Journal of
Oceanography and Marine Research

Ahmadov et al., J Oceanogr Mar Res 2018, 6:3
DOI: 10.4172/2572-3103.1000183

Research Article Open Access

Influence of Nanoparticles on the Yield of Embryos of the Roe Rainbow Trout (*Oncorhynchus mykiss* Walbaum) in the Fermentation Process

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Abstract

Nanoparticles can get into the aquatic environment at different stages of the life cycle of water organisms and these interactions can cause an urgent need for the biochemical balance of the organism and the adaptation of the species to the changing environment. The goal of the current research is to investigate the effect of nanoparticles of Fe_3O_4 (20-30 nm) and Al (18 nm) on the processs of fertilization of sexual cells of rainbow trout (*Oncorhynchus mykiss* Walbaum) in the aquaculture condition and the effects of embryonic developmental stages after fertilization, as well as absorption of nanoparticles by feedstuffs and entry into the food chain and investigating possible bioaccumulation. According to the results of the experiments it was concluded that, when Fe_3O_4 (20-30 nm) and Al (18 nm) nanoparticles are added in roe of rainbow trout prior to fermentation, the percentage of fertilization of roe was higher, and the release of free embryos was higher than in the control variants. Fe_3O_4 (20-30 nm) nanoparticles have a stimulating effect at amount of 0.05 g (2018) and Al (18 nm) nanoparticles at the amount of 0.005 g (2018). It can be assumed that these nanoparticles have a catalytic effect on spermatozoid acrosomes, accelerate energy activity, and eventually increase the activity of spermatozoids, which leads to an increase in the rate of fertilization of the roe.

Keywords: Embryos release; Fermentation; Nanoparticles; Rainbow trout; Roe; Sperm

Introduction

The trout farming is a new aquaculture industry in Azerbaijan. Trout aquaculture, as one of the most progressive and perspective directions of artificial fish breeding, is currently being developed in more than 100 countries around the world. Due to its high adaptability to the environment in which it lives, relatively short incubation period of roes, its rapid growth rate and for some other features the rainbow trout (*Oncorhynchus mykiss* Walbaum) is one of the most widely grown fish species in the world today for commercial purposes [1-3].

There are many approaches to increase the productivity of trout farming which among them application of nanotechnology is a new and interesting field in this activity. The application of engineered nanomaterials and nanoparticles in industry, electronics, food technology, agriculture, medicine and consumer goods over the recent decades significantly increased their likelihood of falling into the environment, including to water, soil and air. Nanoparticles can cause different physiological effects in different aquatic organisms. In aquatic environments, nanoparticles can significantly change their structure, shape, and size as a result of aggregation, solubilisation or adsorption phenomena [4,5]. Physiological effects of nanoparticles on the aquatic organisms depends their type, size, concentration and exposition time. There are data that nanoparticles can diffuse into the organisms of aquatic invertebrates (amoebae, infusoria, cladocerans) and accumulates in organs [6]. In the experiments of Zhu and his colleagues shows that TiO_2, Al_2O_3, and ZnO nanoparticles may accumulate in the gut of the daphnia kept in suspensions of nanoparticles for 48 h. The accumulation time of nanoparticles depends on their type. For example TiO_2 rapidly accumulated over 12 h, whereas their excretion was slowed and a considerable part of these nanoparticles still remained in the daphnia body 72 h later. They compared the toxicity of these nanomaterials with their bulk counterparts using zebrafish embryos. Through examining the morphology, hatching and survival rates of embryos exposed to suspensions of these materials, ZnO was found to be the most toxic one that exerted hatching delay, skin ulceration. The metal based nanoparticles can cause different effect at different stages of the life cycle and these interactions can cause an urgent need for the biochemical balance of the organism and the adaptation of the species to the changing environment [6,7]. For example, at higher concentration (e.g. 1 mg/L of one particular nanoparticle) physical injuries such as gill edema, thickening of lamellae and embryonic injuries and high mortality are reported. At lower concentrations (5-50 μg/L), sub-lethal effects including physiological changes in Na^+K^+-ATPase activity, membrane lipid oxidation, chromosomal aberrations and aneuploidy could be observed, but no mortality. Osmoregulation in migratory fish depends on Na^+K^+-ATPase. According to the studies, nanoparticles can decrease Na^+K^+-ATPase in gill, however, its effect on fish migration, has not been considered [8].

Results of some experiments suggests that some nanometals can be more acutely toxic to some fish than dissolved forms. For example, juvenile zebrafish have a 48 h LC_{50} of about 0.71 and 1.78 mg/L for nano and dissolved forms of Cu respectively [9].

The fate of nanoparticles in water ecosystems, their interactions with biotic and abiotic components, and their potential in the creation of negative cases have not yet been fully investigated, and these uncertainties create anxiety over the risks to human and environmental health [6,10]. Thus, the study of the eco-toxicity of nanomaterials in the aquatic environment is special urgency.

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Received September 11, 2018; **Accepted** October 03, 2018; **Published** October 11, 2018

Citation: Ahmadov IS, Sadıqova NA, Agayeva NJ, Mamedov CA (2018) Influence of Nanoparticles on the Yield of Embryos of the Roe Rainbow Trout (*Oncorhynchus mykiss* Walbaum) in the Fermentation Process. J Oceanogr Mar Res 6: 183. doi: 10.4172/2572-3103.1000183

There is insufficient information in the scientific literature on rapid development of nanotechnology and its impact on hydrocele, particularly to embryonic development stages, which are more sensitive to external environmental factors in connection with its application to various fields. Therefore, the main purpose of the current research is to investigate the effect of nanoparticles of Fe_3O_4 (20-30 nm) and Al (18 nm) on sexual cells (sperm, egg cell) of rainbow trout (*Oncorhynchus mykiss* Walbaum*)* before fermentation in the aquaculture process and the effects of embryonic developmental stages after fermentation, as well as absorption of nanoparticles by feedstuffs and entry into the food chain and investigating possible bioaccumulation.

Material and Methods

The material of the research was carried out in January-March of 2017-2018 at Gabala salmonid fish hatchery named after G.Yusifov of the Republic of Azerbaijan. In the course of the experiment, mature male and female individuals were grouped and labeled according to gender dimorphism, and their length, mass and fullness factor (Fulton) were determined. The roe was taken from the sexually mature egg-laying fish by smoothing softly abdomen with thumb and placed in porcelain dishes with smooth surface according to number of options in experiment. 1 gram of roe sample was taken in each egg-laying fish used to calculate the number of roe and overall productivity and fixed in 4% formalin solution after determining the number of roe pieces.

The fermentation of the roe was carried out in a "dry" manner according to the accepted method [1,3]. The quality of the sperm extracted from males was primarily visualized and the activity of spermatozoids in the samples taken was determined on 5 points scale by adopted methodology for the case using the optic binocular microscope by magnifying them for 400 times. Fe_3O_4 (20-30 nm) and Al (18 nm) nanoparticles were used in experiment and purchased from Skyspring Nanomaterials Inc., USA, Houston TX. The sexual cells of rainbow trout (sperm, egg cell) and roe were exposed on nanoparticles in various amounts (0.0001 g, 0.001 g, 0.005 g and 0.05 g), as well as added to the fermented roe before fertilization and their effect on embryonic development stages was studied under microscope. Every day in the incubation process, dead embryos were removed from the apparatus in every version, was noted in special book and the free embryos were then counted.

Results

For the investigation the impact of nanoparticles on the fermentation process the experiments were conducted in three variant. In the first variant the sperm of rainbow trout were processed with nanoparticles before fermentation and then fertilized. In second variant before fermentation the roe of rainbow trout were processed with nanoparticles and then fertilized. In third variant the nanoparticles added to the fermented material of rainbow trout. The percentage of fermentation of roes and subsequent release of free embryos were calculated. The results of the experiments were compared with control. Experiments were conducted in two repeats (in 2017 and 2018 years).

First, was investigated the effect of Fe_3O_4 nanoparticles with sizes 20-30 nm and concentration of 0.0001 g and 0.001 g on 100 g of roe. As in first variant Fe_3O_4 nanoparticles was added to sperm of rainbow trout before fermentation and then fertilized. The sperm of rainbow trout was taken from fishes which have length 49 cm and mass 1530 g. In the result we showed that the percentage of fermentation of roes and subsequent release of free embryos for control (59.12%) was relatively high and was 61.65% and 71.45% respectively Table 1 and Figure 1. It was results of 2017 years experiments. As shown in Figure 1 the release of free embryos in this case was significantly higher than in control variant. In the Table 2 and Figure 2 shown the release of free embryos during impact of Al nanoparticles with sizes 18 nm on the sperm of rainbow trout before fertilization. In the case of Al nanoparticles the concentrations were same as Fe_3O_4 nanoparticles that is 0.001 g and 0.0001 g on the 100 g of roe. The results of Al nanoparticles were more interesting than with Fe_3O_4. Since at low concentrations (0.0001 g) the yield of roe was 98.66% whereas in control this was just 59.19% (Table 2 and Figure 2).

In the next experiments we increased concentration of nanoparticles about 50 time. In this case the approach was same, the ferment (sperm) of rainbow trout was processed with nanoparticles before fertilization and then roe was fertilized. The result of these experiments are given in Tables 3, 4 and Figures 3, 4. As shown in Figure 3 during processing of sperm of rainbow trout by Fe_3O_4 nanoparticles with 0.05 g concentration the release of roe was 92%. It was 10% more than in control. The same results were obtained with the Al nanoparticles, the release of roe was 93.7%. The results of these experiments say that even if we increase the nanoparticle concentration 50 times, we get the same high yield of rainbow trout roe. In the next experiments we used such concentration of nanoparticles for the processing of roe and fertilized materials.

In the second variant of experiments the roe of rainbow trout were processed with nanoparticles before fertilization. In these case also have used the 0.05 g and 0.005 g Fe_3O_4 and Al nanoparticles. Nanoparticles were added to the roe of rainbow trout, was mixed and five minutes later they produced fertilization. The results of these experiments were shown in the Tables 5, 6 and Figures 5, 6. While Fe_3O_4 nanoparticles were added in roe of rainbow trout in amount of 0.005 g and 0.05 g before fermentation, the release of free embryos in control variant were 35.37%, the total number of deaths was higher, 64.63%. But in test variant the release of free embryos in 0.05 g Fe_3O_4 was 46.64% and dead embryos were 53.36% i.e the number of dead embryos decreases. Same tendency was with concentration of 0.005 g Fe_3O_4 nanoparticles.

When are added to the roe of rainbow trout 0.05 g and 0.005 g Al nanoparticles before fermentation the release of free embryos was 54.1% and 73.8% and the number of dead embryos was 45.49% and 26.2% respectively. In the control variant these data were relatively low in control and was 64.63% and 35.37% respectively Table 6 and Figure 6.

Finally in the third version of experiments nanoparticles were used after fertilization. In this variant also have used the 0.05 g and 0.005 g Fe_3O_4 and Al nanoparticles. Nanoparticles were added to the fertilized materials of rainbow trout, was mixed and calculated release of embryos. The results of these experiments were shown in the Tables 7, 8 and Figures 7, 8. Adding to the fermented material of rainbow trout 0.05 g and 0.005 g Fe_3O_4 nanoparticles after fertilization the release of free embryos was 75.35% and 72.99% and the number of dead embryos was 24.65% and 27.01% respectively. In the control variant these data were relatively low in control the release embryos was 35.37% but the number of dead embryos was 64.63% (Table 7 and Figure 7).

Similar results were obtained when 0.05 g and 0.005 g of Al nanoparticles were added to the fermented roe of rainbow trout, the release of free embryos was 74.18% and 65.35% respectively. The number of dead embryos was 25.82% and 34.65% respectively Table 8 and Figure 8.

Amount of Fe_3O_4 (20-30 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,001	49	1530	100	2000	71,45	1429	28,55	571
0,0001	49	1530	100	2000	61,65	1233	38,35	767
Control	49	1530	100	1260	59,12	745	40,88	515

Table 1: Release of roe during impact of Fe_3O_4 nanoparticles on the ferment (sperm) of rainbow trout.

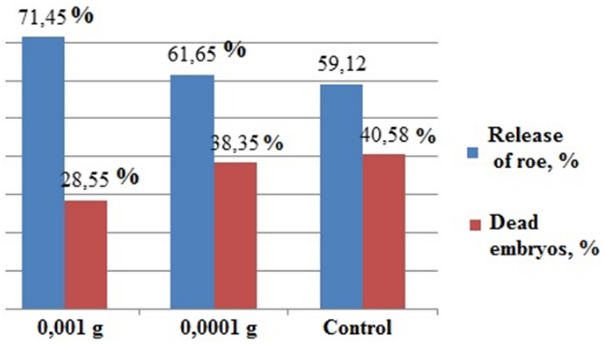

Figure 1: Release of roe during impact of Fe_3O_4 nanoparticles on the ferment (sperm) of rainbow trout.

Amount of Al (18 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,001	33	545	100	3000	59,46	1784	40,54	1216
0,0001	33	545	100	3000	98,66	2960	1,34	40
Control	49	1530	100	1260	59,12	745	40,88	515

Table 2: Release of roe during impact of Al nanoparticles on the ferment (sperm) of rainbow trout.

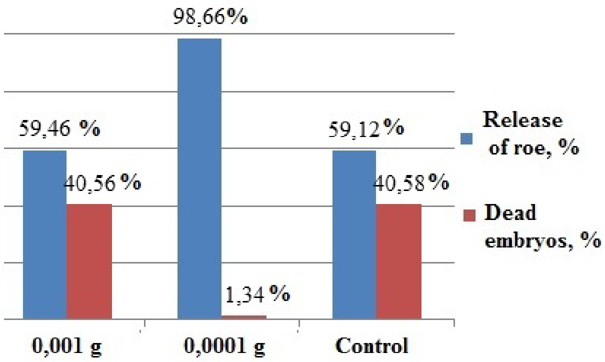

Figure 2: Release of roe during impact of Al nanoparticles on the ferment (sperm) of rainbow trout.

Amount of Fe_3O_4 (20- 30 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,05	54	2070	50	350	92,0	322	8,0	28
Control	54	2070	50	350	82,0	287	18,0	63

Table 3: Release of roe during impact of Fe_3O_4 nanoparticles on the ferment (sperm) of rainbow trout.

Amount of Al (18 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,005	54	2070	50	350	93,7	292	6,3	58
Control	54	2070	50	350	82,0	287	18,0	63

Table 4: Release of roe during impact of Al nanoparticles on the ferment (sperm) of rainbow trout.

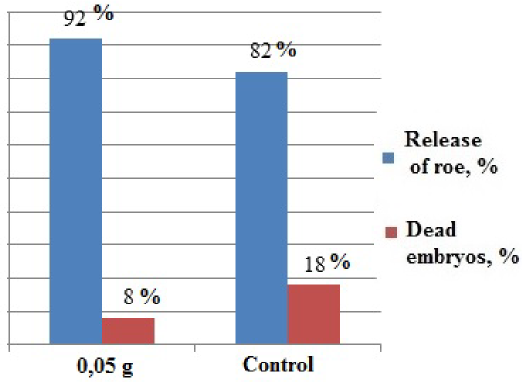

Figure 3: Release of roe during impact of Fe_3O_4 nanoparticles on the ferment (sperm) of rainbow trout.

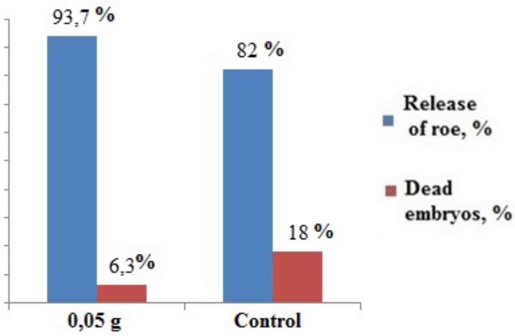

Figure 4: Release of roe during impact of Al nanoparticles on the ferment (sperm) of rainbow trout.

Amount of Fe_3O_4 (20-30 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,05	32	430	50	1300	46,64	606	53,36	694
0,005	44	1090	50	1000	44,1	441	55,9	559
Control	44	1090	38,3	766	35,37	272	64,63	494

Table 5: Release of roe during impact of Fe_3O_4 nanoparticles on the roe of rainbow trout.

Amount of Al (18 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,05	32	500	50	1300	54,1	703	45,49	597
0,005	32	540	50	1400	73,8	1033	26,2	367
Control	44	1090	38,3	766	35,37	272	64,63	494

Table 6: Release of roe during impact of Al nanoparticles on the roe of rainbow trout.

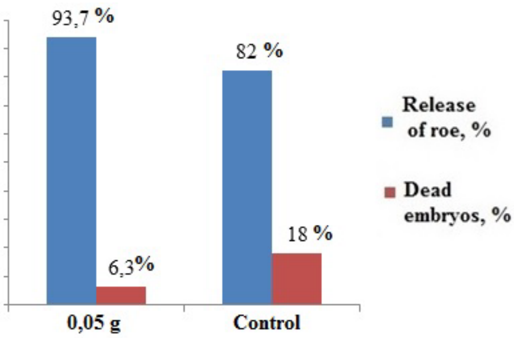

Figure 5: Release of roe during impact of Fe_3O_4 nanoparticles on the roe of rainbow trout.

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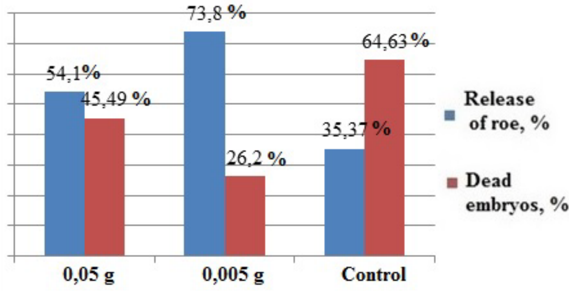

Figure 6: Release of roe during impact of Al nanoparticles on the roe of rainbow trout.

Amount of Fe_3O_4 (20-30 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,05	32	430	50	1300	75,35	979	24,65	321
0,005	44	1090	50	1000	27,01	270	72,99	730
Control	44	1090	38,3	766	35,37	272	64,63	494

Table 7: Release of roe during impact of Fe_3O_4 nanoparticles on the fermented roe of rainbow trout.

Amount of Al (18 nm), g	Length of the fish L, cm	Mass of fish P, g	Amount of roe, g	Total number of roe, pcs	Release		Number of dead	
					%	pcs	%	pcs
0,05	32	500	50	1300	74,18	964	25,82	336
0,005	29	420	50	1350	65,35	882	34,65	468
Control	44	1090	38,3	766	35,37	272	64,63	494

Table 8: Release of roe during impact of Al nanoparticles on the fermented roe of rainbow trout.

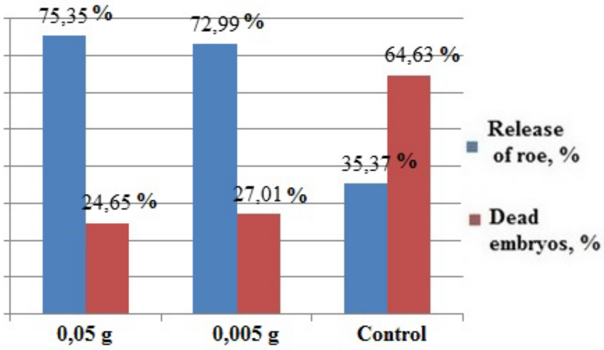

Figure 7: Release of roe during impact of Fe_3O_4 nanoparticles on the fermented roe of rainbow trout.

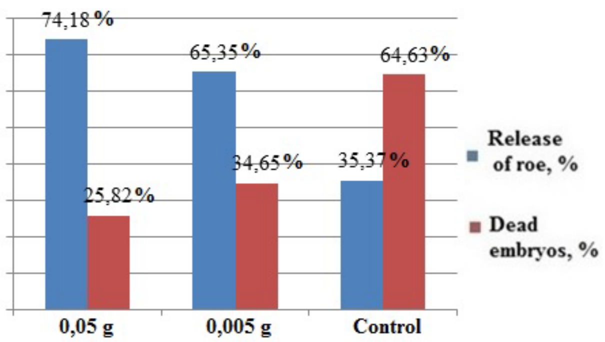

Figure 8: Release of roe during impact of Al nanoparticles on the fermented roe of rainbow trout.

Discussion

Fish play an important roles in the food chain of water ecosystems, therefore often they are used for toxicological monitoring in the environmental studies, because fishes even respond to low concentration of xenobiotics and can rapidly absorb toxic substances [11]. Last years, for rapid evaluation of nanoparticles toxicity the zebrafish (Danio rerio) was a simple model [12] for such kind of investigations. In most cases toxic effects of nanoparticles were observed in different water animals including fishes. Nanoparticles can cause biochemical, physiological and morphological responses in fish which results toxic effects. Using both fishes from sea and sweet waters as model organisms, the toxic effects of nanoparticles are studied comparatively, and the main purpose of these studies is to determine the risk of nanomaterials [13,14]. Bioaccumulation of nanoparticles, its distribution and localization in organisms, removal of tissues and their mechanism of action are intensively studied [7,15-18].

Overall the data suggests that aqueous SWCNTs are a respiratory toxicant, neurotoxic or cardiovascular effects of SWCNTs that may alter fish behavior, SWCNTs increases the risk of mortality [19], TiO_2 nanoparticles could pose toxicity to fish [20]. During exposition fish to different nanoparticles Xiong et al. [21] observed toxicological effects, gill histological alteration, which can compromise vital functions such as, for example, gas exchange. Campos-Garcia et al. [22] have observed hypertrophy and hyperplasia of the epithelial cells, dilation of capillaries and aneurism of the lamellae on influence multiwalled carbon nanotubes.

Based on scientific literature [7,13,22-27] a number of research studies have identified the effect of various nanoparticles on hydroceles, the high biological activity of some of the metals' nanoparticles, the acceleration of metabolism and the increased resistance of the organism to natural factors, growth rate and exterior symptoms. It has been noted that in addition to the high bactericidal properties of metals in the form of nanoparticles, it also has less toxicity and are not accumulated in the body because they are used as microelements in the metabolism process [28].

It should be noted that there is no information in the scientific literature on the study of the effects of Fe_3O_4 (20-30 nm) and Al (18 nm) nanoparticles on sexual cells (sperm, egg cell) of rainbow trout (*Oncorhynchus mykiss*), as well as on its fermented roe. Danio rerio (1822) from sweet water fishes was used as a model object to study the effects of nanoparticles on the embryonic development process. In the course of the experiments, *Danio rerio* embryos were exposed to impact of CeO_2 and silver (Ag) nanoparticles (LC_{50}) for 96 hours, their morphological changes at the embryonic development stages, the release of free embryos and the percentage of deaths depending on the amount of nanoparticles [13,14] were studied. It was noted that in pure conditions, CeO_2 nanoparticles did not cause complications in the development of embryos of *Danio rerio,* but the increase in the amount of silver (Ag) nanoparticles resulted in an increase of death rates of embryos and number of crippled embryos.

According to the results of the research, when Fe_3O_4 (20-30 nm) and Al (18 nm) nanoparticles are added in roe of rainbow trout prior to fermentation, the percentage of fertilization of roe was higher, and the release of free embryos was higher than in other variants. Fe_3O_4 (20-30 nm) nanoparticles have a stimulating effect at amount of 0.05 g and Al (18 nm) nanoparticles at the amount of 0.005 g. It can be assumed that these nanoparticles have a catalytic effect on spermatozoa acrosomes, accelerate energy activity, and eventually increase the activity of spermatozoids, which leads to an increase in the rate of fertilization of the roe.

It should be noted that rainbow trout *(Oncorhynchus mykiss)* was brought to Azerbaijan since 1981, its generative fund has been formed and exploited for many years. Azerbaijan's salmonids fish hatcheries used by producers were formed in plant conditions for many years. In such a situation, the high rate of relationships between individuals leads to the occurrence of the inbreeding. That is why the percentage of deaths among small fries at the fish growing process is high, in many cases the quality of the producers' sexual products is low and the new generation emerging from them is intolerant to the various environmental effects. There are statistical data on this in connection with hatchery's activity for many years.

Conclusion

Toxic effects were observed in the work of many scientists who investigated the effect of nanoparticles on fish. However, in our experiments, we observed the positive effects of iron and aluminum nanoparticles on the fertilization of fish. For example, the addition of 0.001 g of iron oxide nanoparticles to the sperm material release of free embryos after fertilization were significantly higher than the in control variant (59.12% in control, 71.45% in nanoparticles). In all cases during our experiments, nanoparticles accelerated the fertilization process. The mechanism of this process unknown and needs addition experiments.

Thus, the obtained results can be used to reduce the losses occurring during the embryonic development stage in the aquaculture process of the salmonid fishes, especially in the rainbow trouts, and to increase overall productivity.

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