

Title: Further studies on vitrification and cryopreservation of the sperm of European eel (*Anguilla anguilla*): investigation on the effect of different protocols

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Period: 2 weeks (24 November, 2014 – 05 December, 2014), Place: Valencia (Spain)

Work during the STSM period

We have collected sperm from European eel (*Anguilla anguilla*) individuals from three groups of aquarias. The fish were sampled 24 h after the weekly hormonal injection (hCG, 1,5 IU/g fish). After collection we have checked the average cell number and the motility with the CASA system.

Sperm was diluted in different ratios (1:9-1:1) with different cooling mediums (Tanaka plus cryoprotectants). We tested combinations of methanol, ethylene glycol (EG) and propylene glycol (PG) in various concentrations (20-40%). The suspension was supplemented with foetal bovine serum (FBS) as a membrane-protector in the concentration of 10%. For all the methods, the sperm suspension was plunged directly into liquid nitrogen without pre-cooling in its vapour. For the vitrification experiments we used straws (for 250 µl of solution) and cryotops (for 2,5 µl of solution). The thawing took place in 40 °C water bath for 5 s in the case of straws, and directly at the activation media in the case of cryotops.

Results

1. Improving the vitrification methods:

According to the motility, morphological and fluorescent staining studies, the most effective vitrification method was reached with the following parameters:

- dilution ratio 1:1 (50% sperm)
- total cryoprotectant 40% (20% methanol, 20% PG)
- 10% FBS
- cryotop as cooling material.

Future plan

We would like to keep in touch in the future to improve the vitrification and cryopreservation methods of the European eel.

