

**STSM SCIENTIFIC REPORT**

**REFERENCE NUMBER:** COST-STSM- FA1205-20660

**COST ACTION:** FA1205

**STSM APPLICANT:** Ibarra Zatarain, Zohar. PhD student. Research & Technology, Food & Agriculture (IRTA), Sant Carles de la R pita, Spain.

**STSM TITLE:** Improvement of gametes quality (eggs-sperm) and optimization of the hormone-based treatments on gonadal development in grey mullet (*Mugil cephalus*).

**Objective of the STSM:**

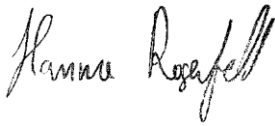
Studying the underlying endocrine status of the reproductive dysfunctions in grey mullet (*Mugil cephalus*) held in captivity in order to optimize hormonal treatments, standardize methods to assess gametes quality and practise ELISAs for measuring hormones in this species.

**Resume of work done and results obtained.**

This internship gave me the opportunity to work with high level specialists in the field of fish reproduction. Thanks to their assistance I was able to: **i)** evaluate *in vivo* the effects of hormonal treatments, **ii)** practise ELISAs to quantify 11-keto-testosterone (11-KT), **iii)** use a computer assisted sperm analysis (CASA) program to analyse sperm quality, and **iv)** analyse genes related to FSH and LH expression by Real-time Polymerase Chain Reaction. To stimulate the onset of gametogenesis mullet breeders were treated with recombinant FSH hormones. Afterwards, fish were injected with LHRH's hormone with addition of metoclopramide to induce the spawning. A total of 27 spawning trials were performed (14 treated fish and 13 control fish). The efficiency of the injections was slightly higher in treated fish than in control fish (42.9% and 38.4%, respectively). The average oocytes diameter, prior and after the hormonal treatments, of FSH treated females ( $571.6 \pm 31 \mu\text{m}$  and  $738 \pm 28 \mu\text{m}$ ) was similar to control females ( $575 \pm 5 \mu\text{m}$  and  $730 \pm 4 \mu\text{m}$ ). FSH treated males showed higher levels of 11-KT in comparison of control males (Figure 1). Moreover, histological analysis proved that testicles of FSH treated males presented an advanced developmental stage than control fish (Figure 2). In accordance to CASA program, it was successfully established for the mullet species (Figure 3A-C). Moreover, it was observed that sperm presented motility after 96 h of being extracted and preserved in the non-activating media (Figure 3D). The results obtained in the present STSM provided a complete description of mullet reproduction and provide new data on the effect of recombinant hormones (FSH) to improve mullet gametogenesis. The obtained result will be practical within the main groups of the AQUAGAMETE Cost Action and may be relevant to aquaculture industry.

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

Dr. Hanna Rosenfeld (Director of National Center of Mariculture and leader of Fish Reproduction Laboratory) certifies that PhD student Zohar Ibarra Zatarain (Research & Technology, Food & Agriculture, Sant Carles de la Rápita, Spain) successfully completed his Short Time Scientific Mission awarded by the Aquagamete COST Action FA1205 between 25<sup>th</sup> of September 2014 to 15<sup>th</sup> of March 2015.



Dr. Hanna Rosenfeld

(Host)



Zohar Ibarra Zatarain

(STSM applicant)

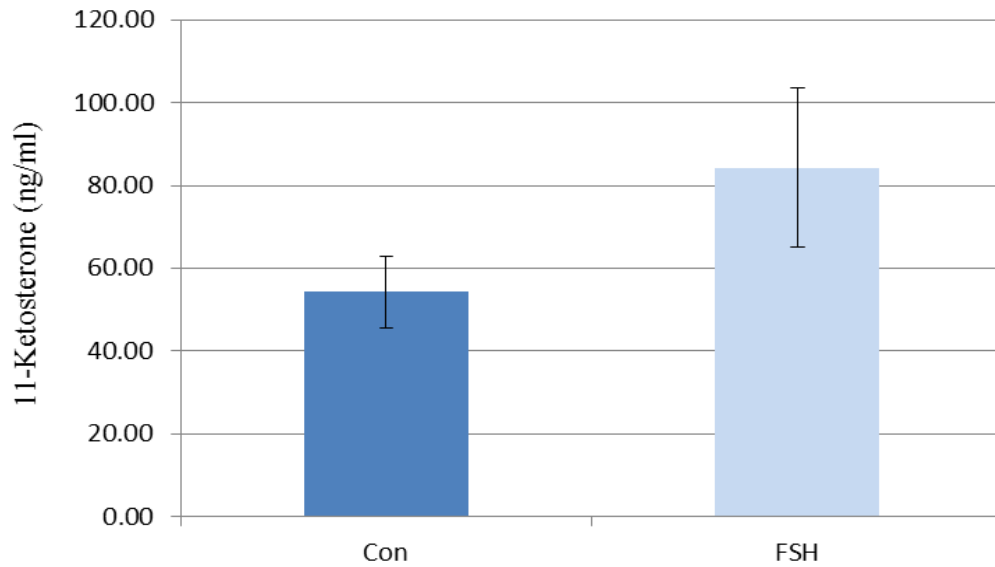


Figure 1. Effects of hormonal treatments on the 11-Ketosterone concentrations in mullet. No significant differences were observed

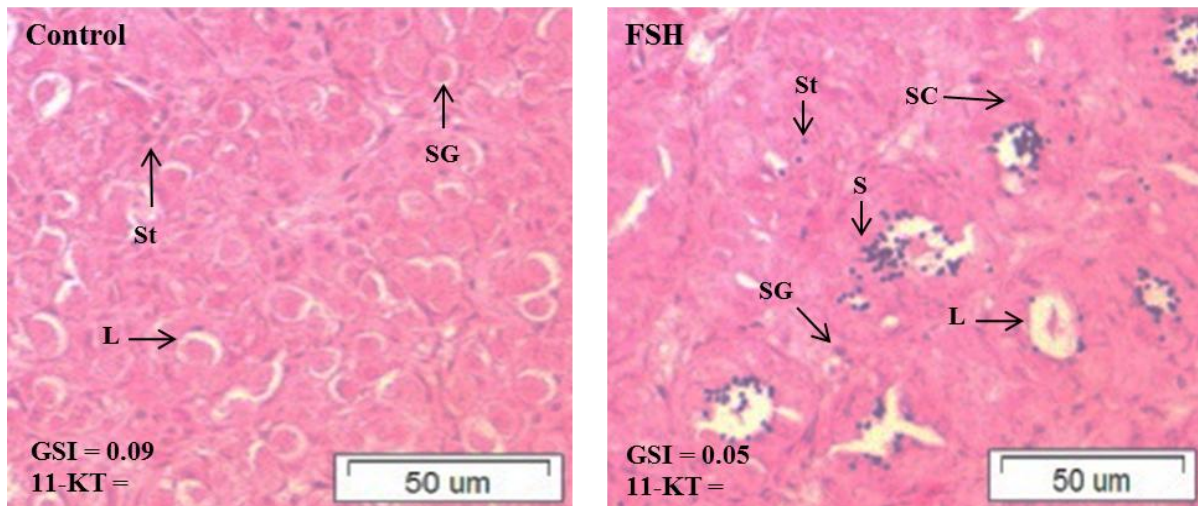


Figure 2. Histological differences between testicular development of control males and those pre-treated with FSH. **St** = Spermatids; **SG**= Spermatogonias; **L**= Lumen; **SC**= Spermatocytes; **S**= Sperm

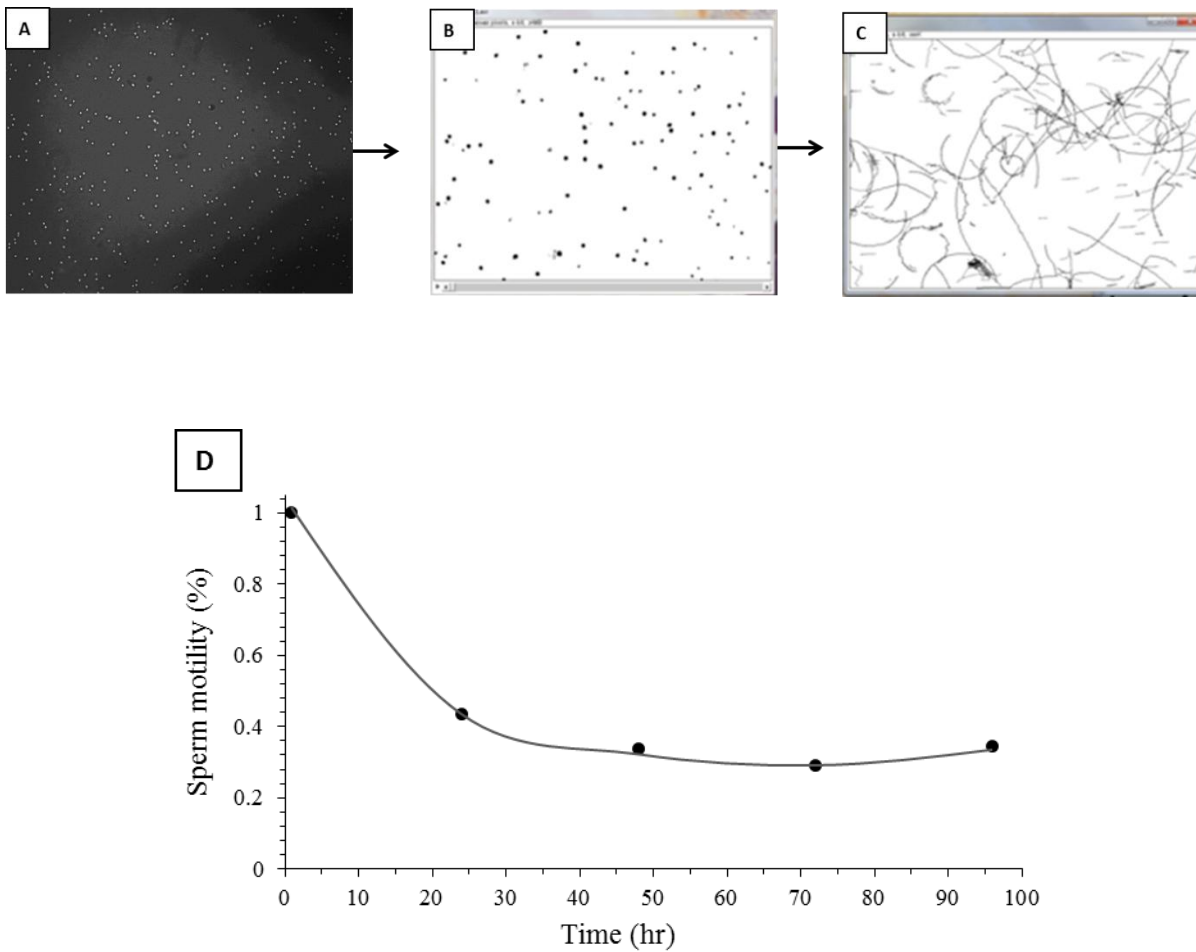


Figure 3. Evaluation of mullet sperm by CASA system. A= extracting background of image; B= sperm cells contrasted in black; C= sperm cell activity and swimming characterization; D= sperm motility (%) along time (0 to 96 hours).