COST Action FA1205: AQUAGAMETE - Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry

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STSM Reference: ECOST-STSM-FA1205-010716-079781
Dates: 01.07.2016. – 15.07.2016.
Location: University of South Bohemia in České Budějovice, Vodňany, Czech Republic
Host: Dr Martin Psenicka

Purpose of the STSM

This STSM was carried out at the University of South Bohemia in České Budějovice, Vodňany, Czech Republic under the supervision of Dr Martin Psenicka, with the main objective of developing techniques for slow-rate freezing of common carp *Cyprinus carpio* testicular tissue.

Description of the work

The main focus of the present STSM was to develop slow-rate freezing procedure for common carp testicular tissue, and afterwards transplant isolated spermatogonia into suitable donors.

For cryopreservation of whole tissue, excised testes were cut into small pieces (0.1 g), equilibrated in cryoprotective media and then frozen in 1.8-ml cryovials while fresh tissue was used as a control. We tested the effects of 5 different cryoprotectants: methanol, DMSO, ethylene glycol, propylene glycol + DMSO and glycerol. Cryopreservation was conducted in a controlled-rate freezer with cooling rates of 1 °C/min. Samples were stored in liquid nitrogen for at least one day. Thawing was conducted in a 38 °C water bath for 40 s. Both control tissue and frozen/thawed tissue were digested using 0.5% trypsin and 40 µg/ml DNase I at room temperature. Percentage of live cells following cryopreservation was determined as the number of recovered cells compared to the controls.

Before transplantation, host zebrafish and goldfish larvae were sterilized by using antisense morpholino oligonucleotide against *dead end* (MO dnd1). In this way we would be able to produce sterile hosts in which only donor-derived germ cells would mature in the hosts following transplantation. During these trials I learned the basics of using MO *dnd1* in sterilization as this is the first time I was included in such a trial.

The main objective of this STSM fits into the working group 2 objectives of the Aquagamete action.

Confirmation by the host institution of the successful completion of the STSM

Dr. Martin Psenicka (USB, Czech Republic) certifies that Zoran Marinović (UNS, Serbia) has completed a Short-Term Scientific Mission (STSM) awarded by the COST Action FA1205 in the period 01.07.2016. – 15.07.2016.

Dr Martin Psenicka
(USB-Host institution)

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(STSM applicant)