Small non-coding RNAs (sncRNAs): Novel biomarkers for assessing sperm fertilizing ability in Eurasian perch

Sperm plays a pivotal role in shaping the phenotype of progeny, with its quality being crucial for achieving high fertilization rates resulting in higher numbers of offspring. There are several sperm quality parameters, such as sperm motility, viability, semen concentration, and DNA integrity, that are currently state-of-the-art indicators for sperm quality. However, none of them directly correlate with fertilization success in Eurasian perch (*Perca fluviatilis*)

Among the various molecules present in the sperm of this species (DNA, RNA, proteins), small non-coding RNAs (sncRNAs) have emerged as the most promising predictive candidates for being molecular biomarkers. These sncRNA biomarkers can significantly enhance selective breeding programs, provide insights into reproductive impairments in fish culture, and ultimately improve our understanding and management of reproductive performance not only in this species but also in other fish species. So far, the usefulness of sncRNA as biomarkers of sperm quality in higher vertebrates has been emphasized, as their expression profiles reflect the functional status and fertilizing capacity of sperm. There is no information available in the literature regarding the use of sncRNA of sperm in predicting reproductive success in Eurasian perch, nor about changes in RNA molecule profiles depending on semen quality. Emerging evidence highlights the capability of sncRNAs as biomarkers for sperm quality, as their expression profiles can reflect the functional status and fertility potential of spermatozoa.

Therefore, the main objective of this project is to identify individual sncRNAs in sperm of Eurasian perch and to examine changes in the sncRNA profile depending on the fertilizing capacity of the sperm. To explore this the following hypothesis is proposed: specific sncRNAs can act as reliable fertility biomarkers for Eurasian perch sperm.

During the implementation of the project, cryopreserved Eurasian perch semen samples (n=20) stored in the semen bank of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences (IRZiBŻ PAS) will be used. These samples have been comprehensively characterized in terms of quality and fertilizing capacity. The first step will involves extracting the total RNA from these cryopreserved semen samples and ensuring that the isolated RNA is free from contamination. Once confirmed, the RNA will be sent for sequencing, focusing specifically on sncRNAs (less than 200 nucleotides). After obtaining the sequencing results, bioinformatic tools will be used to process the data into a readable format. Next, we will perform a weighted gene correlation network analysis to identify any positive or negative correlations with fertilization success data already available in our database. This will allow us to determine whether sncRNAs can be used as fertility biomarkers.

The identification and utilization of sncRNA biomarkers can enhance selective breeding programs and improve our understanding of reproductive failures, thereby contributing to better management and conservation not only Eurasian perch, but also other freshwater fish species.