Final report for Short-Term Scientific Mission (COST Action: FA1205 – Aquagamete)
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Project data

Title: RNA isolation from fish spermatozoa
Beneficiary: Edina Garai, Szent István University - Department of Aquaculture
Host: Ferenc Müller, University of Birmingham - Department of Medical and Molecular Genetics
Period: from 01/02/2016 to 29/04/2016 Place: Birmingham (United Kingdom)

Purpose of project

1. Optimization of zebrafish sperm preparation methods suitable for RNA isolation (trizol, liquid nitrogen etc.).
2. Learning an effective method for RNA purification from small amounts of zebrafish sperm.
3. Learning a suitable method which allows testing the quality of isolated RNA.

Work during the STSM period

1. Collection and preparation methods: We have collected sperm from 16 zebrafish *(Danio rerio)*. First we anesthetized the fishes (with MESAB), after we used a forceps and capillary tube to collect the sperm. We could collect (1-2 µl) of sperm from each individual. Sperm was kept on ice during the preparation method. After collection, the samples were divided depending on the preparation method (1-2 µl sperm with 100 µl trizol, 1-2 µl undiluted sperm from one fish only in liquid nitrogen). All tubes were frozen in liquid nitrogen and kept at –80°C.
2. RNA isolation methods: For comparison, we have tried RNA isolation from different samples (frozen with trizol and undiluted sperm). As a start, 100 µl trizol was added to undiluted samples after thawing. RNA isolation from different samples was performed with homogenization as well as without homogenization. After these, the same RNA isolation method was use in all different samples (general RNA isolation protocol with trizol – http1). As a control 10 hpf embryos were used. Results were added to a database.
3. Testing the quality of the isolated RNA: We have checked the success of different methods on agarose gel and by a nanophotometric analyser, and sometimes by Bioanalyzer. If we have got the suitable quality and quantity of RNA, we have made cDNA with reverse-transcriptase PCR (RT-PCR). Primers were designed on the junction of the exons of chosen genes, on order to avoid wrong transcription in case of DNA contamination. We have chosen genes which are represented only in the skin and blood and one which is related only to the testis.

Results

1. Preparation methods: We could collect little volume of sperm because of the small body size of these fish. The samples have looked clear visually, however the third step (cDNA preparation) have not proved this (see below at step 3). This step need further development.
2. RNA purification method: We could isolate different volumes of RNA from different samples (see Figure 1). Isolations with homogenization were much more effective than isolations without homogenization. This is a very important step in the protocol. We did not find any difference between the efficiency of isolation from sperm frozen with trizol and sperm frozen without any solution.
3. Testing the quality of the isolated RNA: The RT-PCR was successful, the primers have worked really well, however we have got products with all primers (with sperm-specific as well as with skin and blood specific). We have assumed that probably there were some blood and urine contaminations in the sperm.

Future plan

We keep in touch certainly with the host institution. We will try to optimize the collection method from sperm without contaminations.

References

http1: http://tools.invitrogen.com/content/sfs/manuals/trizol_reagent.pdf

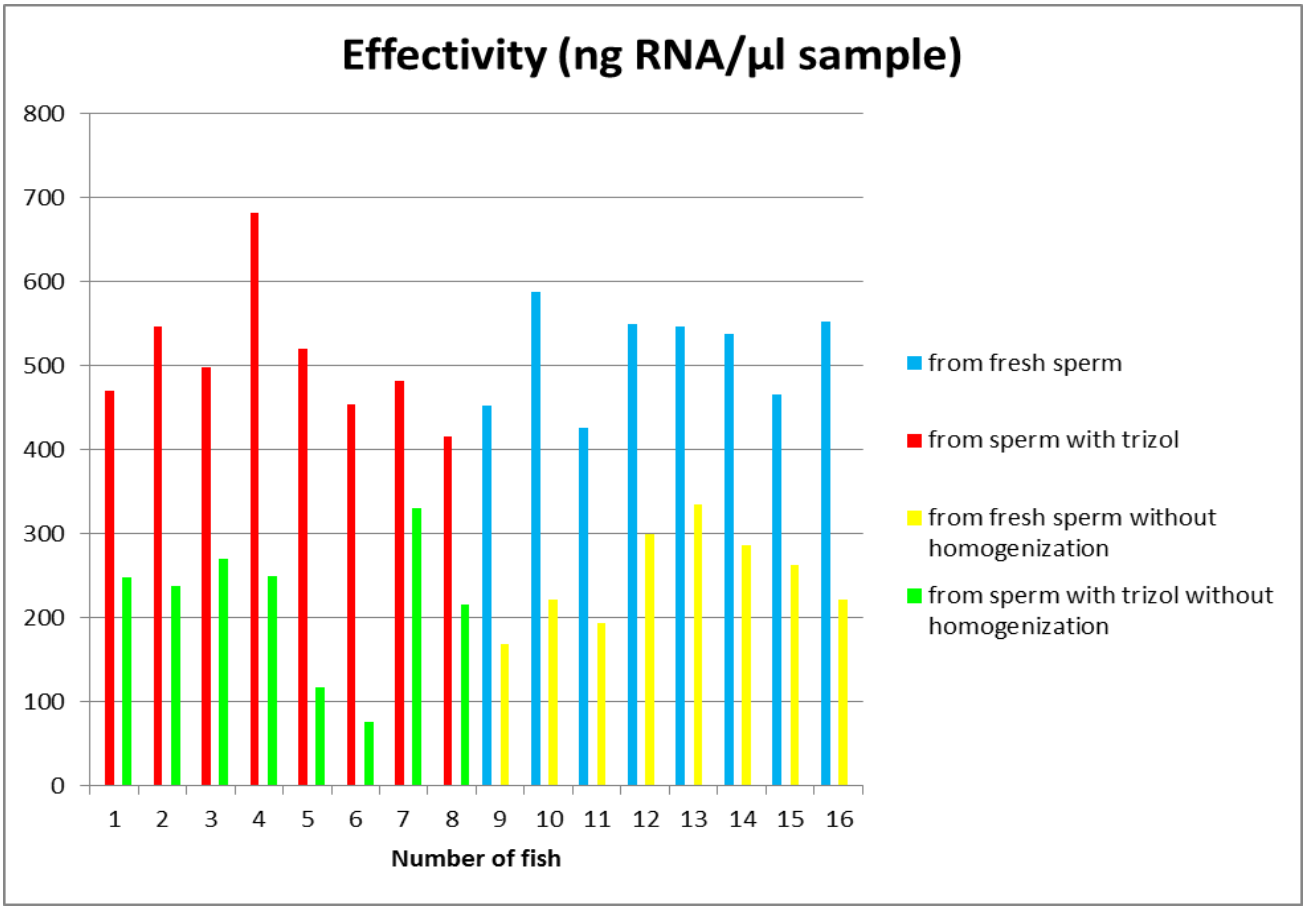

Figure 1.: The effectivity of RNA isolation from zebrafish sperm with different samples and methods

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