

Ultrastructure of the New Spermatogonia of Ohrid Trout (*Salmo Letnica* Ka R.) In the Postspawning Period

I. Tavciovskaja-Vasileva

Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje

The new spermatogonial population on ultrastructural level of Ohrid trout (*Salmo letnica* K a r.) in postspawning period has been analysed. The new spermatogonial population in Ohrid trout is represented by different generation of spermatogonia, the primary hypertrophied spermatogonia of type A and secondary spermatogonia of type B, of smaller dimensions. Its multiplication starts in course of the postspawning period and is a representative of the new spermatogenesis for the following year.

Key words: Ohrid trout (*Salmo letnica* K a r.), testis, ultrastructure, spermatogonia of type A and B.

Introduction

In previous investigations which concern the ultrastructure of the new spermatogonial generation in different Teleostei there are many explanations.

In *Oryzias latipes* the structure of the “nuages” particles during the differentiation of the spermatogonia was especially investigated by Clerot [2] and Hamaguchi [3].

In *Ciprinus carpio* L. an intensive mitosis of spermatogonia of type B was noticed by Billard et al. [1].

The ultrastructural characteristics of the spermatogonial population with *Salmo gairdneri* was described by Hurk et al. [4]; Scott & Sumpter [6] and generally about the trout by Loir [5].

Material and Methods

Testes of sexually mature Ohrid trout (*Salmo letnica* K a r.) males caught in Ohrid Lake in a period of 3 years (1993–1996) have been analysed. Analyses have been done with electronic microscope. Small parts of testes 1-2 mm big have been used for electronic microscopy. The material has been fixed according to following procedure: Immediately after the tissue sections have been taken, they are fixed in

3% glutaraldehyde and then conserved in 0.1 M phosphate buffer. After adequate fixation the material has been submitted to postfixation in 1% osmium tetroxide (OsO₄). In the further treatment the material has been washed in phosphate buffer, dehydrated in series of acetone and uranyl acetate. The tissue parts have been infiltrated with Durcupan ACM mixture, mixture of acetone-Durcupan, Durcupan No 1, Durcupan No 2, first in Durcupan No 2 and polymerised. For the ultrastructural analysis, ultrathin sections of 40-60 nm thickness have been prepared, with the help of glass knives, on Reichert-Yung: Ultracut" ultramicrotome, installed on copper grids, contrasted with uranyl acetate and lead citrate. The sections have been observed on Tesla BS 500 and OPTON (Zeiss) EM 109 electronic microscope.

Results

The new spermatogonia of Ohrid trout (*Salmo letnica* K a r.) come from latent spermatogonia located in the wall of the seminiferous lobules individually or in groups, the number of which increases a lot in the postspawning period and is a representative of the new spermatogenesis, i. e. the new reproductive cycle.

On the ultrathin sections in the cytoplasm of some spermatogonia of type A we have noticed presence of thick particles, i. e. complex of mitochondria ("nuages") of perinuclear location (Fig. 1) which represents a characteristic material for the germinative cells.

In contrast of these spermatogonia in the wall of some seminiferous lobules spermatogonia of the second generations can be seen, i. e. groups of spermatogonia of small dimensions, that is of smaller diameter of the nucleus and presence of more heterogeneous chromatin, i. e. secondary spermatogonia of type B (Fig. 2).

On the ultrastructural level completely well can be noticed that the new spermatogonial generation is represented by young cells. These young cells possess darker cytoplasm (Fig. 3, 4, 5) in which mitochondria with electron dense matrix and lamellar crusts (Fig. 5) in phase of formation, can be noticed.

In the cytoplasm of some spermatogonia "lamellae annulate" (Fig. 4), a lot of ribosomes and polyribosomes (Fig. 2, 5) can be noticed, which points to an intensive synthesis of proteins which is characteristic of the young cells which grow up.

These cells have a clearly seen nucleus with prominent nucleolus (Fig. 3, 4, 5, 6).

It is noticed that spermatogonia which are organised in groups (cysts) at ultrastructural level are connected among themselves with desmosomes (Fig. 7, 8).

Discussion

Our results which concern the ultrastructure of the new spermatogonial generation of Ohrid trout (*Salmo letnica* K a r.) correspond with the findings of other authors, as well as our previous results which concern the same or other teleost species [7, 8, 9].

The ultrastructural characteristics of the spermatogonial population with *Salmo gairdneri* was described by H u r k et al. [4]; S c o t t & S u m p t e r [4] and generally about the trout by L o i r [5].

Our investigations of the spermatogonial population in Ohrid trout (*Salmo letnica* K a r.) point to intensive mitotic divisions of spermatogonia of type B, especially in the period of regeneration.

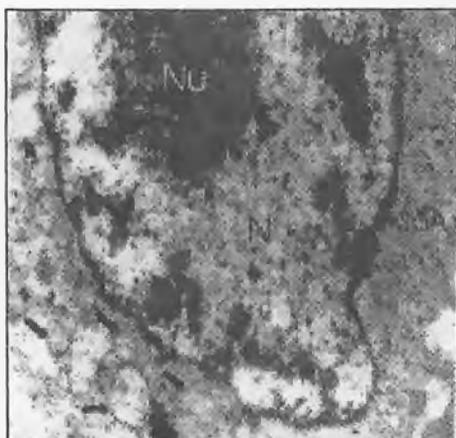


Fig. 1. A part of spermatogonium of type A (SpA) with prominent nucleus (N), nucleolus (Nu) and "nuages" particles with perinuclear location (arrows). Ultrathin section ($\times 12\ 000$)

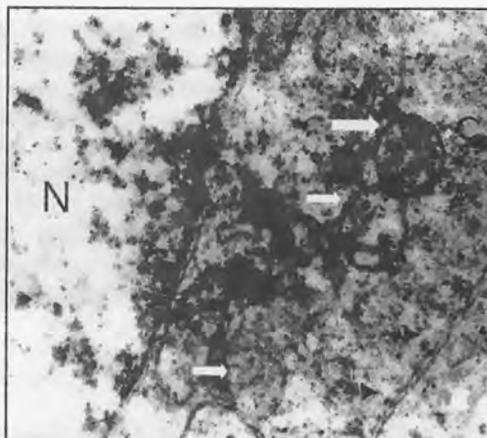


Fig. 2. A part of nucleus (N) and darker cytoplasm (C) of young spermatogonium. Mitochondria (arrows) with electron dense matrix and tubular crists in the phase of formation, ribosomes and polyribosomes (small arrows). Ultrathin section ($\times 20\ 000$)

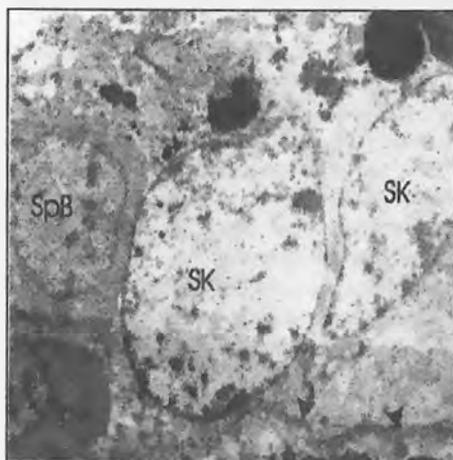


Fig. 3. Two Sertoli cells (SK) in degeneration. Two spermatogonia of type B (SpB). Visible basal lamina of the lobule (small arrows). Ultrathin section ($\times 4\ 400$)

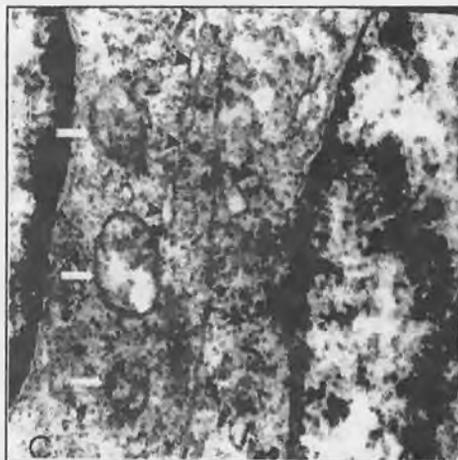


Fig. 4. A part of nucleus (N) and cytoplasm (C) of two young spermatogonia with mitochondria (arrows) and "lamellae anulate" (small arrows). Ultrathin section ($\times 12\ 000$)

In *Cyprinus carpio* L. an intensive mitosis of spermatogonia of type B was noticed by Billard et al. [1].

In the cytoplasm of this 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 Clerot [2] and Hamaguchi [3].

Also, these particles with the spermatogonial population of Dojran perch (*Perca fluviatilis macedonica* K a r.) were noted by Tavciovaska-Vasileva (1992, 1994).

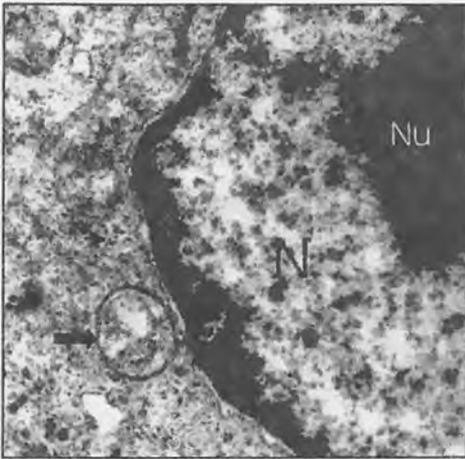


Fig. 5. A part of young spermatogonium (Sp) with nucleus (N) and prominent nucleolus (Nu). In the cytoplasm presence of ribosomes and polyribosomes (small arrows), mitochondrion with lamellar crusts which are in the phase of formation (big arrow). Ultrathin section ($\times 20\ 000$)

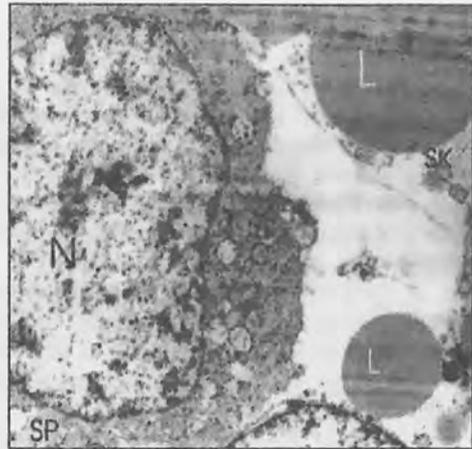


Fig. 6. Young spermatogonium (Sp) with visible nucleus (N) and darker cytoplasm (C) with mitochondria (small arrows). A part of Sertoli cell (SK) in degeneration with big lipids (L). Ultrathin section ($\times 7\ 000$)

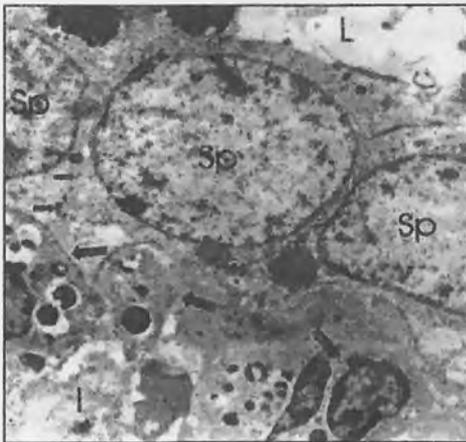


Fig. 7. Spermatogonia (Sp) connected among themselves with desmosomes (small arrows). Presence of polymorphonuclears (neutrophils) (big arrows) in the interstitium (I). Visible lumen (L) of the lobule. Ultrathin section ($\times 4\ 400$).

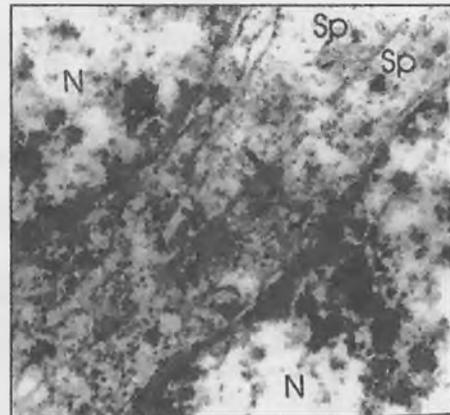


Fig. 8. A part of two spermatogonia (Sp) connected with desmosomes (arrows). Visible nuclei (N). Ultrathin section ($\times 3\ 000$)

Conclusions

The ultrastructural characteristics of spermatogonial population of Ohrid trout (*Salmo letnica* K a r.) in the postspawning period can be defined like this:

1. "Nuages" particles with perinuclear arrangement can be noticed in the cytoplasm of spermatogonia of type A.

2. The young spermatogonia of type B possess darker cytoplasm in which mitochondria with with electron dense matrix and lamellar crusts which are in the phase of formation, can be noticed.

3. In the cytoplasm of some spermatogonia "lamellae anulate", a lot of ribosomes and polyribosomes can be noticed, which points to an intensive synthesis of proteins which is characteristic of the young cells which grow up.

4. The young spermatogonia have a clearly seen nucleus with prominent nucleolus.

5. The spermatogonia which are organized in cysts are connected among themselves with desmosomes.

References

1. Billard, R., C. Weil, K. Bieniarz, T. Mikolajczyk, B. Breton, P. Epler, M. Bongoussa. Testicular and some hormonal changes during the first four years of life in the mirror carp, *Cyprinus carpio* L. — *J. Fish Biology*, 41 (3), 1992, No 3, 473-487.
2. Clerot, J. C. Les groupements mitochondriaux des cellules germinales des poissons Teleosteens Cyprinides. I. Etude ultrastructurale. — *J. Ultrastruct. Res.*, 54, 1976, No 3, 461-475.
3. Hamaguchi, S. The structure of the germinal dense bodies (nuages) during differentiation of the male germ line of the Teleost, *Oryzias latipes*. — *Cell Tiss. Res.*, 248, 1987, 375-380.
4. Hurk, R. Vanden, J. Peute, J. A. J. Vermeij. Morphological and enzyme cytochemical aspects of the testis and vas deferens of the rainbow trout, *Salmo gairdneri*. — *Cell Tissue Res.*, 186, 1978, 309-325.
5. Loir, M. Trout Sertoli and germ cell in primary culture: I. Morphology and ultrastructural study. — *Gamete Res.*, 24, 1989, 151-169.
6. Scott, A. P., J. P. Sumpter. Seasonal variations in testicular germ cell stages and plasma concentration of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. — *Gen. Comp. Endocrinol.*, 73, 1989, 46-58.
7. Tavciovsk a-Vasileva, I. Histološka struktura na semenikot na dojranskata perkija (*Perca fluviatilis macedonica* K a r.) vo periodot po mrestenjeto. — *Magisterski trud*, Skopje, 1992.
8. Tavciovsk a-Vasileva, I. Spermatogonijalna degeneracija kako propraten fenomen na spermatogenezata vo postmrestitelniot period kaj dojranskata perkija (*Perca fluviatilis macedonica* K a r.). — *God. Zb., Biol., Skopje*, 47, 1994, 189-198.
9. Tavciovsk a-Vasileva, I. Komparativni strukturni i ultrastrukturni karakteristiki na semenicite kaj *Salmonidae* (Pisces: Teleostei) od Ohridskoto Ezero vo postmrestitelniot period. — *Doktorska disertacija*, Skopje, 1999.