

ULTRASTRUCTURAL VIEW OF THE MACROPHAGES OF TESTES OF TWO OHRID SALMONS, OHRID TROUT (*SALMO LETNICA* KAR.) AND OHRID BELVICA (*ACANTHOLINGUA OHRIDANA*)

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ULTRASTRUKTURNI IZGLED MAKROFAGA U TESTISIMA DVE OHRIDSKE PASTRMKE, OHRIDSKE PASTRMKE (*SALMO LETNICA* KAR.) I OHRIDSKE BELVICE (*ACANTHOLINGUA OHRIDANA*)

Abstrakt

U radu je data uporedna ultrastrukturalna analiza makrofaga testisa Ohridske pastrmke (*Salmo letnica* Kar.) i Ohridske belvice (*Acantholingua ohridana*) u periodu posle mresta. U semeniferim režnjevima ovih vrsta sa degenerisanim Sertolijevim materijalom može se opaziti prisustvo makrofaga. Oni se nalaze ili u zidu režnjeva, ili u njihovoj šupljini. Makrofagi poseduju karakterično produbljeno jedro. Neki makrofagi imaju proširen perinuklearni prostor. Makrofagi imaju segmentirano jedro, što je karakteristično za sve ćelije i citoplazmu bogatu fagolizozomima. Njihovo prisustvo u semenim režnjevima kada teče intenzivna involucija tj. degeneracija Sertolijevih ćelija može se opaziti po njihovoj eventualnoj fagocitozi, odnosno eliminaciji nekrotičnog materijala koji potiče iz Sertolijevih ćelija.

Ključne reči: *Ohridska pastrmka (Salmo letnica Kar.), Ohridska belvica (Acantholingua ohridana), testis, makrofagi, period posle mresta*

INTRODUCTION

In literature there are data wich describe macrophages as phagocytotic elements of testes in many Teleost fish (Chavez-Pozo et al., 2005, 2007; Hales, 2002; Hedger, 2002; Lahnsteiner & Patzner, 1990; Liarte et al., 2007; McClusky, 2005; Micale et al, 1987;

Sepulcre et al., 2002; Young, 2001). Also macrophages was noticed in Chondroichthyes, in the spotted ray (*Torpedo marmorata*) by Prisco et al. (2003). There are literature data which poin to the participation of the macrophages (besides Sertoli cells) in the elimination of the sperm residues with some species of Teleostea in the period after the spawning. This phenomenon with *Salvelinus fontinalis* was described by Henderson (1962), with *Rutilus rutilus dojranensis* K a r . by Dimovska (1965), with *Oryzias latipes* by Gresek et al. (1973) and also was stated with other Vertebrata, for example with swan (*Cygnus olor*) by Breucker (1978). The presence of macrophages with *Preca fluviatilis macedonica* K a r . in the period after the spawning was pointed out by Dimovska et al. (1986/87), Tavciovaska-Vasileva (1992). According to Loir et al. (1995) with trout the interlobular macrophages can participate in the reinitiation of the spermatogonial proliferation. Macrophages was noticed in Salmonidae, in rainbow trout (*Salmo gairdneri*) by Billard et al. (1983); Scott & Sumpter (1989); Van den Hurk et al. (1978). The presence of these phagocytotic elements also was noticed with Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) in the period after the spawning by Tavciovaska-Vasileva (1999, 2001), Tavciovaska-Vasileva & Rebok (2004). The presence of macrophages is connected with the phagocytosis of Sertoli cells, because they are present in interstitium, as well as in the seminiferous lobules.

MATERIAL AND METHODS

Testes of sexually mature Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) males caught in and Ohrid Lake were 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 by the 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 tetraoxid (OsO₄). In the further treatment the material has been washed in phosphate buffer, dehydrated in series of acetone and uranil acetate, and after that it has been dehydrated in dry acetone. The tissue sections have been infiltrated with Durcopan ACM mixture, a 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 nm tickness have been prepared, with the help of galss knives, on Reichert-Yung "Ultacut" ultramicrotome, installed on copper nets, contrasted with uranil acetate and lead cytrate. The sections have been observed on Tesla BS 500 and OPTON (Zeis) EM 109 electronic microscope. The microphotographs for electronic microscopy have been photographed with Agfa Scientia EM Film 23056/6, 5 x 9 cm, ORWO NP 20 panchromatic 120, KODAK 120 and made on Agfa Papirtone Paper P1-3.

RESULTS

In the seminiferous lobules of Ohrid trout (*Salmo letnica* Kar.) where Sertoli degenerated material is present, it is characteristic that there are macrophages located in the wall of the lobules, in their lumen or in the interstitium (Fig. 1). The macrophages possess a characteristic deepened nucleus. Some macrophages have widened perinuclear space (Fig. 2).

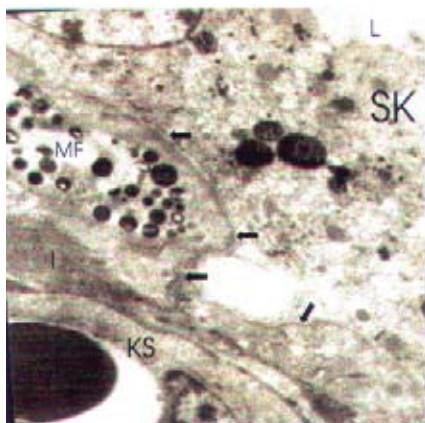


Figure 1. A part of the cytoplasm of Sertoli cell (SK) in degeneration. A part of the lumen (L) of the lobulus. Developed interstitium (I) with macrophage (MF) and blood vessel (KS). Well seen basal lamina (arrows) of the lobulus. Ultrathin section, x 4.400.

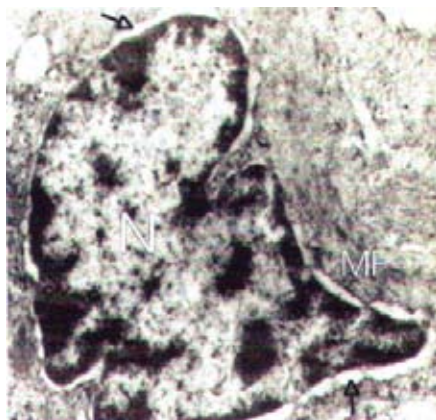


Figure 2. Macrophage MF with deepened nucleus (N) with widened perinuclear space (arrows). Ultrathin section, x 12.000

The presence of macrophages in seminiferous lobules in the period of intensive degeneration, i. e. involution of Sertoli cells shows that these phagocyte elements probably participate in elimination (phagocytosis) of Sertoli necrotic material. Macrophages can be noticed in the lobules with the Sertoli degenerated material. With Ohrid belvica (*Acantholingua ohridana*) the macrophages have been noticed in the wall of the lobules, as well as in the lumen. At ultrastructural level we can see that macrophages possess a segmented nucleus (Fig. 3) which is characteristic for these cells, and a cytoplasm rich with phagolysosomes (Fig. 4, 5). Their presence in the seminiferous lobules when intensive involution, i. e. degeneration of Sertoli cells goes on, can be connected with their eventual phagocytosis, i. e. elimination of the necrotic material, which originates from the Sertoli cells.

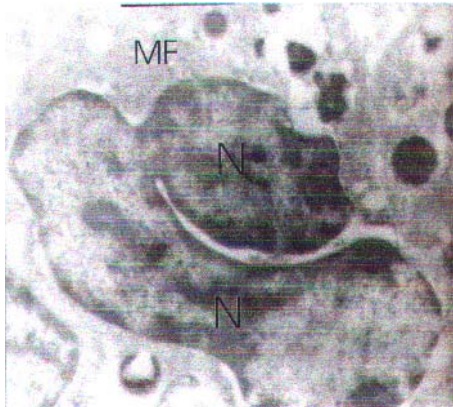


Figure 3. Macrophage (MF) with segmented nucleus (N). Ultrathin section, x 12.000.

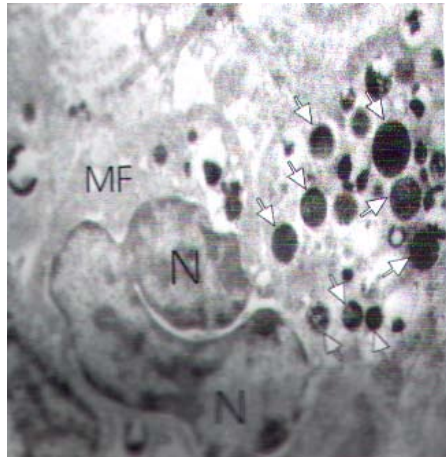


Figure 4. Macrophage (MF) and cytoplasm rich with phagolysosomes (arrows). Ultrathin section, x 7.000.

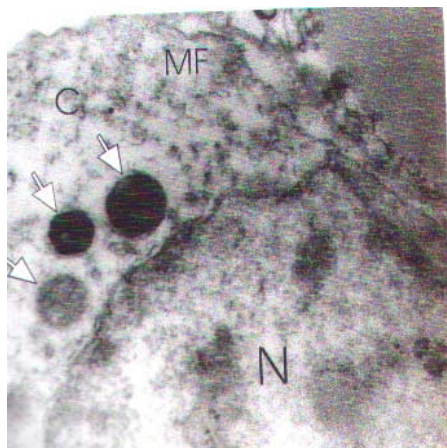


Figure 5. A part of macrophage (MF) with nucleus (N) and cytoplasm (C) with phagolysosomes (arrows). Ultrathin section, x 12.000.

DISCUSSION

In the testes of these two analysed species Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) the presence of macrophages have been stated on the level of interstitium, as well as in the wall or in the lumen of the seminiferous lobules by Tavciovaska-Vasileva (1999, 2001), Tavciovaska-Vasileva & Rebok (2004). Macrophages was noticed in Salmonidae, in rainbow trout (*Salmo gairdneri*) by Billard et al. (1983); Scott & Sumpter (1989); Van den Hurk et al. (1978). Their presence can point to their possible participation in the elimination of the Sertoli necrotic material. This phenomenon in *Salvenilus fontinalis* was pointed out by Henderson (1962), *Rutilus rutilus dojraneensis* Kar. (Dimovska, 1965), *Oryzias latipes* (Gresek et al., 1973), *Perca fluviatilis macedonica* Kar. (Dimovska et al, 1986/87; Tavciovaska-Vasileva, 1992). In literature there are data wich describe macrophages as phagocytotic elements of testes in many other Teleost fish (Chavez-Pozo et al., 2005, 2007; Hales, 2002; Hedger, 2002; Lahnsteiner & Patzner, 1990; Liarte et al., 2007; McClusky, 2005; Micale et al, 1987; Sepulcre et al., 2002; Young, 2001). Also macrophages was noticed in Chondroichtyes, in the spotted ray (*Torpedo marmorata*) by Prisco et al. (2003). It is also stated with other Vertebrata, for example with swan (*Cygnus olor*) by Breucker (1978). Because of the fact that they appear in the interlobular interstitium, as well as in the wall and in the lumen of the seminiferous lobules when degeneration, i. e. involution of Sertoli cells happens, their presence can be connected with phagocytosis of the residues which originate from Sertoli necrotic (degenerated) material. With the trout, according to Loir et al. (1995) the interlobular macrophages can participate in reinitiation of the spermatogonial proliferation.

CONCLUSION

With the cytological analysis based on ultrastructural findings of the testes of Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) we can state that macrophages can be noticed in the wall and in the lumen of some seminiferous lobules, and their role can be connected with the elimination of Sertoli necrotic material.

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