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**Application of density gradient centrifugation for obtaining highly motile spermatozoa from carp and trout cryopreserved sperm**

Nowadays, cryopreserved sperm is used in environmental protection programs and animal husbandry. In fish, cryopreservation is primarily used as a method of conservation of endangered species. Sperm cryobanking has already been used as one of the directions of activity in the National Programme on Conservation of Farm Animal Genetic Resources in the Czech Republic. In addition, the application of fish sperm cryobanking has the applied potential in aquaculture for reducing the number of male broodstock, prevention of desis transfer, and conservation of genetic material required for breeding programs.

Various factors limit the implementation of cryopreservation in practice. Cryopreservation of sperm is always accompanied by damaging factors, e.g., due to an osmotic shock during mixing sperm with a cryoprotective extender as well as caused by significant changes in the cellular environment (like pH, ionic composition, and medium osmolality) appearing due to ice crystal formation. In the process of freezing and thawing, various cell components can be damaged, which leads to changes in motility parameters. And because the most important work of spermatozoa is dilivery of genetic material to the egg. We will mainly investigate changes in the percent of motile cells and another parameter of cell movement.

The present study will look for optimal parameters for separating the fraction of motile cells from cryopreserved sperm of rainbow trout and common carp. The selection of fish models for the study is based on the assumption that common carp and rainbow trout are the main aquaculture species for inland aquaculture in many countries.

The object for our study will be cryopreserved sperm from adult males of common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Sperm samples will be prepared before the experiment during the natural breeding season for each species. During the summer school project, samples will be thawed according to existing protocols, and sperm suspension will be added on top of the Percoll gradient collum and centrifuged. After obtained fraction will be washed from Percoll in an artificial seminal fluid. Samples before and after separation will be video-recorded using a digital camera coupled with a negative phase-contrast microscope. Video records will be analyzed using the CASA (Computer-Aided Sperm Analysis) plugin for ImageJ. The obtained data will be statistically analyzed and used to improve freezing protocols further.