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Location: Departmenf for Animal Science, Biotechnical Faculty of Ljubljana, University of Ljubljana, Domžale, Slovenia
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STSM report

This STSM was carried out at the Department for Animal Science, Biotechnical Faculty of Ljubljana, University of Ljubljana, Domžale, Slovenia in a collaboration with Dr Simona Sušnik Bajec. The main aims of this STSM were developing a slow-rate freezing procedure for testicular tissue of brown trout *Salmo trutta* and transplantation of spermatogonia from fresh and freeze-thawed tissue into rainbow trout larvae.

Two cryopreservation experiments were conducted. In the first experiment, four different cryoprotectants (methanol, DMSO, glycerol and ethylene glycol) were used in the concentration of 1.3 M. In the second experiment, cryoprotectant with the best results from the first experiment was used to test different concentrations. In this case, DMSO displayed the highest viability values and therefore, we tested three concentrations (1 M, 1.3 M and 1.6 M) of this cryoprotectant on cell viability. Freezing procedure was conducted by using CoolCell boxes which enabled cooling rates of -1 °C/min from 4 °C to -80 °C after which the samples were plugged into liquid nitrogen. Thawing was conducted in a 10 °C water bath until samples were not thawed (approximately 2 minutes).

For both experiments small pieces of testicular tissue (W: 0.01-0.02 g) were used. Samples were cryopreserved in 1.8-ml cryovials. Fresh tissue pieces were used as a control. Both control and frozen/thawed tissue were digested using 2 mg/ml collagenase and 10 µg/ml DNase at room temperature. Percentage of live cells following cryopreservation was determined as the number of recovered cells compared to the controls.

Transplantation of both fresh and cryopreserved spermatogonia of brown trout to rainbow trout larvae was done. Larvae were anesthetized prior handling and in each was injected 2-4 µl of cell suspension (approximately 20.000 cells) stained by PKH-26. Success of the transplantation procedure will be checked two months after transplantation.

AQUAGAMETE

Dr Simona Sušnik Bajec
(STSM host)

AQUAGAMETE

Dr Jelena Lujić
(STSM applicant)