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Cytogenetic monitoring of freshwater aquaculture in Ukraine

Cytogenetic control of fish chromosomal apparatus, it integrity and presence of structural and quantitative mutations are an integral part of the genetic examination of farm animals breeding resources. This is the relevance of the introduction of cytogenetic tests in fish farming which aimed to determining the nature of the influence of exogenic and endogenic factors on the fish genome. Specimens of freshwater aquaculture are characterized by the different phenotypic and genotypic peculiarities, growth rate, fecundity, search ability, cold resistance, resistance to infectious diseases. Consequently, in fisheries are necessary complex knowledge of variability of populations genetic structure, level of somatic and generative mutagenesis, resistance to infectious diseases for creating of high-productive brodstock of fish. For diagnostic and prognostic purposes are necessary to perform the research for detection genomic and chromosomal instability.

Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. Research reports maintained that it can be applicable to freshwater and marine fishes and that gill cells are more sensitive than the hematopoietic cells to micronucleus inducing agents [1]. According to Fagr [2], the incidence of micronuclei in fish and other aquatic life serves as an index of these types of damage and counting of micronuclei is much faster and less technically demanding than scoring of chromosomal aberrations [3, 4]. That is why cytogenetic control of fish chromosomal apparatus, presence of genomic mutations is necessary for genetic examination of freshwater fish of Ukraine. The purpose of our work is to determine the level of cytogenetic indicators of fish from different regions of Ukraine. For cytogenetic analysis has been used micronucleus test and analysis of frequency of apoptosis of blood cells.

Two groups of scaled and framed carps from fish farm “Sumyrybgosp” of Sumy region and one group of starlet (*Acipense Ruthenus*) from ≪Odessa Sturgeon Breeding Complex≫ LLC, Vylkove, Odessa region has been sampling. Peripheral blood is obtained from the dorsal vessels of each individual by vertically puncturing of sterile syringe. The MN assay was performed as per the protocol of Davydov O.N. and Temnyhanov Y.D. [5] but with own modifications. Blood smears were made onto grease-free precleaned and marked slides by droping two drops of 0,6 % NaCl and one drop of blood. The slides were air-dried for 24h. After fixation in pure methanol

for 30 min, the slides were allowed to air-dry and stained by the method of Romanowsky with standard Giemsa solution for 40 min. Slides were made for each fish and scored 3000 cells using oil-immersion under a light microscope (Primo Star Zeiss, 100/1.25). There were counted the occurrence frequency of cytogenetic indicators (erythrocytes with micronuclei (EMN), lymphocytes with micronuclei (LMN), binuclear lymphocytes (BNL) and apoptosis). Obtained results were expressed as ppm (‰). Statistical analysis was performed using the Student's t-distribution.

It has been investigated, that group of framed carp from fish farm "Sumyrybgosp" was characterized by the highest level of erythrocytes with micronuclei (4.8 ± 0.3 ‰), lymphocytes with micronuclei (3.2 ± 0.3 ‰), binuclear lymphocytes (1.5 ± 0.1 ‰) compared with group of scaled carp from this farm which characterized: EMN (3.2 ± 0.2 ‰), LMN (2.4 ± 0.1 ‰) and BNL (1.1 ± 0.1 ‰). Statistically significant intergroups differences were find out by the frequency of EMN ($P < 0.05$) and LMN ($P < 0.05$). According to our results of analyses of cytogenetic damages in erythrocytes and lymphocytes, we can conclude, that the immune system of framed carp more sensitive to the influence of genotoxins in water comparatively with scaled carp. One more important indicator of cytodifferentiation of fish cells is apoptosis. According to researchers O. Varga and V. Ryabov, apoptosis is the way of genetic defective cells death. Therefore, next step of our investigation was a comparative analysis of apoptosis frequency of framed and scaled carps. It has been established that a group of framed carp was characterized not only highest level by the results of micronucleus test and by the level of frequency of apoptosis (4.0 ± 0.2 ‰) comparatively with scaled carp (2.3 ± 0.2 ‰). Statistically significant intergroups differences ($P < 0.01$) has observed. Therefore, as to our opinion, the comparatively high level of apoptosis in a group of framed carp from fish farm "Sumyrybgosp" was the result of eliminating of genetic defective cells by this way and testifies that this breed of carp more sensitive to the effects of the environment genotoxins.

The results of cytogenetic analysis of starlet demonstrated that this fish were characterized not higher level of erythrocytes with micronuclei (EMN) (3.6 ± 0.5 ‰). The frequency of lymphocytes with micronuclei (LML) was (2.8 ± 0.3 ‰). The values of binuclear lymphocytes was at the level (1.9 ± 0.2 ‰). The presence of binuclear lymphocytes at the level of 1.9 ± 0.2 ‰ indicates about a little effect of direct-acting mutagens on the mitotic apparatus. According to the frequency of apoptosis, the starlet group was characterized by the following values (4.7 ± 0.5 ‰). This indicator testifies that a significant number of genetically defective cells were eliminated by programmed cell death, which made it possible to control normal cellular homeostasis in the studied group of starlet.

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