8th Call for STSM - Scientific Report

COST STSM reference number: ECOST-STSM-FA1205-010616-078352
Period: from 01-06-2016 to 30-06-2016
COST action: FA1205 (Assessing and improving the quality of aquatic animal gametes to enhance aquatic resources. The need to harmonize and standardize evolving methodologies, and improve transfer from academia to industry)
STSM applicant: David G. Valcarce (Molecular Biology Dpt and INDEGSAL; University of León, Spain)
Host: Marta F. Riesco (Aquaculture Research Group *AQUAGROUP*; Centre of Marine Sciences of the University of Algarve, Portugal)
STSM Topic: Optimization of cryopreservation protocols in *Solea senegalensis* sperm

Purpose of the STSM

The aim of this project was to evaluate a cryopreservation protocol in terms of viability in Senegalese sole (*Solea senegalensis)* sperm. Within the diversification of Southern European Aquaculture, Senegalese sole is one of the most promising species. There are some reproductive problems related to males born in captivity (F1 generation), which hinder industrial production. The absence of natural reproduction addresses to the use of artificial fertilization methods, involving gametes collection, sperm classification by motility and cryopreservation for best sperm samples storage.

Work during the STSM period

The first period of the stay was dedicated to learn the handling of Senegalese sole individuals and obtaining sperm from breeders.

For the experimental assay, sperm was collected from wild captured males (n=3) and F1 born in captivity males (n=6). The motility of each sample was analysed and two subgroups were created within F1 samples: a) low motility samples (% of motile cells $\leq$ 30%) and b) high motility samples (% of motile cells $\geq$ 45%). Samples from wild captured animals had good motility scores (over the 60%) and they were used as a control. All the ejaculates were split into two: 1) a fresh aliquot and 2) a cryopreserved aliquot. Cryopreservation-thawing protocol was performed following the guidelines published by Rasines and colleges (Rasines et al., 2013) for *S. maximus*. This species extrapolation was performed due to the absence of a published optimal cryopreservation method for *S. senegalensis* sperm. The viability analysis was performed for both fresh and cryopreserved samples staining with 1.5 µM propidium iodide (PI). Each ejaculate was diluted (1–2 million spermatozoa/mL) and after 10 min in ice and darkness, the stained samples were evaluated by flow cytometry. Forward scatter and side scatter (FSC/SSC) were used to distinguish sperm population from other events. Once the spermatozoa were gated, they were classified in two cell populations: non-viable (PI^+) and viable (PI^-) sperm, negative for IP. A total of 10,000 events will be counted for each sample.

The results showed good viability percentages in fresh samples for the three studied groups. However a statistical significant differences were found in cryopreserved samples in both F1 groups (high and low motility samples) being low motility cryopreserved samples more damaged by the freezing-thawing protocol. The results indicate that different molecular defects, still to study, may affect these samples making them prone to suffer different types of damage resulting in worse motility scores.

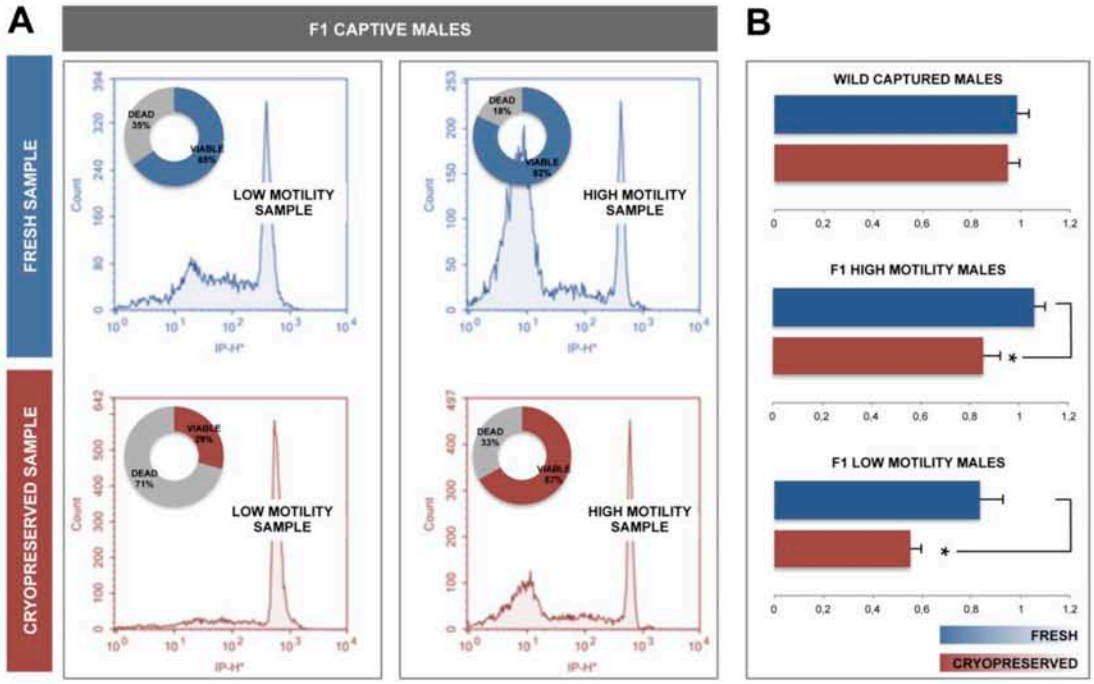

Figure 1. Sperm viability results after flow cytometry analysis. A. Examples of cytograms and percentages of viable (PI^-) *vs* dead (PI^+) spermatozoa from *S. senegalensis* F1 males (low motility and high motility samples) **B.** Viability comparison (normalized %) between fresh and cryopreserved samples in the three studied groups: wild captured males (WT CONTROL), F1 males with high motility sperm (F1 HIGH MOTILITY) and F1 males with low motility sperm (F1 LOW MOTILITY). PI: propidium iodide. Results are shown as mean values ± SD. Asterisks show statistical significant differences ($p<0.05$).

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

Dr. Marta F. Riesco (Post-Doctoral Researcher in the Aquaculture Research Group *AQUAGROUP*; Centre of Marine Sciences of the University of Algarve, Portugal) certifies that David G. Valcarce (PhD student in the Molecular Biology Dpt and INDEGSAL; University of León, Spain) has completed a Short-Term Scientific Mission (STMS) awarded by the COST Action FA1205 AQUAGAMETE during the pediod 01.06.2016-30.06.2016.

Marta F. Riesco, PhD
(Host institution)

David G. Valcarce, MSc
(STSM applicant)