EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

AQUAGAMETE

8th Call for STSM-Scientific Report

COST STSM reference number: COST-STSM-FA1205-30871

Period: 2016-04-01 to 2016-04-30

COST action: FA1205

STSM applicant: Ms Yevhen Horokhovatskyi, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic

Host: Prof. Andrzej Ciereszko, Polish Academy of Sciences, Institute of Animal Reproduction and Food Research, Department of Gamete and Embryo Biology, Olsztyn, Poland

STSM Topic: Changes in fish sperm proteins after cryopreservation

Purpose of the STSM

This STSM was carried out at the Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research under the supervision of Prof. Andrzej Ciereszko, with the main objective to investigate protein content in sturgeon (Acipenser ruthenus) sperm after cryopreservation applying Percoll density gradient separation.

Work during the STSM period

The main focus of the present STSM was to investigate damaging effect of cryopreservation procedure to fish sperm only of surviving spermatozoa. For obtaining only survived sperm fraction Percoll density gradient centrifugation was applied as a simple and safe technique for separation of spermatozoa. After sperm collection half volume of sperm was frozen-thawed and live spermatozoa were separated using Percoll density gradient centrifugation. During all steps of experiment, sperm quality was controlled by optical phase contrast microscope and flow cytometry live/dead sperm viability (SYBR Green / Propidium Iodide). Sperm motility and viability of spermatozoa following separation was > 99%. Sperm fractions were washed with artificial seminal fluid and from resulting pellets of spermatozoa the proteins were extracted. Before the proteomic analysis by 2D-DIGE (two-dimensional difference in-gel electrophoresis), protein lysate containing approximately 500 µg of sperm proteins were processed using a Clean-Up Kit. The protein concentration before and after the cleaning procedure was measured by a Coomassie (Bradford) Protein Assay Kit. Then, aliquot of 50 µg of protein from each sample was labeled with CyDye DIGE Fluor minimal dyes. During first dimension, differentially labeled samples were mixed together according to the special scheme, loaded onto IPG strips with passive rehydration and separated by isoelectric focusing. After isoelectric focusing, the strips were equilibrated in SDS equilibration buffer and transferred to precast DIGE gels. A second dimension of

electrophoresis was performed in an Ettan Dalt-Six apparatus for 16 h. After electrophoresis, the gels were scanned with a scanner using the parameters suggested by the manufacturer for 2D-DIGE experiments. The scanned images were analyzed with DeCyder Differential In Gel Analysis version 5.02 software in order to quantify the fluorescent intensities of the spots. Only protein spots with P-value < 0.05 by t-test analysis that showed at least a 1.2 fold increase or decrease in their relative intensities were considered to be proteins present in different levels of abundance. The first results shown that proteins of survived spermatozoa after cryopreservation are not so different from proteins of native sperm. We can suppose that, the survived spermatozoa after cryopreservation are not undergo to the damaging effect of cryopreservation.

This stay intensified the research cooperation between our Institutes and contributed to the future plans to continue the work started. Our future plans include identification of significant different spots with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry (MS). The practice and knowledge obtained during this Short-Term Scientific Mission is highly important for my PhD as well as for future carrier.

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

Prof. Andrzej Ciereszko (IARFR PAS, Poland) certifies that Yevhen Horokhovatskyi (RIFCH, Czech Republic) has completed a Short-Term Scientific Mission (STSM) awarded by the COST Action FA1205 AQUAGAMETE during the period 01.04.2016 – 30.04.2016.

Prof. Andrzej Ciereszko MSc.
(Host institution)

Yevhen Horokhovatskyi
(STSM applicant)