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Histological and Ultrastructural Structure of Testes of Salmonidae from Ohrid Lake in the Period of Regeneration

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This article contains the description of the period which follows the period after the spawning, the transitional period or period of regeneration, which is characterised by the presence of spermatogonial generation for the following anual reproductive cycle. In this period of Salmonidae there is regeneration of the spermatogenetic process. The further multiplication of the spermatogonia is more intensive and is shown by numerous mitosis of spermatogonia. The seminiferous lobules are completely filled with two generations of spermatogonia, larger spermatogonia of A type and smaller spermatogonia of B type.

Key words: Salmonidae, Ohrid Lake, period of regeneration, Sertoli cells, spermatogonial generation, histology, ultrastructure.

Introduction

There are many data in literatute which refer to the changes that occurred in the testes in different species of Teleostei during the annual reproductive cycle. These changes are related to the histological and functional characteristics of the testes of Teleostei. The studies which concern the germinative generation of the testes has a dominant place in the previous investigations which treat the annual reproductive cycle at different species of Teleostei, seasonal changes which occured during the year [11, 12]. As to the Salmonidae in literature there are data about the annual reproductive cycle in natural and in experimental conditions [9, 1, 2, 7, 8, 4]. There are few data in the literature about the testes of Salmonidae from Ohrid lake in the period of regeneration [15, 16]. Some authors think that the new spermatogonial generation originate from spermatogonia which are in latent condition before the spawning ("resting spermatogonia") [14, 17, 3, 18, 19, 20]. In experimental conditions the spermatogonial proliferation in the trout was analysed by L o i r [8]. In *Oryzias latipes* a more contemporary analysis and classification concerning different generations of the germinal cells was given by H a m a g u c h i [5] and K a n a m o r i et al. [6] and in *Anguilla japonica* by S a k s e n a et al. [13].

Material and Methods

Testes of sexualy mature Salmonidae males caught in Ohrid Lake were analysed by means of light and electron microscopy. For the light microscopy small parts of testes have been taken immediately after the decapitation of the alive samples then fixed in Bouin fixative and 4% neutral formaline and embedded in parafin. The section are 5 μm thin stained by Hemalaun-Eozin method. The microphotographs for light microscopy have been taken with Wetzlar Ortholux microscope, camera Ortxomat. Small parts of testes 1-2 mm in size have been used for electronic microscopy. For the ultrastructural analysis, ultrathin sections of 40-60 m thickness have been used and later contrasted with uranil acetate and lead cytrate. The sections have been observed on Tesla BS 500 and OPTON (Zeis) EM 109 electronic microscope.

Results

In Salmonidae during the period of regeneration the spermatogonial generation is characteristic for the following annual reproductive cycle. In this period of Salmonidae there is a regeneration of the spermatogenetic tissue. Namely, the further multiplication of the spermatogonia, the new spermatogonial wave is more intensive and is shown by numerous mitosis of spermatogonia (Fig. 1). The seminiferous lobules are completely filled with different generations of spermatogonia. Some of them are larger in size with bright cytoplasm, visible cell borders, a well seen euchromatic nucleus, with emphasised counours, bigger diameter, periferally located chromatin and nucleolus with central position. These cells

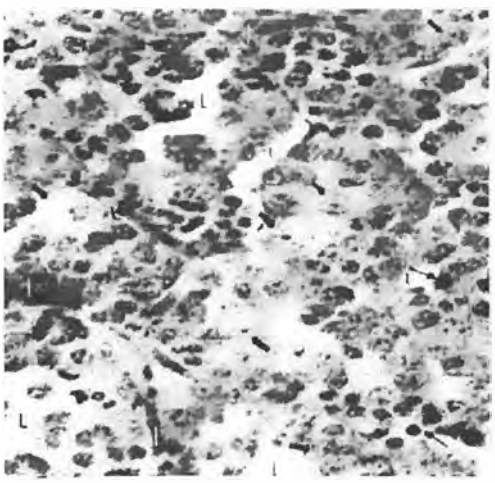

Fig. 1. A part of testis of Salmonidae in the period of regeneration. Presence of different generations of spermatogonia of A and B type (thick black arrows). Presence of spermatogonia in degeneration with pycnotic nuclei (thin black arrows) Accompanying precursor Sertoli cells in contact with the spermatogonia (thin black arrow). Visible lumen (L) of the seminiferous lobules. Interstitium (I) with blood vessels. Hemalaun-Eozin, ×45

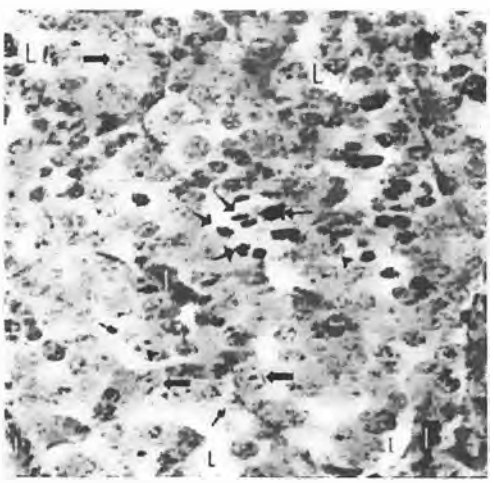

Fig. 2. A part of testis of Salmonidae in the period of regeneration. Presence of different generations of spermatogonia of A and B type (thick black arrows). Some spermatogonia of A type with two nuclei (small black arrows). Noticible mitosis of spermatogonia (pointed black arrows). Presence of spermatogonia in degeneration with pycnotic nuclei (thin black arrow). Accompanying precursor Sertoli cells in contact with the spermatogonia (thin black arrow). Visible lumen (L) of the seminiferous lobules. Interstitium (I) with blood vessels. Hemalaun-Eozin, ×45

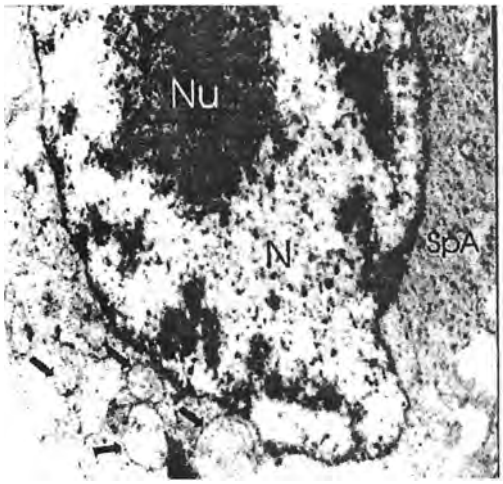

Fig. 3. A part of spermatogonium of A type (SpA) with clearly seen nucleus (N), nucleolus (Nu) and "nuages" particles with perinuclear location (arrows). Ultrathin section, ×12 000

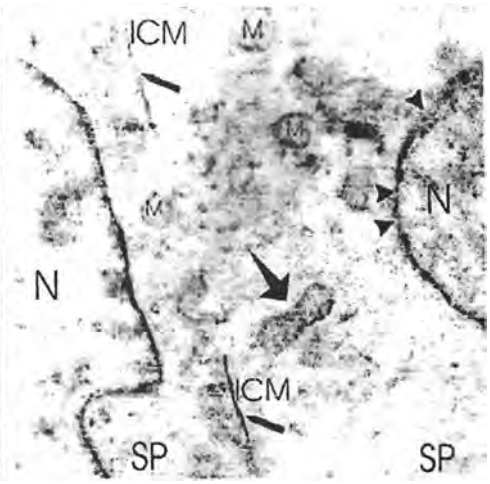

Fig. 4. A part of two neighboring spermatogonia connected among themselves with intercellular bridges (ICM) (black arrows). A part of nucleus (N) with prominent nuclear pores (small black arrows). In the cytoplasm mitochondria (M), some of them in the course of division (big black arrow). Ultrathin section, ×12 000

are spermatogonia of A type. (Figs. 1, 2). In some spermatogonia of A type two nucleoli may be noticed, which shows that these spermatogonia are preparing for division (Fig. 1). In the cytoplasm of some spermatogonia of A type we have noticed presence of thick particles, complex of mitochondria ("nuages") with perinuclear location that represent a characteristic material for the germinative cells (Fig. 3). It was seen at ultrastructural level that spermatogonia which are organised in cysts are interconnected by intercellular bridges (Fig. 4). The prominent pores of the nuclear membrane was noticed and mitochondria in process of division can be found (Fig. 4). Except these spermatogonia of A type, spermatoginia of B type also are observed and they have smaller dimensions (Figs. 1, 2) compared to the spermatogonia of type A. They have smaller diameter of the nucleus and presence of more heterogenous chromatin. In the course of this period the number of spermatogonia of B type progressively increases (Figs. 1, 2). The population of spermatogonia of B type are the most sensitive stage and they are responsible for initiation of the spermatogenesis for the next year, initiating rapid begining of maturation of the germinal cells.These young cells possess darker cytoplasm (Figs. 6, 7) in which ribosomes and polyribosomes can be noticed (Fig. 7) which points to an intensive synthesis of proteins, which is a characteristic of the young cells which grow up. These cells have a clearly seen nucleus with prominent nucleolus (Figs. 6, 7). In the cytoplasm of some spermatogonia "anulate lamele" can be noticed (Fig. 6) and mitochondria with lamelar crusts (Fig. 7) which are in the phase of formation. The spermatogonia of type B are connected among themselves and with the neighbouring Sertoli cells with desmosomes (Fig. 8). In the cytoplasm of these cells also a "nuages" particles can be noticed.. It is noticed that spermatogonia which are organised in cysts at ultrastructural level are connected among themselves with desmosomes. During the period of regeneration of Salmonidae, spermatogonia in degeneration can be observed, that is manifested by hyperchromatic characteristic of the nuclei or karyopyknosis has already occurred with them (Figs. 1, 2). Accompanying precursor Sertoli cells may be observed in contact with the spermatogonia of A and B type (Figs. 1,

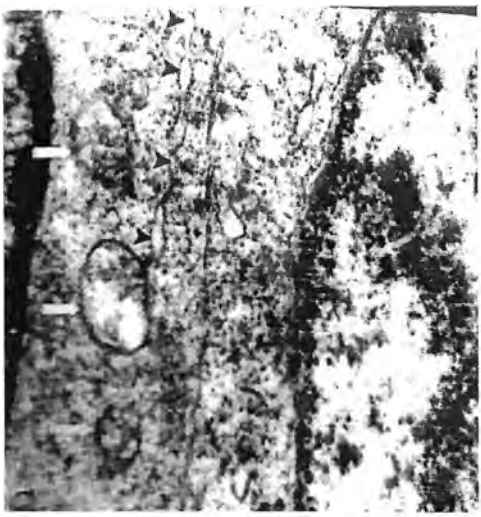

Fig. 5. A part of nucleus and cytoplasm of two young spermatogonia with mitochondria (arrows) and "anulate lamele" (small arrows). Ultrathin section, ×12 000

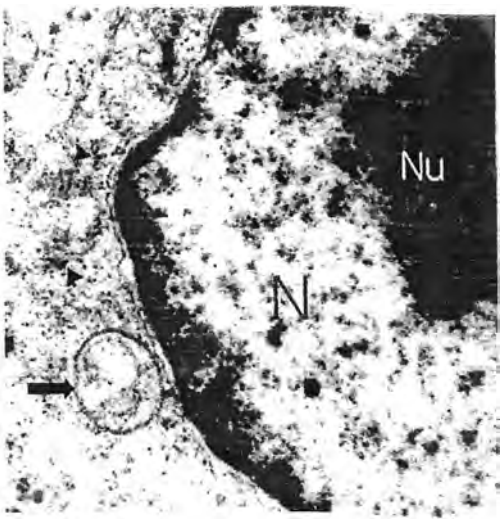

Fig. 6. A part of spermatogonium (Sp) with nucleus (N) and prominent nucleolus (Nu). In the cytoplasm presence of ribosomes and polyribosomes (small arrows), mitochondrium with lamelar crusts in the phase of formation (big arrow). Ultrathin section, ×20 000

2). These precursor Sertoli cells possess a nucleus with triangle or oval shape and a visible nucleolus. In the central part of the seminiferous lobules there is a visible lumen (Figs. 1, 2). The interlobular interstitium of Salmonidae in the period of regeneration is considerably reduced in comparison with the one of the period after the spawning. Although reduced, it

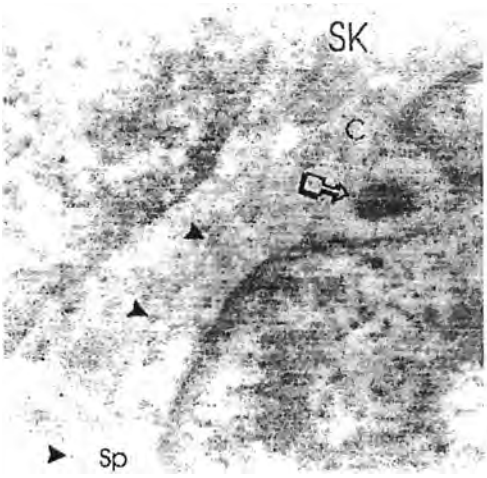

Fig. 7. A part of one Sertoli cell (SK) and one spermatogonium (Sp) connected among themselves with three desmosomes (small black arrows). In the cytoplasm (C) of the spermatogonium presence of "nuages" particles (big white arrow). Ultrathin section, ×12 000

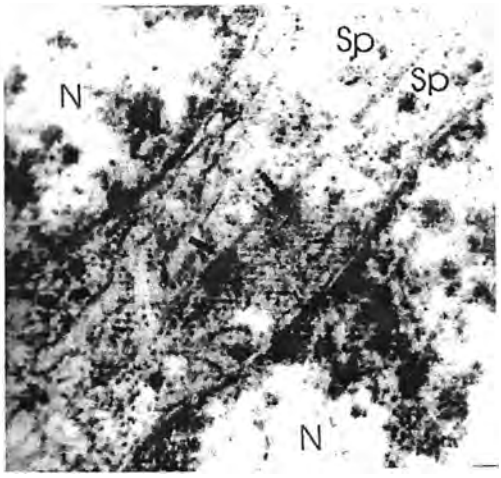

Fig. 8. A part of two spermatogonia (Sp) connected with desmosomes (arrows). Clearly seen nuclei (N). Ultrathin section, ×30 000

is characterised by a rich vascularisation in this period (Figs. 1, 2). In Salmonidae in the period of regeneration tunica albuginea, similary to the interstitium, is considerably reduced in comparison with the one of the period after the spawning.

Discussion

The new spermatogonial generation in Salmonidae which appears in the period after the spawning becomes more representative. The presence of nondiferentiated accompanying precursor Sertoli cells also can be observed [15, 16, 17]. In our preliminary investigations the period of regeneration in Dojran perch (*Perca fluviatilis macedonica* K a r.) [14], Ohrid trout (*Salmo letnica* K a r.) [16] and Ohrid belvica (*Acantholingua ohridana*) [15] was analysed. As to the family of trout (Salmonidae) in literature there are data about annual reproductive cycle in natural [9, 1, 2, 7, 10, 4] as well as in experimental conditions [8]. There are many data in the literature about new spermatogonial generation which has been initiated in the period after the spawning. Reproductive cycle with different species of Teleostei was studied by P a t z n e r and S e i w a l d [11]. Our results concerning ultrastructure of the new spermatogonia of Ohrid belvica (*Acantholingua ohridana*) correspond to the findings of other authors, as well as our previos results on the same or other teleost species [17]. The ultrastructural characteristics of the spermatogonia in the trout was described by L o i r [8]. Our investigations of the spermatogonia in Ohrid trout (*Salmo letnica* K a r.) showed active mitotic divisions of spermatogonia of type B, especially in the period of regeneration. In *Cyprinus carpio* L. an intensive mitosis of spermatogonia of type B was noticed by B i l l a r d et al. [1]. In the cytoplasm of salmonid fish from Ohrid Lake presence of "nuages" particles with perinuclear location were noticed, which is characteristic material for the germinal cells. The structure of "nuages" particles during the differentiation of spermatogonia in *Oryzias latipes* was investigated by H a m a g u c h i [5]. Also, these particles with the spermatogonia of Dojran perch (*Perca fluviatilis macedonica* K a r.) were noted by T a v c i o v s k a - V a s i l e v a [14]. In Ohrid trout (*Salmo letnica* K a r.) "nuages" particles were observed by T a v c i o v s k a - V a s i l e v a [17]. As to the spermatogonial proliferation which occurred in later phase of the spermatogenesis there are many data in the literature [11, 12]. In Ohrid trout (*Salmo letnica* K a r.) the new spermatogonial generation which appears in the period after the spawning becomes more representative. It initiates the following reproductive cycle and originates from rare single spermatogonia. We can see cysts located in the wall of the seminiferous lobules which until the spawning remain in latent condition. Their intensive multiplication starts immediately after the spawning and as a result their number progressively increases, especially in the period of regeneration. In the previous investigations there is different interpretation of the new spermatogonial generation, in different species of Teleostei. Some authors think that new spermatogonial generation of Teleostei originates from spermatogonia which are in latent condition before the spawning ("resting spermatogonia") [14, 17, 3, 18, 19, 20]. As to the source of the primordial germinal cells, at Teleostei, one hypothesis was suggested [12] — there is a reserve of latent, primordial germinal cells in the wall of the lobules during the year. During every annual cycle they underwent mitosis, but only one part enters the spermatogonial cycle. The other cells serve as a source of germinal cells for the following spermatogenesis. In some species of Teleostei, the spermiation is followed by presence of spermatogonia which are latent type of germinal cells. In other species the spermatogenetic activity occurred, but it has been stopped on a level of type A or B spermatogonia. The spermatogonia type A are the most stable stage in which the development of germinal cells has been stopped, while the spermatogonia type B are the most sensitive stage and they are responsible for initiation of the spermatogenesis

for the next year, initiating rapid procees of maturation. During this period the number of B spermatogonia progressively increases. The spermatogonial proliferation in the trout in experimental conditions was analysed by L o i r [8]. There are significant differences in the interpretation which concern the spermatogonial proliferation in different species of Teleostei. As to the new spermatogonial generation in the period after the spawning with Ohrid trout (*Salmo letnica* K a r.) different generations of spermatogonia have been noted, larger in size spermatogonia of A type and smaller spermatogonia of type B. More contemporary analysis and classification of different generations of spermatogonia in *Oryzias latipes* was suggested by H a m a g u c h i [5] and K a n a m o r i et al. [6]; in *Anguilla japonica* by S a k s e n a et al. [13]. Also, in the testes of Dojran perch (*Perca fluviatilis macedonica* K a r.) presence of different generations of spermatogonia (A and B type) was noted by T a v c i o v s k a - V a s i l e v a [14]. In Ohrid trout (*Salmo letnica* K a r.) the presence of nondifferentiated Sertoli cells has been seen [16, 17, 18].

Conclusions

1. In the period of regeneration in Salmonidae the seminiferous lobules are comletely filled with diferent generations of spermatogonia, larger in size spermatogonia of A type and smaller spermatogonia of B type.

2. The further multiplication of the spermatogonia, the new spermatogonial wave is more intensive in this period, manifested by numerous mitosis, especially in spermatogonia of type B. They are the most sensitive stage that is responsible for initiation of spermatogenesis for the next year, initiating rapid process of maturation.

3. In the cytoplasm of some spermatogonia of type A and B "nuagess" particles with perinuclear location can be noticed.

4. In the cytoplasm of the spermatogonia, besides nucleus with prominent pores of the nuclear membrane, a lot of ribosomes, polyribosomes and mitochondria (some of them in the course of division) can be noticed.

5. The spermatogonia of type B (organised in cysts) are connected among themselves and with the neighbouring Setoli cells with desmosomes and intercellular bridges.

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