STSM Scientific Report

COST Action: FA1205

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Topic: Whole mount *in situ* RNA hybridization (WMISH) using the In Situ Pro VS robot

Aims:

I. To learn the Whole mount *in situ* RNA hybridization (WMISH) technique using the In Situ Pro VS robot in sturgeon embryos and gonads.

II. To learn cloning of candidate cDNA from sturgeon gonads to optimize the protocol of the WMISH technique that needs to be applied on sturgeons' samples.

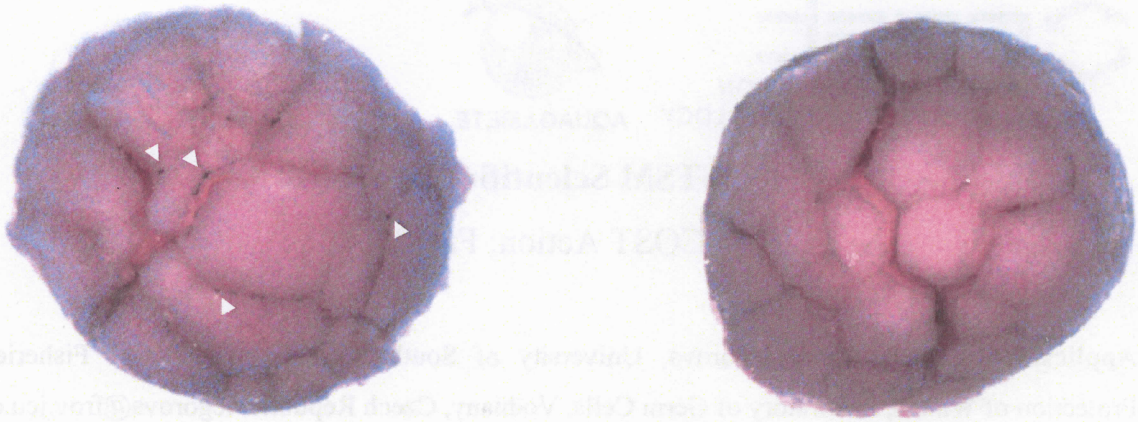
Work carried out:

During my stay in the Testicular Physiology and Puberty laboratory (INRA) under the supervision of Dr. Jean-Jacques Lareyre (Principal Investigator) and Edouard Curran (PhD student) were performed 3 WMISH which were done manually and 1 WMISH using the In Situ Pro VS robot (Intavis) in sturgeon embryos and gonads. *Vasa* and *dnd* sense and antisense riboprobes were tested. Protocol for WMISH was modified and improved. In addition, we disigned primers using partial cDNA sequences (*piwil2* and *translin*) deduced from the transcriptome of sterlet or russian sturgeon's developing gonad. The molecular cloning of the corresponding cDNA was also successfully initiated.

Results

We investigated the expression pattern of *vasa* and *dnd* RNA in sturgeon's *(Acipencer Ruthenus)* embryos from the 1 cell stage to 9 days of development. The signal was obtained from 2 cells stage embryos up to larvae. The most interesting fact was founded that clumps of *vasa*-transcripts were distributed in all parts of embryo (animal pole, vegetal pole, inside of embryos the signal was also obtained).

To compare results between work manually and using In Situ Pro VS robot, we can mention that quality of embryos is much higher because embryos are not exposed to destruction.



A B

Pic 1. Expression pattern of *vasa* transcript in 1K stage in sturgeon's *(Acipenser ruthenus)* embryos (vegetal pole). Note the staining of clumps (white arrows) using the antisense probe (A) but not the sense probe (B).

The cDNA encoding for *piwil2* and *translin* were successfully amplified from gonadal total RNA and the molecular cloning is in progress.

Confirmation by the host institution

Dr. Jean-Jacques Lareyre, Head the Testicular Physiology and Puberty research team at the INRA Institute (Rennes, France) certifies that Viktoriia Iegorova visited his laboratory as a guest researcher from 1st to 30th of October 2015 thanks to a grant awarded by Short Term Scientific Mission, COST Action FA1205.

Rennes, France, 30.10.15

Dr. Jean-Jacques Lareyre
(Host institution)

M.Sc. Viktoriia Iegorova
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