

**MACROMOLECULAR CHANGES OF SWORDFISH OOCYTE AT DIFFERENT DEVELOPMENTAL STAGE: NEW INSIGHT FROM FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY (FTIRM)**

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## **Introduction:**

The swordfish is an important commercial species with an extensive seasonal migration and a circumglobal distribution. It is a gonochoristic species, females are multiple spawners with asynchronous ovary. Limited research has been published on the reproductive biology of swordfish from Mediterranean sea. As reported in the literature, five different stages can be observed on the base of morphological features, but macromolecular information about cytoplasmic and ultrastructural changes are still lacking.

To date, Fourier Transform Infrared Microspectroscopy (FTIRM) is a powerful technique to analyze the macromolecular chemistry of cells. Using multivariate procedures, it is possible to generate chemical maps that provide at the same time and on the same sample, unique biochemical and ultrastructural information. Since several reports applied spectroscopic studies to evaluate biochemical changes associated with oocyte growth and maturation, in this study we applied for the first time FTIRM, to achieve specific macromolecular fingerprint of swordfish oocyte at different developmental stages.

## **Methods**

Fishes were fished by Italian fleets operating in central Mediterranean Sea. FTIRM measurements were carried out by using a Bruker VERTEX 70 interferometer coupled with a Hyperion 3000 Vis-IR microscope equipped with a bidimensional Focal Plane Array (FPA) detector. FTIRM measurements were acquired on oocytes at six different developmental stages: primary, alveoli cortical, early vitellogenic, late vitellogenic, mature and atretic oocytes.

## **Results and Discussion:**

The topographical distribution of lipids, proteins, phosphates and carbohydrates within oocytes at different developmental stages was assessed. In particular, previtellogenic oocyte were characterized by a protein-rich cytoplasm, with central oil globules containing short-chain, unsaturated lipids exclusively, and cortical alveoli rich in glycoproteins. During vitellogenic phase, the oocytes showed small yolk granules around the peripheral cytoplasm, composed of glycoproteins, and proteins rich in tyrosine, glutamate and aspartate. By exploiting FTIRM analysis, it was possible to focus on macromolecular changes of vesicles containing vitellogenin when they

blend with lysosomal ones, or when they cross Zona Radiata (ZR) and plasma membrane. FTIRM analysis let also to obtain macromolecular and ultrastructural information about ZR that becomes already evident in previtellogenic stages, increasing in dimension and changing in composition in late vitellogenic and mature oocytes (with the inner layers mainly proteic and the outer richer on glycoproteins). In addition, the central oil globules change in shape and lipid composition (in terms of unsaturation levels and length of chain). Finally, analysing atretic oocytes it was possible to point out macromolecular changes, in terms of secondary structures and composition, occurring to proteins of ZR and cytoplasm and the increase of saturation levels of membrane lipids.

### **Conclusions:**

FTIR analysis let obtain deeper insight into the macromolecular and ultrastructural characterization of swordfish oocytes.

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