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OBSERVATION OF RED BLOOD CELL ALTERATIONS IN TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*)

Short communication

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Abstract. The subject of this study was investigation of red blood cell alterations in diploid and triploid rainbow trout (*Oncorhynchus mykiss*). The triploid rainbow trout were obtained through the application of heat shock of 26.5°C for 20 min to eggs 20-min following fertilization. The diploids were not subjected to shock. The 3N and 2N fingerlings of rainbow trout were reared under the same condition (environment, feeding). Fish ploidal level was determined by erythrocyte size measurement and the number of active nucleoli in epithelial gill cells (NORs). Triploidy was induced in 80.86% in heat shocked treatment. It was found that the percentage of erythrocytes with segmented nuclei in triploid fish was 13.52% and was significantly higher ($P < 0.01$) than that in diploid fish (1.06%). In addition amitosis of red blood cells only seen in triploids (0.57%). It seems that increase of nuclear segmentation is one of the important characteristics for triploid rainbow trout.

Key words: amitotic cell, red blood cell, rainbow trout, triploid, segmented nuclei

INTRODUCTION

Triploidy induction is an effective way in salmonid fish cultivation. Triploids have three sets of homologous chromosomes in their somatic cells instead of the normal diploid number of two [Smith and Benfey 2001]. This results in the abnormal pairing of chromosomes during meiotic reduction divisions, and the resultant inability of most triploid germ cells to complete gametogenesis and produce euploid gametes. Thus, triploids typically show impaired gonadal development and are sterile and therefore metabolic energy is diverted from reproduction to somatic growth [Benfey 1999]. They have also reduced if not eliminated other problems associated with sexual development.

Because of polyploidy induction, some physiological alterations may be resulted in some fish species. Blood cells structure has some differences between diploid and triploid Salmonids.

For example, total blood hemoglobin and mean cellular hemoglobin concentration were lower in triploid Atlantic salmon as compared with diploids [Benfey and Sutterlin 1984]. There was no significant difference in total blood hemoglobin between diploid and triploid brook trout (*Salvelinus fontinalis*), although total number of erythrocytes was lower and cell size was larger in triploids [Benfey and Biron 2000]. Erythrocyte size was larger in triploid rainbow trout but total erythrocytes and their hemoglobin content was lower as compared with diploids [Suresh and Sheehan 1998]. In this research, some structural abnormalities like erythrocyte amitosis and nuclei segmentation was observed during the examination of blood smears. The objective of this research was accurate determination of these alterations and their probable effect on erythrocyte functions in diploid and triploid rainbow trout (*Oncorhynchus mykiss*).

MATERIAL AND METHODS

Triploid rainbow trout were obtained through the application of heat shock of 26.5°C for 20 min to eggs 20-min post fertilization. Diploids (control) received no heat shock, and fingerlings were reared at the same nutritional and environmental conditions. Fish ploidy level was determined by erythrocyte size measurement [Perovic et al. 2003] and by counting of gill epithelial cells active nucleoli (NORs) [Philips et al. 1986]. Blood samples for the smears were taken from the caudal vein of 42 triploids and 35 diploids, fixed in 95% methanol for 3 minutes, left to air-dry and stained with 10% Giemsa solution for 15 minutes. One hundred erythrocytes per slide were studied at 400* magnification to determine the frequency of cell amitosis and cells with segmented nuclei. Statistical evaluation was carried out in SPSS using non-parametric t-test

RESULTS

Efficiency of triploidy induction was 80.86% in heat shocked treatment. The number of erythrocytes with nuclear segmentation was significantly higher ($P < 0.05$) in triploids than in diploids (13.52% in triploids and 1.06% in diploids). These nuclei were divided into two rather equal parts (Fig. 1 a). Amitosis of erythrocytes (Fig. 1 b) was only seen in triploid fish (0.57%).

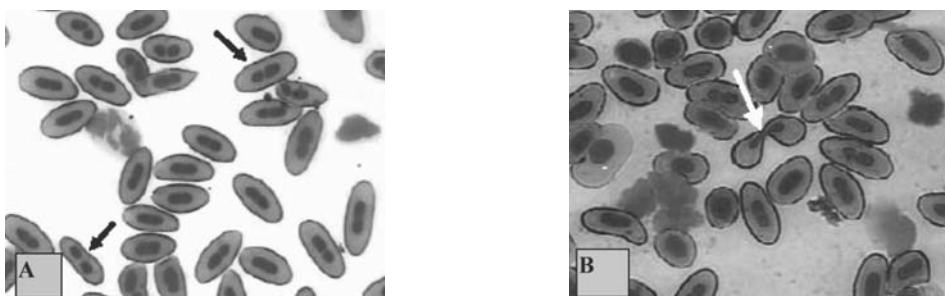


Fig. 1 a. Erythrocytes with segmented nuclei (black arrow) in triploid rainbow trout

Fig. 1 b. Amitosis of red blood cell (white arrow) in triploid rainbow trout

Rys. 1a. Erytrocyty z segmentowanym jądrem (czarna strzałka) u triploidów pstrąga tęczowego

Rys. 1 b. Amitoza krwinki czerwonej (biała strzałka) u triploidów pstrąga tęczowego

DISCUSSION

The effect of triploidy induction through the application of heat shock to rainbow trout eggs (26.5°C for 20 min) 20 min post fertilization was 80.86% that correspond entirely with findings of Wagner [2002]. Erythrocyte nuclear segmentation has been reported in brook trout *Salvelinus fontinalis* [Benfey 1999]. Własow et al. [2004] showed that the frequency of erythrocytes with segmented nuclei (19.1%) was significantly higher in triploid brook trout than in diploids ($P < 0.05$). They also observed a low percentage of amitosis cells in triploid brook trout. In present research on diploid and triploid rainbow trout, the number of erythrocytes with segmented nuclei was significantly ($P < 0.01$) higher in triploids. Also, a percentage of 0.57 amitosis cells were observed in triploid erythrocytes.

Structural changes in triploid erythrocytes may disrupt some of their functions such as the ability of gas transportation. Because of entirely the same nutritional and environmental conditions in this research, it is hypothesized that the increase of abnormalities in triploid erythrocytes may not be due to these conditions. These changes have also been reported in triploid brook trout [Własow et al. 2004] produced by hydrostatic pressure shock and thus shock technique can not make these abnormalities. This phenomenon has presumably direct relation to increased ploidy level.

On the other hand, red blood cells with divided nuclei have been observed in coho salmon (*Oncorhynchus kisutch*) due to the lack of folic acid in its diet [Smith 1968]. The presence of folic acid is essential for normal formation of red blood cells. The lack of this vitamin in fish can also result in the decrease of total number of erythrocytes, so observation of these abnormalities in triploids may be due to disruption in function of gene replicates related with folic acid synthesis or metabolism in DNA molecule. It is necessary to design some more experiments to document this finding.

According to Lehmann and Stürenberg [1975] segmentation of nuclei in erythrocytes of salmonids fish is the first step of red blood cells amitosis. Due to amitotic division of cells the number of cells increases. In addition in small cells (diploid fish) the relation of cell surface to cell volume is more profitable to gas exchange than in large erythrocytes of triploids.

It seems that increase of nuclear segmentation in erythrocytes is one of the important characteristics for triploid rainbow trout.

The segmentation of nuclei in red blood cells of examined triploid rainbow trout we can consider as a process of improving conditions for good function of these cells.

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WYSTĘPOWANIE ZABURZEŃ W ERYTROCYTACH TRIPLOIDÓW PSTRĄGA TĘCZOWEGO (*Oncorhynchus mykiss*) (doniesienie)

Streszczenie. Analizowano zmiany strukturalne w krwinkach czerwonych diploidalnych i triploidalnych pstrągów tęczowych (*Oncorhynchus mykiss*). Triploidy uzyskano poprzez zastosowanie szoku termicznego (26,5°C przez 20 min) na jaja w 20. minucie po zaplemnieniu. Diploidy nie były poddawane szokowi termicznemu. Narybek pstrąga tęczowego: 3N i 2N hodowano w tych samych warunkach środowiskowych i żywienia. Ploidalność ryb oceniono przy użyciu pośrednich wskaźników ploidalności – pomiaru wielkości erytrocytów i liczności aktywnych jąder w jądrach komórkowych nabłonka skrzelii (NOR). W wyniku manipulacji genomowej uzyskano 80,86% triploidów. Zaobserwowano, że w krwi triploidów udział erytrocytów z jądrem segmentowanym wynosił 13,52% i był istotnie wyższy ($P < 0,01$) w porównaniu z występującym u ryb diploidalnych (1,06%). Amitozę krwinek czerwonych obserwowano jedynie u triploidów (0,57%). Wzrost udziału erytrocytów z dzielącym się jądrem wydaje się być ważną cechą triploidalnych pstrągów tęczowych.

Słowa kluczowe: amitotyczna komórka, erytrocyt, pstrąg tęczowy, segmentowane jądra, triploid

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