Extracellular vesicles – potential mediators in the functioning of fish male reproductive system

Fish reproduction determines both the survival of species in the wild and the profitability of aquaculture. Environmental stress, infections, and poor farming conditions disrupt these processes, threatening biodiversity and causing economic losses estimated at millions of dollars annually. Our project focuses on the role of small extracellular vesicles (EVs), microscopic structures secreted by cells, in regulating key processes occurring in the male reproductive system of fish. These vesicles act as carriers of proteins, lipids, and genetic material, making them important mediators of immune processes, stress responses, and reproductive functions. EVs are present in all biological fluids, such as blood and semen, where they serve as crucial messengers in intercellular communication. In mammals, EVs are known to improve semen quality and support immune defense mechanisms; however, their specific role in fish reproduction remains largely unexplored. By studying these vesicles, we aim to fill this knowledge gap and determine how EVs influence sperm function, immune response, and stress adaptation. The research will be conducted on two species of the carp family: common carp (*Cyprinus carpio* L.) and zebrafish (*Danio rerio*). Carp is a key species in global aquaculture due to its economic value, while zebrafish, as a model laboratory organism, provides unique insights into cellular and molecular processes. The combination of these two species allows the creation of a complementary research system that will enable a comprehensive understanding of the role of EVs in fish physiology. The project includes several key components. First, we will isolate and characterize EVs from carp blood plasma and seminal plasma to determine their protein composition, miRNA content, and identify specific markers. Next, we will investigate how stress and infections caused by bacteria and viruses alter the protein and miRNA profiles of EVs. An important aspect of this research is to determine how EVs derived from the seminal plasma and blood plasma of infected and stressed carp males affect sperm motility, viability, and resistance to oxidative stress—factors that directly influence reproductive success. Additionally, we will analyze whether immune system cells, particularly carp macrophages, secrete EVs and what impact these vesicles have on semen quality in both carp and zebrafish. The project also has practical potential, as EVs may serve as non-invasive biomarkers for monitoring fish health, enabling fish farmers to detect stress and diseases earlier, thereby improving animal welfare. Thanks to an interdisciplinary approach utilizing advanced proteomic and molecular techniques, our project will not only deepen knowledge about fish reproduction and immunity but also provide tools supporting sustainable and efficient aquaculture practices.