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Structural and ultrastructural characteristics of tunica albuginea of Salmonidae of Ohrid lake during the postspawning period

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This study concerns the structural and the ultrastructural characteristics of tunica albuginea of two salmonid fishes of Ohrid Lake, Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) during the postspawning period. For this purpose testes of sexually mature Ohrid trout and Ohrid belvica males caught in Ohrid Lake in a period of 3 years (1993/1996) have been analysed. Analyses have been done with light and electronic microscope. Tunica albuginea is noted on the surface of the testes in both species. In the prespawning period it is slightly developed, highly extended and very thin. The presence of fibroblasts, elastic fibrils and rare myoid elements (oriented in parallel with the surface of testes) has been noticed at the level of its joining stroma. The surface mesothelium is also highly extended. It is characteristic that tunica albuginea of the testes in both species immediately after the spawning (an early postspawning period) becomes more visible, because it is considerably hypertrophic. At its level three integral components can be clearly seen: surface mesothelium, joining stroma with a great number of elastic fibrils and myoid elements. In both species in the period of regeneration tunica albuginea, similarly to the interstitium, is considerably reduced compared with the one of the postspawning period.

Key words: Ohrid trout, testis-tunica albuginea, hystological and ultrastructural analysis.

Introduction

Tunica albuginea of the testes with its integral components (mesothelium, joining stroma with elastic fibrils and myoid elements) of *Perca fluviatilis* L. in the postspawning period was analysed in 1927 by Kulaev. Data about tunica albuginea of Perca fluviatilis macedonica Kar. are presented by Tavciovska-Vasileva [3].
Tunica albuginea of Salmonidae in the postspawning period as part of our previous investigations of the testes of Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) has been analysed by Tavciovska-Vasileva [4].

Material and Methods

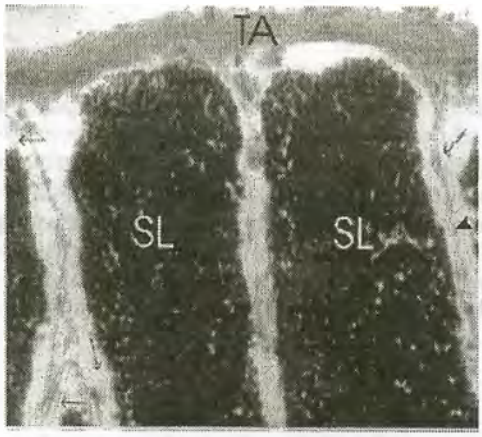
Testis of sexually mature Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) males caught in Ohrid Lake in a period of 3 years (1993/1996) have been analysed. Analyses have been done with light and electronic microscopes. For the light microscopy parts of testes have been taken immediately after the decapitation of the alive samples, and fixed in Bouin fixative and 4% neutral formaline. Standard paraffin technique has been used for the preparation of microscopic slides. The sections are 5 μm thin and they are stained according to the following 3 methods: Hemalaun-Eozin method, Floranten method and modification of Mallory by Heidenhain (Azan method). The microphotographs for light miroscopy have been taken with Leitz-Wetzlar Ortholux microscope, camera Otrhomat. The histological analysis of the material has been done on the paraffin sections, as well as on semithin ones, prepared according to the following procedure: 1. The sections with the thickness 0,5—1 μm have been cut on Reichert-Yung "Ultracut' ultramicrotome, using glass knives for electronic microscopy. 2. The sections have been stained by the method of permanent monochromatic staining with toluidine blue.The microphotographs of the semithin slides have been done using Leitz-Wetzlar Ortholux microscope, camera Otrhomat. The photodocumentation of paraffin and semithin preparations has been done using Kodak Ektapress Multi Gold II PJM-36 slides, with blue filter. Small parts of testes 1-2 mm big have been used for electronic microscopy.The material has been fixed according to following procedure: 1. Immediately after the tissue sections have been taken, they are fixed in 3 % glutaraldehyde and then conserved in 0,1 M phosphate puffer. 2. After adequate fixation the material has been subunited to postfixation in 1 % osmium tetraoxid (OsO_4). 3. In the further treatment the material has been washed in phosphate puffer, dehydrated in series of acetone and uranil acetate, and after that it has been dehydrated in acetone. 4. The tissue sections have been infiltrated with Durcopan ACM mixture, mixture of acetone-Durcopan, Durcopan No 1, Durcopan No 2. The same fixation has been used for semithin sections. For the ultrastructural analysis, ultrathin sections of 40-60 μm tickness have been prepared and contrasted with uranil acetatae and lead cytrate.The sections have been observed on Tesla BS 500 and ORTON (Zeis) EM 109 electronic microscopes.

Results

Ohrid trout (*Salmo letnica* Kar.)

Prespawning period

Tunica albuginea covers the surface part of the testes. In the prespawning period it is slightly developed, highly extended and very thin (Fig. 1). Fibroblasts, elastic fibrils and rare myoid elements (oriented in parallel with the surface of testes) have been noticed at the level of its joining stroma. On the surface of the tunica albuginea the mesothelium is also highly extended (Fig. 2). Tunica albuginea, i.e. its integral components are well noticed on semifine slides, as well as on the slides examined by light microscope (Fig. 3).



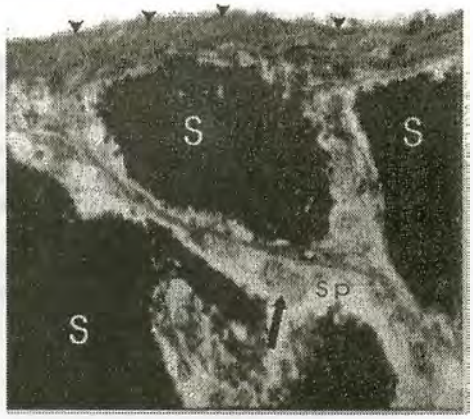


Fig. 1. Maximally dilated seminiferous lobules (SL) filled with sperm cells. On the surface well seen tunica albuginea (TA). Sertoli cells with endotheliomorphic appearance (arrows). Floranten (×45)

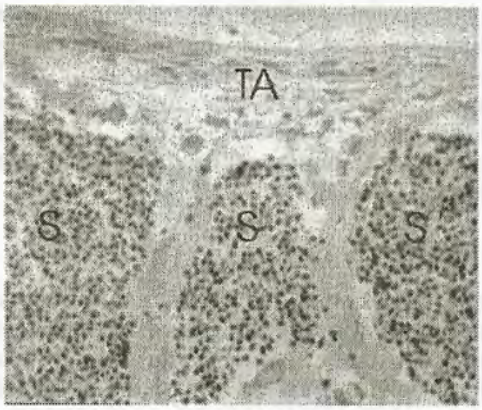


Fig. 2. Tunica albuginea with surface mesothelium (small arrows). Seminiferous lobules filled with sperm cells (S). Rare, single spermatogonia (Sp) in latent (dormant) condition (arrow). Floranten (×45)

Fig. 3. Surface part of the testis with tunica albuginea (TA) with colagenous, elastic fibrils, fibroblasts and rare myoid elements. Seminiferous lobules filled with sperm cells (S). Semithin section, toluidine blue (×40)

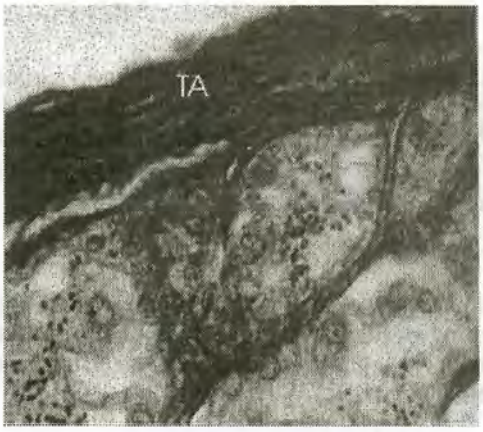


Fig. 4. On the surface of the testis maximally hipertrophic tunica albuginea (TA). Hemalaun - Eozin (× 45)

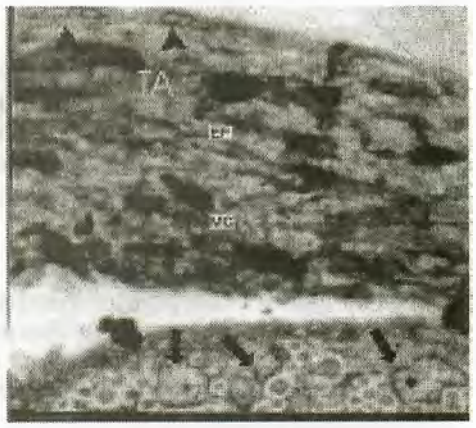


Fig. 5. Maximally hypertrophic tunica albuginea (TA) with surface mesothelium (small arrows), big number of elastic fibrils (EF), myoid cells (MC). Sertoli cells (arrows) with polymorphic nucleus. Semithin section, toluidine blue (×100)

Postspawning period

In the postspawning period all the integral components of testes, including the tunica albuginea, the latent (dormant) spermatogonia, i. e. the germinative component of testes, Sertoli cells as somatic elements of the seminiferous lobules and the interlobular interstitium undergo changes. All these changes cause a completely histological appearance as well as histoarhitectonic of testes. It is characteristic that tunica albuginea of the testes in Ohrid trout (*Salmo letnica* Kar.) immediately after the spawning (an early postspawning period) becomes more visible, because it is considerably hypertrophic (Fig. 4, 5). Its three integral components can be crearly seen: surface mesothelium (Fig. 5), joining stroma with a great number of elastic fibrils and myoid elements. Tunica albuginea is especially well noticed on semithin slides (Fig. 5).

Period of regeneration

In order to emphasise the histological characteristics of the tunica albuginea of testes in Ohrid trout (*Salmo letnica* Kar.) in the postspawning period, a partial analysis of it is done in the period which follows the postspawning period, and which as to its reproductive activity may be determined as transitional period or period of regeneration [1]. In the period of regeneration tunica albuginea is considerably reduced in comparison with the one of the postspawning period. In this period on the surface of the testes it is slightly developed, highly extended and very thin. Fibroblasts, elastic fibrils and rare myoid elements have been noticed in its joining stroma.

Ohrid belvica (*Salmothymus ochridanus* Steind.)

In belvica, similarly to the trout, in postspawning period all the integral components, including the tunica albuginea, the germinative component of testes, i. e. the latent (dormant) spermatogonia, the somatic Sertoli cells and the interlobular interstitium undergo changes. All these changes, in Ohrid belvica, cause a completely changed histoarhitectonic of the testes.

Prespawning period

On the surface of the testes in Ohrid belvica (*Salmothymus ochridanus* Steind.) tunica albuginea can be noted. In the prespawning period it is slightly developed, highly extended and very thin (Fig. 6, 7). Presence of fibroblasts, elastic fibrils and rare myoid elements (oriented in parallel with the surface of testes) has been noticed at the level of its joining stroma. The surface mesothelium is also highly extended (Fig. 6).

Postspawning period

Tunica albuginea in the postspawning period becomes more visible and thicker (Fig. 8) compared with the one of the prespawning period. On the surface of the tunica albuginea mesothelium can be seen in this period, while in its joining stroma there are elastic fibrils (Fig. 9) and myoid elements (Fig. 10).

Period of regeneration

In the period of regeneration tunica albuginea of the testes of Ohrid belvica (*Salmothymus ochridanus* Steind.) similarly to Ohrid trout, is considerably reduced in com-

Fig. 6. Maximally dilated tunica albuginea (TA) with surface mesothelium (small arrows). In the subcapsular region latent (dormant) spermatogonia (arrow). Seminiferous lobulus with sperm cells (S). Hemalaun - Eozin (×40)

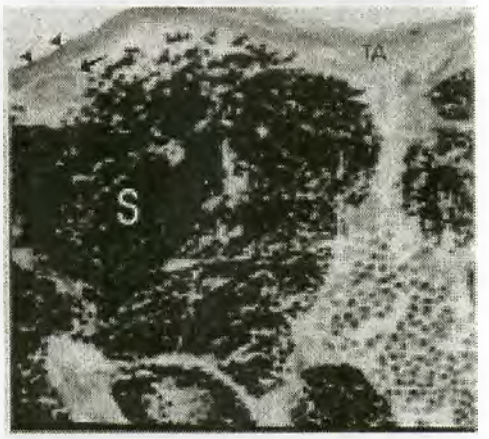


Fig. 7. On the surface of the testis well seen tunica albuginea (TA). Seminiferous lobulus (SL) with sperm cells (S). Sertoli cells with endotheliomorphic appearance (thin arrows). Presence of single or in cysts spermatogonia (arrows) with accompanying precursor Sertoli cells (small arrows). Floranten (×40)

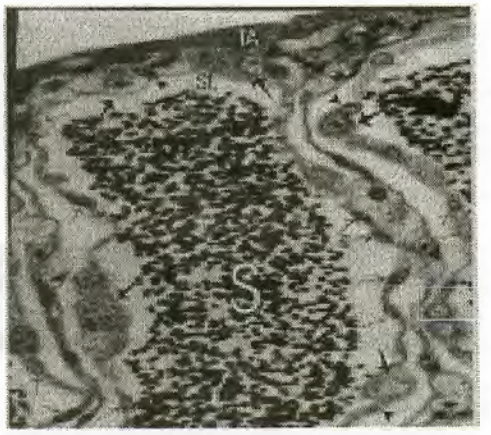
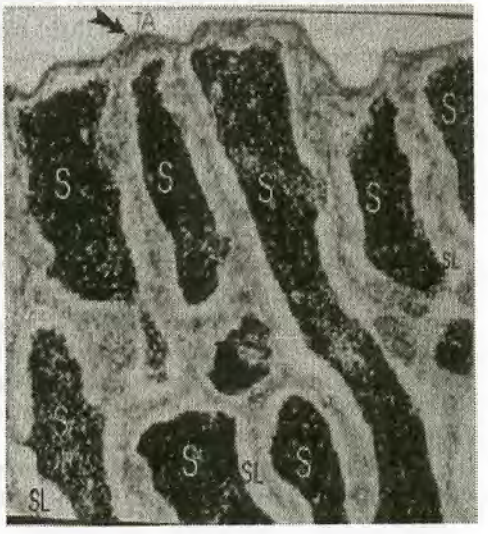


Fig. 8. Subcapsular region of testis. Seminiferous lobules (SL) filled with sperm cells (S) (during the spawning). On the surface of the testis well seen tunica albuginea (TA) (arrow). Hemalaun - Eozin (×10)



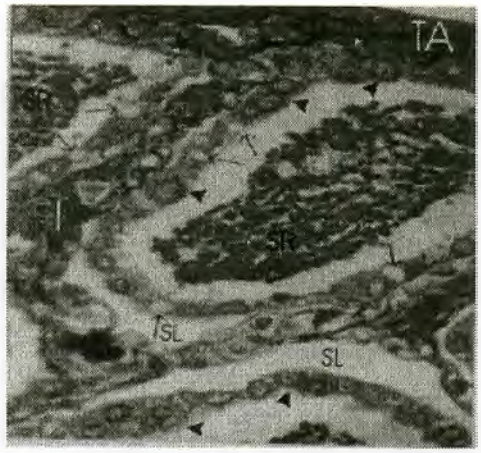


Fig. 9. Hypertrophic tunica albuginea (TA) with elastic fibrils (small white arrows). Seminiferous lobules (SL) with sperm residues (SR). Sertoli cells with lipid vacuoles (black arrows) of various dimension. Presence of spermatogonia in the wall of the lobules (small black arrows). Vascularised interstitium (I), with present collagenous fibrils in it. Azan, (×40)

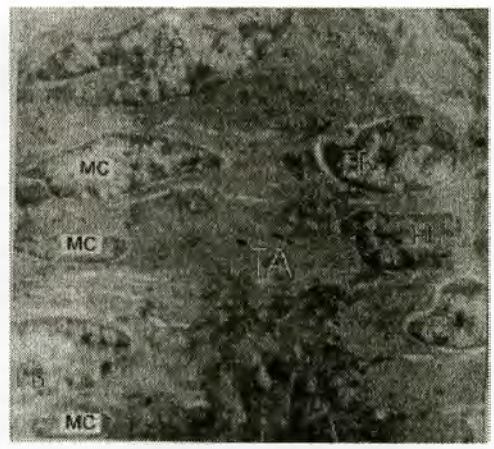


Fig. 10. A part of tunica albuginea (TA) with fibroblasts (FB) and myoid cells (MC). Ultrathin section, (×4400)

parison with the one of the postspawning period. Tunica albuginea of Ohrid belvica in the period of regeneration is slightly developed, highly extended and very thin. Its joining stroma is not rich to the cellular components. Elastic fibrils, rare fibroblasts and single myoid elements have been noted.

Discussion

The structural and ultrastructural analysis made on the testes of the two salmonid species from Ohrid Lake, i.e. Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) in the postspawning period shows certain characteristics which give a remarkable histological picture of the testes in this period.

In the postspawning period noticeable changes are seen on a level of the tunica albuginea, and the most remarkable changes have been noted in the seminiferous lobules, i.e. Sertoli cells as a somatic component of the same lobules and the interlobular interstitium. All these changes during the postspawning period happen successively.

The changes especially those which happen in the final phase of the postspawning period, sufficiently reorganize the histoarhitectonic of the testes, in comparison with the one of the prespawning period.

Following successively the characteristic changes which happen on a level of the testes in the postspawning period in both analysed salmonid species from Ohrid Lake, Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) we have found out that the postspawning period is a period of complete reorganisation of the testes.

Tunica albuginea in the postspawning period in both species is noticably thicker, which makes possible its three integral components, mesothelium, joining stroma

rich with elastic fibrils and myoid elements to be clearly seen. With these integral components tunica albuginea in *Perca fluviatilis* L. was described by Kulaev [2] and in Dojran perch (*Perca fluviatilis macedonica* Kar.) by Tavciovska-Vasileva [3].

Tunica albuginea in Salmonidae with similar integral components has been analysed in our previous research work.

Conclusion

On the basis of the cytological analysis made on the testes of Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Salmothymus ochridanus* Steind.) in the postspawning period, we can conclude the following: Tunica albuginea in postspawning period is very well developed and on its level we can clearly see its three laminar structure, i.e. presence of mesothelium, joining stroma rich in elastic fibrils and myoid elements.

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