

COST Action FA1205: AQUAGAMETE - 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

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**Dates:** 16.3.2015 – 15.4.2015

**Location:** Centre for Marine Sciences, University of Algarve, Faro, Portugal

**Host:** Dr Elsa Cabrita

### **Purpose of the STSM**

This STSM was carried out at the Centre of Marine Sciences, Faro Portugal under the supervision of Dr Elsa Cabrita, with the main objective of isolation of spermatogonial stem cells (SSCs) by using specific molecular markers and under flow cytometer.

### **Description of the work**

In order to isolate SSCs we first had to digest the testicular tissue. We have tested several different protocols including different enzymes and different incubation times. Digested tissue was afterwards centrifuged and resuspended. Viability of cells was verified with Syber green/propidium iodide (live/dead staining) under a fluorescent microscope as well as with flow cytometer. More than 90% of testicular cells were identified as live cells.

Flow cytometer was used for analyzing single cell suspensions as well as for cell sorting. We were able to identify spermatogonia based on their morphology. More than 95% of these cells were identified as live cells. During these trials I learned basics of using flow cytometry, selecting gates and fluorescence compensation.

In order to isolate SSCs based on their specific markers, we first identified the expression of two markers (GDNF family receptor  $\alpha 1$  and nanos 2) in the testis within cell suspension as well as on histological slides by immunohistochemistry. Cell suspensions were then analyzed under the flow cytometer, gates were set in order to isolate green fluorescing cells, and this was conducted with the cell sorter.

The main objective of this STSM fits into the working group 2 objectives of the Aquagamete action. Future collaboration will be established between Dr Elsa Cabrita and my group. Obtained results are planned to be presented at the 5th International Workshop on the Biology of Fish Gametes and published later in a peer-reviewed journal.

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

Dr Elsa Cabrita (CCMAR\UAlg) certifies that Zoran Marinović (Faculty of Novi Sad, Serbia) has completed a Short-Term Scientific Mission awarded by the COST Action FA1205 in the period 16.3.2015 – 15.4.2015.



Dr Elsa Cabrita  
(CCMAR/UAlg -Host institution)



Zoran Marinović  
(STSM applicant)