Key words:* Sertoli cells, testes, Salmonidae, Ohrid Lake, spermatogenesis, ultrastructural characteristics.

## Introduction

The number of authors having described the structural and functional characteristics of Sertoli cells in different Teleostei species is noticeable (Billard; Nicols & Graham; Gresik et al.; Dimovska et al.) [1, 10, 4, 2]. However, literature data about the changes in the postspawning period in different species of Teleostei, i.e. changes which occur immediately after the spawning, and even later, are less (Billard; Tavciovska-Vasileva; Tavciovska-Vasileva & Dimovska) [1, 12, 16]. The studies about

the annual reproductive cycle in natural and experimental condition in Salmonidae are also relatively (Hurk et al.) [6]. The Sertoli cells were analysed in the period after the spawning when their phagocytotic role was remarkable (Hurk et al.) [6]. The lack of literature data concerning the testis (Tavciovska-Vasileva & Dimovska) [16], especially the Sertoli cells as somatic components of the seminiferous lobules of testes of the two species of Salmonidae from Ohrid Lake (Tavciovska-Vasileva; Tavciovska-Vasileva & Rebok) [13, 14, 15, 17, 18, 19], has motivated this research. On the other hand, the two species of Salmonidae from Ohrid Lake were chosen as an object of research because of their big economic significance for the Ohrid Lake and due to the fact that they represent a relic and endemic species of this lake.

## Material and Methods

Testes of 100 sexually mature male Salmonidae, i.e. 50 sexually mature male of Ohrid trout (*Salmo letnica* Kar.) and 50 sexually mature male of Ohrid belvica (*Acantholinqua ohridana*) caught in the Ohrid Lake were analysed by electronic microscopy. Small parts of testes, 1-2 mm, were used. The material was prepared using the following procedure: immediately after obtaining tissue specimens, they were fixed in 3% glutaraldehyde and then conserved in 0, 1 M phosphate buffer for 12 hours. After adequate fixation, the material was submitted to postfixation in 1% osmium tetraoxide ($OsO_4$). Further, the material was washed in phosphate buffer, dehydrated in series of acetone and uranil acetate, and then dehydrated in dry acetone. The tissue sections were infiltrated with Durcopan ACM mixture, mixture of acetone-Durcopan, Durcopan No 1, Durcopan No 2, fit in Durcopan No 2 and polymerised. For the ultrastructural analysis, ultrathin sections of 40-60 ηm were prepared using glass knives, on Reichert-Yung "Ultracut" ultramicrotome, installed on cooper nets and contrasted with uranyl acetate and lead citrate. The sections were observed on Tesla BS 500 and OPTON (Zeis) EM 109 electronic microscope. The microphotographs for electronic microscopy were obtained on Agfa Scientia EM Film 23056/6,5 × 9 cm, ORWO NP 20 panchromatic 120, Kodak 120 and made on Agfa papirtone Paper P1-3.

## Results

In the period after the spawning the most important changes in testes of Salmonidae occurred on the level of Sertoli cells, being in the structure of seminiferous lobules as their somatic components. Compared to the period before spawning in which Sertoli cells were characterised with squamous appearance, as the process of involution of seminiferous lobules continued, in the period after the spawning they gradually lost the squamous form, increased their dimensions and acquired polymorphic nuclei. The presence of lipid vacuoles of different sizes was evident in their cytoplasm, especially well seen on ultrathin sections (Fig. 1). At an ultrastructural level a nucleus with prominent nucleolus could be seen in the Sertoli cells' cytoplasm (Fig. 2). On the surface of the nucleus there was a nuclear cover (Fig. 3). Mitochondria with lamellar and tubular crysts (Fig. 4), vesicles of SER and lysosomes could be observed (Fig. 5). Also, at an ultrastructural level, the cell membrane between the adjacent Sertoli cells (Fig. 6), the basal lamina of the seminiferous lobules themselves (Fig. 7), as well as interdigitations between the Sertoli cells were clearly noticed (Fig. 8). One of the functions of Sertoli cells is phagocytosis of the sperm residues. The pre-

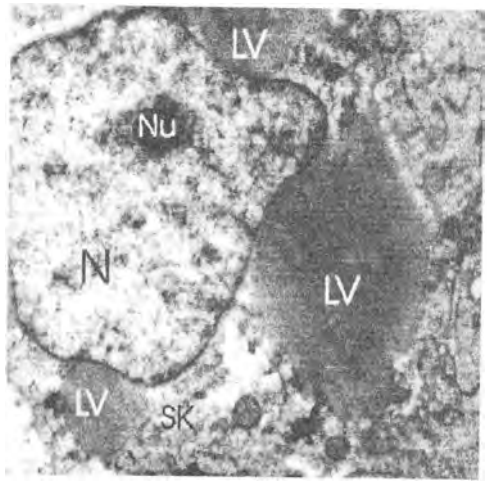


Fig. 1. A part of Sertoli cell (SK) with well seen nucleus (N) and nucleolus (Nu), presence of big lipid vacuoles (LV). Ultrathin section (× 7000)

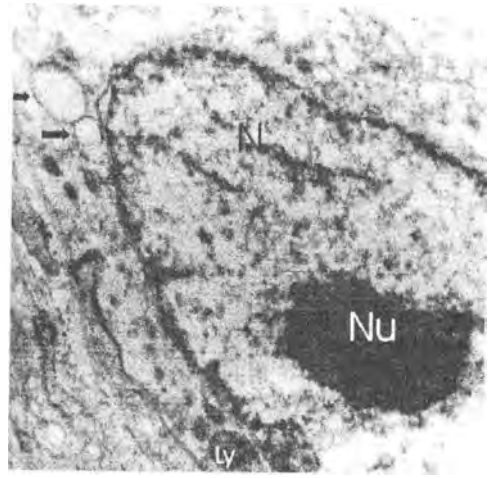


Fig. 2. A part of cytoplasm of Sertoli cell with well visible nucleus (N), prominent nucleolus (Nu), vesicles of SER (black arrows) and lysosomes (Ly). Ultrathin section (× 12 000)

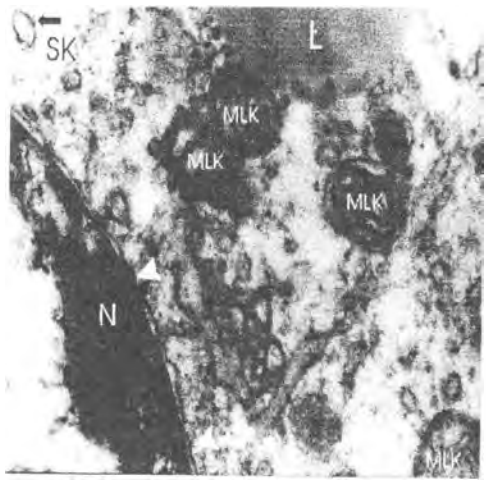


Fig. 3. Cytoplasm of Sertoli cell (SK) with mitochondria with lamellar crysts (MLK), vesicles of SER (black arrow), lipid droplets (L) and nucleus (N) with nuclear membrane on its surface (white arrow). Ultrathin section (× 20 000)

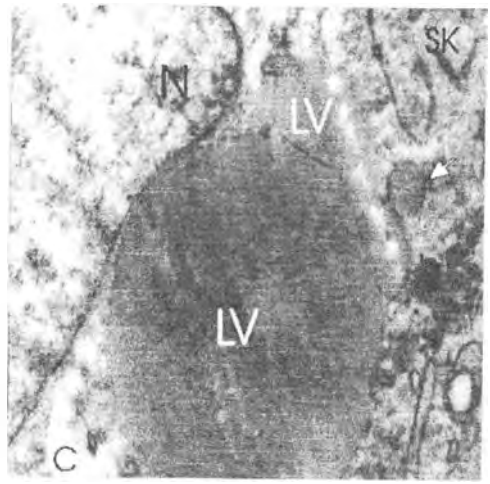


Fig. 4. A part of nucleus (N) and cytoplasm (C) of Sertoli cell (SK) with big lipid vacuoles (LV), mitochondria with tubular crysts (white arrow). Ultrathin section (× 12 000)

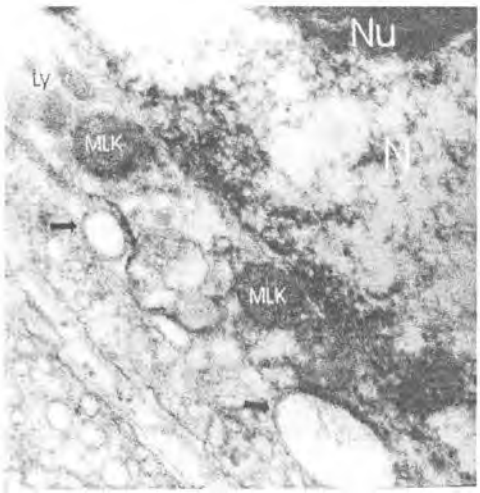


Fig. 5. A part of Sertoli cell with well visible nucleus (N), prominent nucleolus (Nu), mitochondria with lamellar crysts (MLK), vesicles of SER (black arrows) and lysosomes (Ly). Ultrathin section (× 20 000)

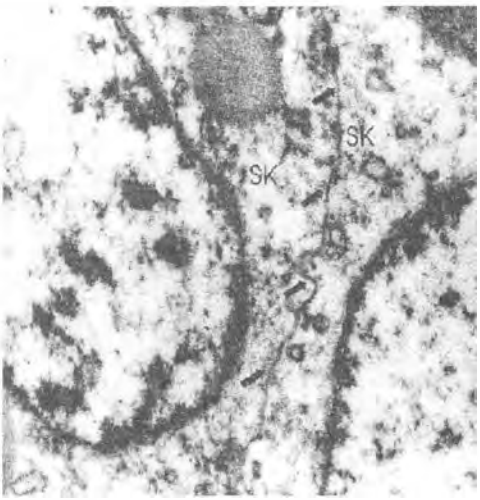


Fig. 6. Clearly visible cell membrane (black arrows) between two adjacent Sertoli cells (SK) Ultrathin section (× 12 000)

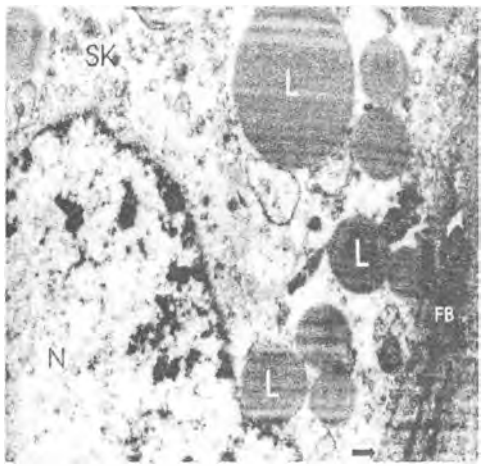


Fig. 7. A part of Sertoli cell (SK). Presence of lipid droplets (L) with different size and well visible nucleus (N). The basal lamina of the lobule (black arrow) and presence of one fibroblast (FB) near the basal lamina are visible. Ultrathin section (× 7000)

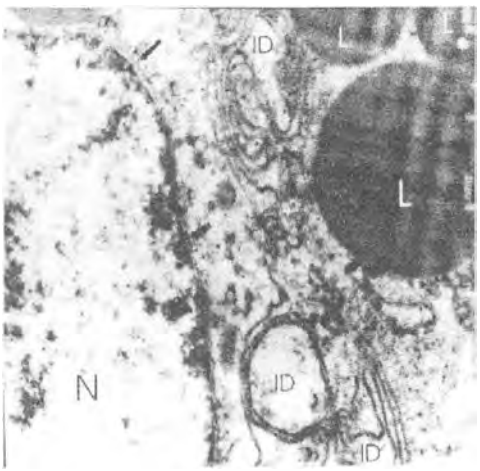


Fig. 8. Interdigitations (ID) between two adjacent Sertoli cells, lipids (L) in the cytoplasm and prominent nucleus (N) with well seen nuclear membrane (black arrows). Ultrathin section (× 12 000)

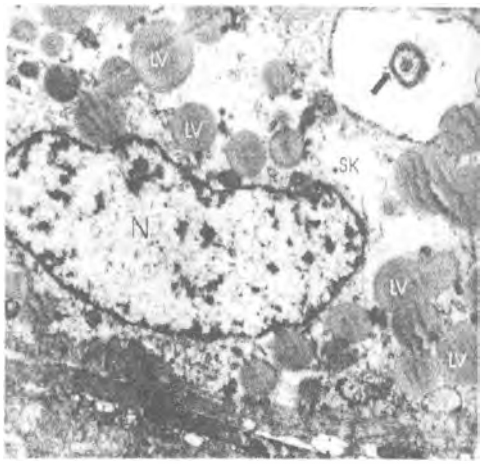


Fig. 9. A part of cytoplasm of Sertoli cell (SK) with well seen nucleus (N) and lipid vacuoles (LV) of different size. Presence of transversally cut fragments of flagellumes of sperm residues (black arrow) Ultrathin section (× 4400)

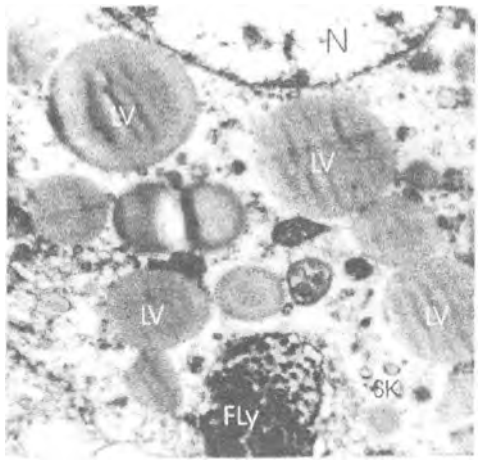


Fig. 10. A part of Sertoli cell cytoplasm (SK) with phagolysosomes (FLy) with sperm residual material. Presence of lipid vacuoles (LV) of different size and a part of nucleus (N) of the Sertoli cell are also visible. Ultrathin section (× 12 000)

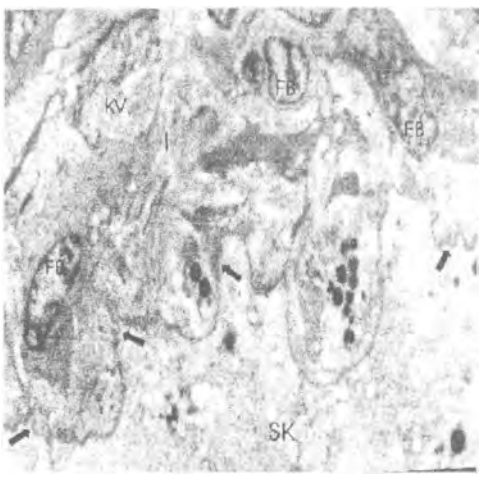


Fig. 11. Well distinguished interstitium (I) with fibroblast (FB) and colagenous fibers (KV). A part of Sertoli cell (SK) cytoplasm in degeneration, is seen, as well as the basal lamina (black arrow) of the lobule. Ultrathin section (× 3000)

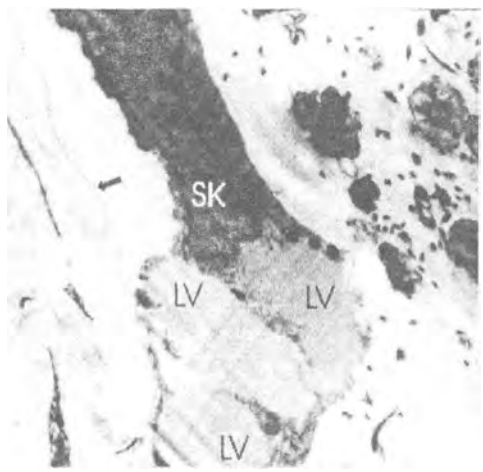


Fig. 12. Sertoli cell (SK) in degeneration. Presence of lipid vacuoles (LV) in the cytoplasm and separation of cytoplasm from basal membrane (black arrow) are visible. Ultrathin section (× 4 400)

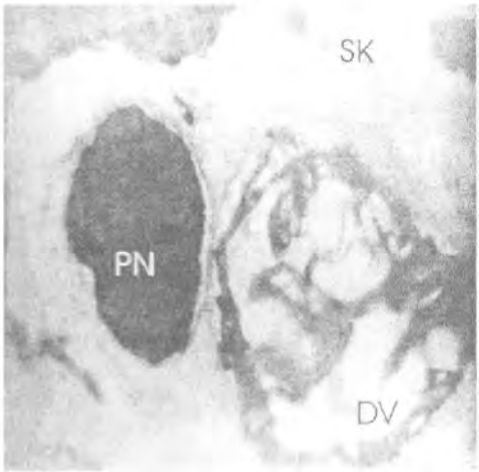


Fig. 13. A part of cytoplasm of Sertoli cell (SK) in degeneration with a pycnotic nucleus (PN) and a digestive vacuole (DV). Ultrathin section (× 12 000)

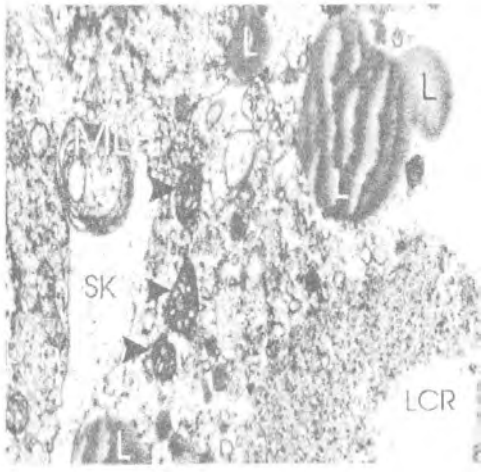


Fig. 14. A part of cytoplasm of Sertoli cell (SK) in degeneration, with lysosomes with "myeline-like" figures (MLF), lysed cytoplasmic regions (LCR), mitohondria in degeneration (black arrows), lipid droplets (L) with different size. A part of one spermatogonium in degeneration (DSp) is shown. Ultrathin section (× 8000)

sence of transversal cut fragments of flagellumes of sperm residues in the cytoplasm of Sertoli cells (Fig. 9) or phagolysosomes with already digested material of sperm origin (Fig. 10) supported this fact. In the later phase of the life cycle of Sertoli cells a more distinct vacuolisation of their cytoplasm could be observed, which caused a degeneration of these somatic cells, characterised by karyopycnosis. The final phases of Sertoli cells' life cycle were followed by exfoliation from the wall of the seminiferous lobules, disintegration and complete destruction of the cells, presence of detritus, i.e. residues in the lumen of the lobules, as well as lysis. Disintegration and destruction of some Sertoli cells which are manifested with torn cell borders, presence of vesicular nucleus or nucleus in pycnosis with emphasized hyperchromatic characteristics, undifferentiated nucleolus were evident on ultrathin sections (Fig. 11). The degeneration of the Sertoli cells was followed by detachment of the nuclear membrane, a process which was well distinguished at an ultrastructural level (Fig. 12). In the cytoplasm of Sertoli cells in degeneration, excluding the presence of pycnotic nucleus, digestive vacuoles, i.e. autophagosomes were noticed, indicative for autophagia occurring on the level of these cells (Fig. 13). On ultrathin sections the degeneration of Sertoli cells was demonstrated by a presence of lysosomes with "myeline-like" figures in their cytoplasm, endoplasmic reticulum in disorganisation, mitochondria with initial signs of degeneration, with widened crysts and thickened matrix, chyaloplasm with granular structure and lysed cytoplasmic regions (Fig. 14). All these changes occurring on the level of Sertoli cells showed their degeneration in the period after the spawning.

## Discussion

The ultrastructural analysis of testes of Salmonidae from Ohrid Lake during the spermatogenetic cycle showed certain features which provided a characteristic histological picture of testes in this period. In the period after the spawning visible changes on the level of seminiferous lobules, especially in the Sertoli cells were observed. All these changes occurred successively. In the initial phase of the period after the spawning sperm residues were still present in the lumen of seminiferous lobules. As changes progressed, degeneration of Sertoli cells took place. The mentioned changes, especially those which happened in the final phase of the period after the spawning, at a sufficient extent, changed the histoarchitectonic of the testes, in comparison with the period before spawning. On the basis of consequent characteristic changes which happened on the level of the testes in the period after the spawning in Salmonidae from Ohrid Lake, we can concluded that this was a period of regeneration of the testes. The seminiferous lobules underwent important transformations in the period after the spawning. As a somatic component of the seminiferous lobules Sertoli cells suffered significant degenerative changes which caused their involution, i.e. involution of seminiferous lobules themselves. This process in Salmonidae repeats every year. The seminiferous lobules and the Sertoli cells themselves, in Salmonidae, are not constant elements of testes, but temporary formations which are formed every year after the spawning. The findings of this study confirmed our preliminary investigations (Tavciovska-Vasileva; Tavciovska-Vasileva & Dimovska; Tavciovska-Vasileva & Rebok) [13, 14, 15, 16, 17, 18, 19] on changes which happen on the level of testes of Salmonidae from Ohrid Lake, i.e. collapsing and disintegration of the lobules, degeneration, i.e. involution of the Sertoli cells, ect. This process was also noted in other Teleostei (Turner; Tavciovska-Vasileva) [20, 12]. Therefore, our results support the difference between mentioned species and mammals, where seminiferous lobules or tubules are constant elements of the testes. There are literature data for different Teleostei species which point out the presence of degenerative changes of Sertoli cells during the period after the spawning. After phagocytosis of the residual bodies by Sertoli cells, they later suffer lipid degeneration, i.e. involution. So, in *Perca Flavescens* Mitch. an involution of the seminiferous tubules in the period after the spawning was described, which in an idirect way points to involution of Sertoli cells, as a unique somatic component of the tubules in this period (Turner) [20], while *Perca fluviatilis* L., (Kulaev) [7] concretely points to some degenerative changes which happen with Sertoli cells in the period after the spawning. Also, similar statements were given about the fate of the Sertoli cells after the finished sexual cycle with *Perca fluviatilis macedonica* Kar. by Dimovska et al. [2] and Tavciovska-Vasileva [12]. After the expulsion of sperm cells in the lumen of tubules, in several species of Teleostei, Sertoli cells suffer lipid degeneration, and probably, finaly are resorbed (Nagahama et al.) [9]. Similarly, it was pointed out that in *Cymatogaster aggregata*, many Sertoli cells suffer degeneration (Gardiner) [3]. The degeneration of Sertoli cells in some species of Atheriniformes, as *Poecilia reticulata* was also described (Billard) [1]. According to Turner [20], the genesis of seminiferous tubules in Teleostei during their embryonic development is similar to that in mammals, but it happens only once in their life, while in Teleostei it repeats every year with the new reproductive cycle. Recently the phenomenon of the life cycle of Sertoli cell has been noted by other authors, not only with Teleostei, but in other low Vertebrata as well (Lofts) [8]. However, the fact is that a small number of authors have dealt with this problem. Relatively few authors have treated the changes which happen immediately after the spawning, and later (Billard, 1970; Tavciovska-Vasileva, 1992;

Tavciovska-Vasileva & Dimovska, 1997) [1, 12, 16]. Our investigation in Salmonidae from Ohrid Lake pointed out that directly after the spawning, similarly to other examined Teleostei, an intensive phagocytosis of sperm residues by Sertoli cells took place. The phagocytic activity of these somatic elements of seminiferous lobules was accompanied at the same time by numerous changes which reflected upon their cytomorphological appearance. Namely, in the prespawning period Sertoli cells are characterised with squamous appearance, whereas in the postspawning period they gradually lost the squamous form and increased their dimensions. The presence of increased number of vacuoles of different size was evident in their cytoplasm. Close to or in contact with these Sertoli cells, as in their cytoplasm numerous sperm residues were edident. In favour of this fact was the presence of transversally and longitudinally cut fragments of flagelumes of sperm residues in the cytoplasm of these cells, later its lysis, which indicated the phagocytotic role of these somatic elements of the seminiferous lobules during this period of the year. In other species of Salmonidae the phagocytotic activity of Sertoli cells in the period after the spawning was reported in *Salmo gairdneri* by Hurk et al. [6]. The phagocytotic activity of Sertoli cells was demonstrated also by the ultrastructural findings of Grier [5]. Gresek et al. [4] noticed presence of philopodia and residual bodies on the level of Sertoli cells in the period after the spawning in *Oryzias latipes*. The presence of philopodia and residual bodies of Sertoli cells has been also pointed out in Poecilidae, *Poecilia latipina* (Pudney & Callard) [11]. The presence of philopodia in Sertoli cells of different species of Teleostei in the period after the spawning was reported in *Cyclostoma nigrofasciatum* (Nicholls & Graham) [10], in *Cymatogaster aggregata* (Gardiner) [3]. In Salmonidae as *Oncorhynchus kisutch* and *Oncorhynchus gorbuscha* the presence of philopodia on a level of Sertoli cells was determined by Nagahama et al. [9].The phagocytotic activity of Sertoli cells in Salmonidae from Ohrid Lake is characterised by subsequent considerable cytological changes, manifested by intensive vacuolisation of the cytoplasm, lipid gegeneration, karyopycnosis, total destruction and delamination, presence of their residues in the lumen of the seminiferous lobules, as well as its lysis, mitochondria with disintegrated crysts, autophagosomes, "myeline-like" structures. All these structural changes point out the degeneration of these somatic cells, i.e. these changes cause their involution and with that the involution of the seminiferous lobules themselves. In other species of Salmonidae similar statement concerning the definitive fate of Sertoli cells in the period after the spawning was given by Hurk et al. [6]. In their study which concerns the testes of *Salmo gairdneri*, Hurk et al. [6] pointed out that in the period of intensive phagocytic activity some Sertoli cells which separate from the wall of the seminiferous lobules could be observed, which cause their degeneration.

## Conclusions

The successive cytological changes based on ultrastructural findings in some regions of testes of Salmonidae from Ohrid Lake during the spermatogenetic cycle, with a special emphasis of Sertoli cells, can be defined like this:

1. Sertoli cells as an integral part of seminiferous lobules suffered considerable changes, changing their cytomorphological aspect. Namely, out of cells with squamous appearance characteristic for the period before the spawning, they gradually increased their dimensions. Lipid vacuoles of different size can be noticed in their cytoplasm while the nuclei acquired a polymorphic form.

2. The close contact of Sertoli cells with the sperm residues, as well as the presence of fragments of their flagellumes in the cytoplasm of Sertoli cells, showed their phagocytic activity.

3. The degenerative changes of Sertoli cells were manifested by extreme vacuolisation, mitochondria in degeneration with widened crysts and thickned matrix, disorganised ER, digestive vacuoles (autophagosomes), "myeline-like" structures and lysed cytoplasmic regions. The above-mentioned changes were followed by karyopycnosis, complete degeneration and delamination of the cells from the wall of the seminiferous lobules, their detritus (Sertoli necrotic material) in the lumen of the lobules and its lysis.

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