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INFLUENCE OF Fe_3O_4 AND AL NANOPARTICLES ON EMBRYONIC GROWTH AND ORGAN STRUCTURES OF COMMON CARP IN CONTROLLED AQUATIC ENVIRONMENTS (*CYPRINUS CARPIO* L.)

Aysel Hajiyeva*, Chingiz Mammadov, Rovshan Khalilov

*Baku State University, 23 Z. Khalilov St, AZ-1148, Baku, Azerbaijan**

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Abstract

This study investigates the effects of iron oxide (Fe_3O_4) and aluminum (Al) nanoparticles on the reproductive cells and embryonic development of common carp (Cyprinus carpio), a key species in global aquaculture. Given the growing application of nanomaterials across industries and their increasing presence in aquatic ecosystems, understanding their impact on fish reproduction and development is critical. The study was conducted in two parts: first, reproductive cells (sperm and eggs) were exposed to varying concentrations (0.0001–0.05 g) of Fe_3O_4 and Al nanoparticles during artificial fertilization. Results indicated that low concentrations (0.0001 g and 0.001 g) of both nanoparticle types enhanced fertilization rates, while higher concentrations significantly reduced embryo viability and increased mortality. In the second part, common carp were fed diets supplemented with Fe_3O_4 nanoparticles (10 mg and 100 mg per 10 g feed) for 7 days. Histological and ultrastructural analyses of the intestinal tissue revealed dose-dependent cytotoxic effects, including microvilli degradation, mitochondrial damage, and nanoparticle accumulation in epithelial and vascular cells. These findings highlight the dualistic nature of nanoparticle exposure—potentially beneficial at low doses but harmful at higher concentrations—underscoring the need for regulated nanoparticle usage in aquaculture. The results provide valuable insights for establishing safe biotechnical standards in fish farming under ecologically sustainable conditions.

Keywords*: common carp; nanoparticles; sexual cells; fertilization of roe; fermentation process*

1. Introduction

Aquaculture has emerged as a critical solution to meet the rising global demand for animal protein, offering a sustainable alternative to overexploited marine fisheries. Among various aquaculture species, common carp (*Cyprinus carpio* Linnaeus, 1758) holds a prominent position due to its remarkable adaptability to diverse environmental conditions, high growth performance, resistance to diseases, and relatively simple breeding techniques [2]. As a result, it has become one of the most cultivated freshwater species globally, especially in regions such as Asia, Eastern Europe, and parts of Africa.

In parallel with advancements in aquaculture, nanotechnology has seen explosive growth in the last two decades, leading to the widespread use of nanoparticles (NPs) in a broad range of industries including

*Corresponding author. Tel.: +994 50 775 00 91
E-mail address: aysel.haciyeva@list.ru

agriculture, cosmetics, medicine, electronics, and water treatment [1, 3]. While the integration of nanomaterials has brought significant innovations, it has also raised substantial concerns about their environmental safety. Nanoparticles, particularly those composed of metal oxides such as iron oxide (Fe_3O_4) and aluminum (Al), are increasingly being detected in aquatic ecosystems due to leaching, industrial discharge, and atmospheric deposition [14]. Their unique physicochemical properties, including high surface area and reactivity, render them more biologically active than their bulk counterparts [7].

Despite these concerns, the ecotoxicological implications of nanoparticles in aquatic environments remain insufficiently understood. The potential for nanoparticles to interact with cellular membranes, induce oxidative stress, disrupt enzymatic activity, and interfere with reproductive physiology has been documented in several aquatic organisms [7]. However, studies focusing on early ontogenetic stages—including gametes and embryos—are still scarce. These developmental stages are particularly vulnerable to toxicants due to their rapid cell division and differentiation, limited detoxification mechanisms, and the high permeability of protective membranes such as the chorion [5, 8, 13]. Furthermore, nanoparticles may not only exert direct cytotoxic and genotoxic effects but could also be bioaccumulated through trophic transfer, thereby posing long-term threats to aquatic food webs and, ultimately, to human health via consumption of contaminated fish [13]. In the context of aquaculture, such risks could compromise reproductive success, larval survival, and overall productivity, undermining the sustainability of fish farming operations [7].

Therefore, this study aims to assess the toxicological effects of Fe_3O_4 and Al nanoparticles on the reproductive cells (sperm and eggs) and embryonic development of common carp under controlled aquaculture conditions [2]. By evaluating different nanoparticle concentrations and their impact on fertilization rates, embryo viability, and mortality, this research contributes to a more comprehensive understanding of nanoparticle interactions with aquatic biota and offers important insights for risk assessment in nanotechnology-integrated environments [7].

2. Materials and Methods

The investigation consists of two parts. Experimental studies of the first part were carried out in June 2021 at the aquaculture facility "Samukh - Fish" in the Republic of Azerbaijan. Sexually mature *C. carpio* individuals were categorized by sex, and their biometric parameters-total length, body weight, and Fulton's condition factor-were recorded.

Gametes were extracted using non-invasive methods. Oocytes were obtained through gentle abdominal pressure and deposited into smooth porcelain containers. From each female, 1 g of eggs was sampled to assess fecundity. Samples were preserved in 4% formalin for further analysis. Spermatozoa were collected from males and evaluated for motility using a five-point scoring system under a Karl Zeiss Axio Imager M2 microscope at 200× magnification.

Fe_3O_4 (20–30 nm) and Al (18 nm) nanoparticles, procured from Skyspring Nanomaterials Inc. (Houston, TX, USA), were introduced into the gametes at graded concentrations (0.0001 g, 0.001 g, 0.005 g, 0.05 g). The exposure took place during dry fertilization procedures. Additionally, the influence of these nanoparticles on post-fertilization embryonic development was monitored using a microscope throughout the incubation period. Dead embryos were recorded daily and removed to prevent fungal contamination [17].

In the other part, a total of 33 one-year-old carp fry were obtained from a fish farm in the Neftchala city of Azerbaijan. The fish were divided into three groups (n=11 per group) and maintained in three separate aquaria (60 L each) under controlled conditions. Group I served as the control, while Groups II and III were fed commercial feed supplemented with Fe_3O_4 nanoparticles at doses of 10 mg and 100 mg per 10 g of feed, respectively. The experiment lasted for 7 days. Fe_3O_4 nanoparticles (10–30 nm, 98+% purity, Skyspring Nanomaterials, USA) were used. Water parameters (temperature: 22–24°C, O_2: 8.2–8.6 mg/L, pH: 7.4–7.6) and feeding regimes were kept constant. At the end of the exposure, fish were dissected, and samples of the small intestine were collected and fixed for light and transmission electron microscopy (TEM). Standard protocols were followed for tissue preparation, sectioning, staining, and imaging [4, 11].

3. Results and discussion

Based on the objectives of the study, a series of experiments were conducted using varying concentrations of Fe_3O_4 (20–30 nm) and Al (18 nm) nanoparticles to evaluate their effects on the fertilization capacity and embryonic development of common carp (*Cyprinus carpio* L.) [2]. The experiments involved exposure of reproductive cells (sperm and eggs) and fertilized roe to nanoparticles in different concentrations (0.0001 g,

0.001 g, 0.005 g, and 0.05 g). Results from the experimental groups were compared against the control group to assess both beneficial and adverse outcomes.

The addition of Fe_3O_4 nanoparticles at lower concentrations (0.0001 g and 0.001 g) to the sperm samples prior to fertilization showed a notable improvement in fertilization success. Specifically, fertilization rates increased to 61.65% and 71.45% compared to the control group's rate of 59.12% [14]. This suggests a potential stimulatory effect at lower concentrations, possibly due to enhanced motility or membrane interaction that facilitates sperm-egg binding.

Similarly, Al nanoparticles at 0.0001 g and 0.001 g concentrations produced an even more pronounced effect. The fertilization rates reached as high as 98.66%, indicating that low levels of Al nanoparticles may promote fertilization processes. However, the 0.001 g Al-treated group showed a moderate fertilization rate of 59.46%, suggesting that the optimal concentration window is narrow and highly dose-dependent [17].

Conversely, higher concentrations of Fe_3O_4 nanoparticles (0.005 g and 0.05 g) introduced to unfertilized roe prior to fertilization resulted in a substantial decrease in embryo viability. Despite the control group maintaining a healthy 35.37% embryo release rate, the treated groups exhibited increased embryonic mortality rates of 53.36% and 55.9% respectively. These findings highlight a toxic threshold beyond which Fe_3O_4 nanoparticles exert detrimental effects, likely due to oxidative stress or disruption of early developmental signaling [7, 10].

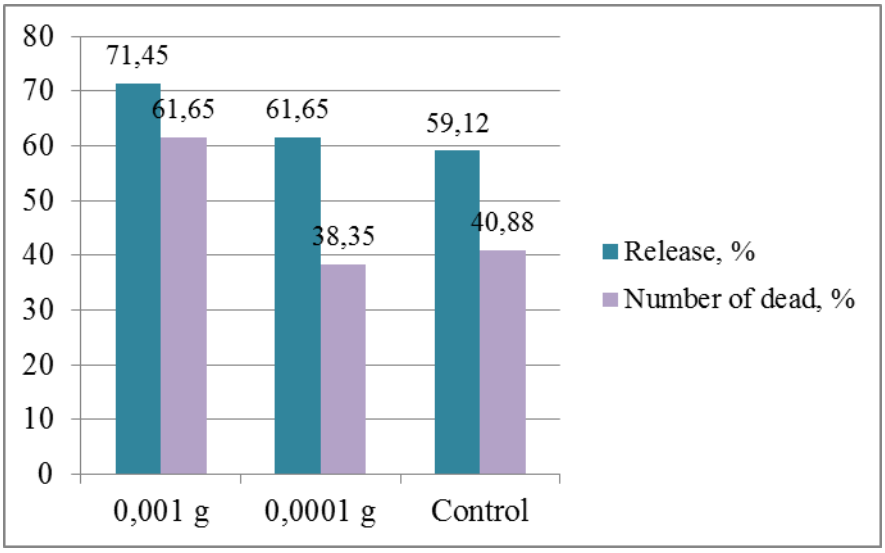


Fig. 1. Including of Fe_3O_4 (20-30nm) nanoparticles to the ferment (sperm) of common carp

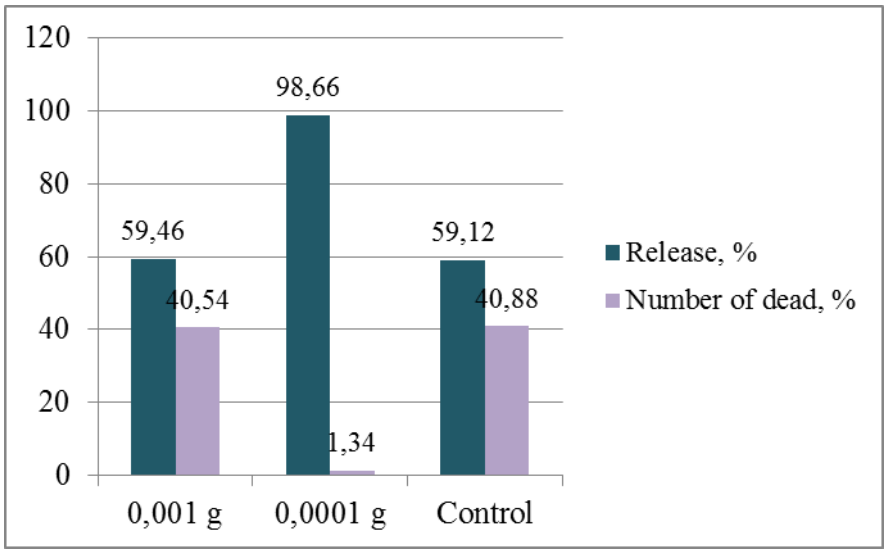


Fig. 2. Including of Al (18nm) nanoparticles to the ferment (sperm) of common carp

Daily monitoring during the incubation period revealed consistent trends in embryo mortality across experimental groups. Embryos exposed to higher nanoparticle concentrations exhibited delayed development, increased malformations, and impaired hatching rates [6]. These effects are consistent with nanotoxicological literature indicating that nanoparticles can induce cellular stress, interfere with normal morphogenesis, and accumulate in vital organs such as the liver and brain during later stages of development [13].

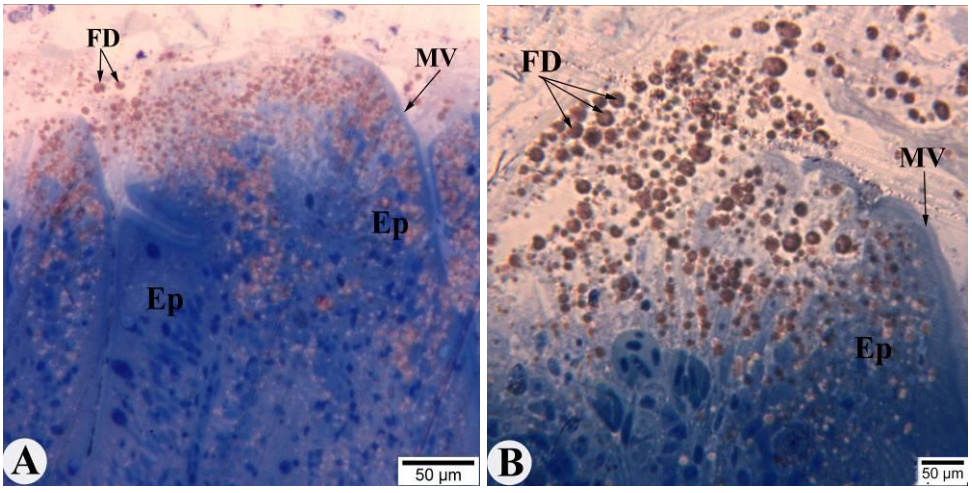
Taken together, the results underscore the dual nature of nanoparticles in aquatic systems: while low concentrations may enhance certain physiological processes, elevated exposure can lead to toxicity and developmental impairment [7]. Further mechanistic studies are required to elucidate nanoparticle interactions with gametes at the molecular level and to define safe exposure thresholds in aquaculture settings.

The histological and ultrastructural analyses revealed significant alterations in the intestinal architecture of Cyprinus carpio juveniles exposed to Fe_3O_4 nanoparticles under aquaculture conditions. Control specimens exhibited normal intestinal morphology, with well-preserved epithelial layers, intact microvilli, and typical submucosal and muscular layers [4]. In the group exposed to 10 mg/day of Fe_3O_4 nanoparticles, mild pathological changes were observed in the apical regions of enterocytes. Light and transmission electron microscopy (TEM) showed disrupted and disorganized microvilli at the epithelial surface facing the intestinal lumen. Fat droplets and cellular debris were observed in the lumen, suggesting early-stage membrane instability and cytoplasmic damage. Mitochondria in these cells displayed partial membrane disintegration and loss of cristae, accompanied by intercellular edema [10, 14].

More pronounced effects were recorded in the group receiving 100 mg/day of Fe_3O_4 nanoparticles. Structural damage extended to all intestinal layers, including the serosa, muscularis, submucosa, and mucosa. Muscle cells exhibited mitochondrial swelling and disintegration of cristae [15]. Neurocytic elements in the muscularis layer showed nuclear membrane thickening and cytoplasmic vacuolization. Endothelial cells lining the blood vessels in the lamina propria were deformed, with notable protrusions into the vascular lumen. The microvilli of enterocytes were either severely shortened or entirely degraded [10].

TEM images confirmed that the majority of internalized nanoparticles were between 10–20 nm in diameter, despite the applied particle size being up to 30 nm. Nanoparticles were traced from the intestinal microvilli into the epithelial cytoplasm, accumulating in mitochondria, lysosomes, and lipid droplets. Further penetration into the lamina propria and vascular endothelial cells was also observed [1, 16].

These findings suggest a dose-dependent cytotoxic and structural impact of Fe_3O_4 nanoparticles on intestinal tissues. The progressive nature of the damage indicates that even short-term exposure to higher nanoparticle concentrations may lead to systemic tissue injury and potential impairment of intestinal function in aquaculture species [2, 14].



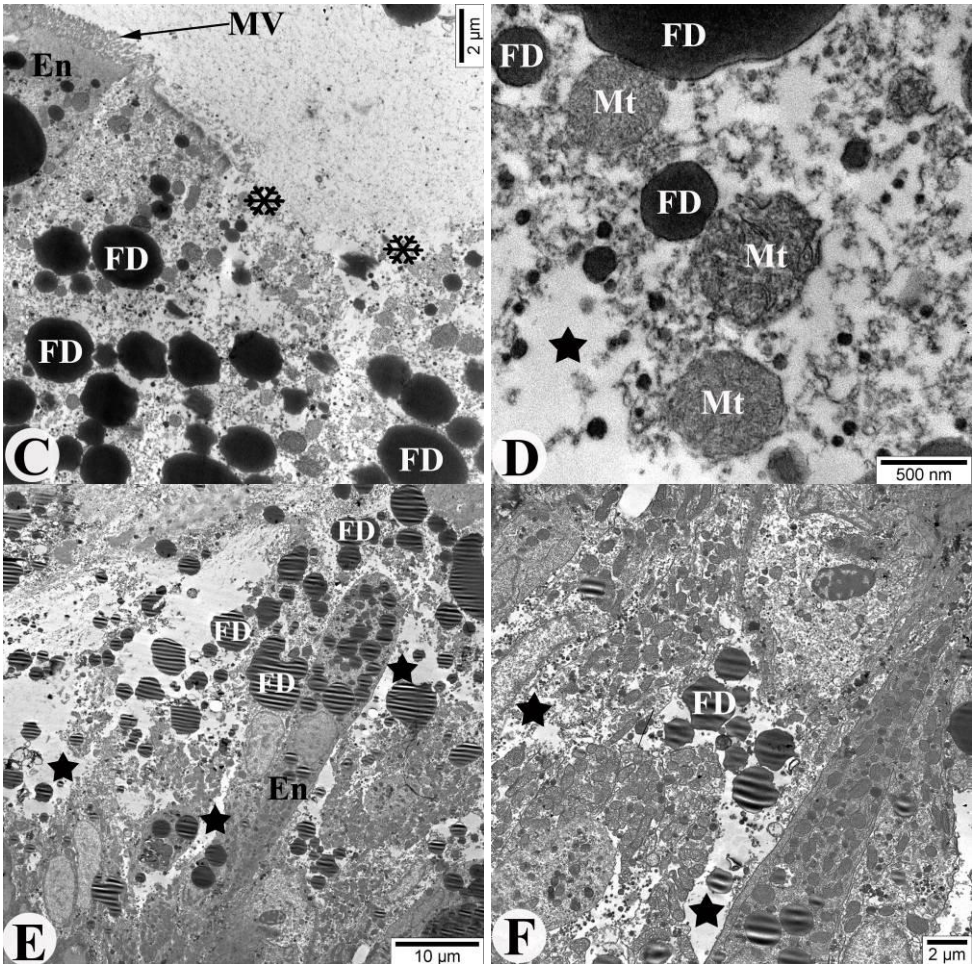


Fig. 2. Changes in the small intestine after exposure to Fe_3O_4 (10 mg) nanoparticles in common carp. A – B – Light microscope image of the mucosa layer of the small intestine, C – F – Electronograms of the epithelial cells and cytoplasmic organelles in the mucosa layer. **Designations:** Ep – epithelium, MV – microvilli, FD – fat droplets, En – enterocyte, Mt – mitochondrion, Star – edema.

4. Suggestions for implementing the results

The results obtained can be used in developing optimal biotechnical standards for the cultivation of fish species of great importance in aquaculture in Azerbaijan from the point of view of food security, in ecologically clean conditions, compliance with sanitary standards, and also for the formation of healthy reproductive stocks [12].

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