Scientific Report

My STSM was carried out in Prof. Weltzien's laboratory in Oslo. The aim of the mission was the application of Fluorescence *In Situ* Hybridization (FISH) to investigate the localization of the mRNA of kisspeptin receptor (kissR2) and the FSH and LH beta subunits in the pituitary gland of European hake (*M. merluccius*). Besides the FISH, also the immunofluorescence on FSH and LH protein was performed to increase the knowledge about the role of this hormones during reproduction of hake.

The first weeks were used to produce and to test the kissR2 probe both in pituitary gland and in the brain of hake. It was labeled by digoxigenin (DIG) and the antibody activated a Tyramide-TAMRA fluorophore. Together with Dr. Fontaine, some steps of the protocol were adapted for this experimental model and for the receptor investigation, for example using different temperatures of hybridization and different concentration of reagents, but, unfortunately, both negative and positive controls of kissR2 showed the same expression. So, in conclusion, one month was not enough to investigate the localization of kissR2 in the pituitary gland of hake.

At the same time FSHβ and LHβ probes for FISH and LHβ and FSHβ antibodies for immunofluorescence were tested in pituitary. The cloning sequences were amplified and labeled with two different RNA labeling mixes; FSHβ with digoxigenin (DIG), and LHβ with fluorescein isothiocyanate (FITC). The antibodies were anti-DIG and anti-FITC, which activated two different fluorophores, Tyramide-Cy5 and Tyramide-FITC, for FSHβ and LHβ respectively. For the immunofluorescence, the antibodies specific for LH and FSH of other teleosts were used and unfortunately only the LHβ antibody worked. The secondary antibody, that bound the primary antibody, was marked by Alexa Fluor®594.

After FISH and immunofluorescence analysis, the data were analyzed by confocal microscopy. The preliminary results showed that the *in situ* hybridization and immunofluorescence protocol was successfully transferred to hake samples.

I would like to thank the Aquagamete that gave me the possibility to go in another laboratory to increase my scientific knowledge. Furthermore I prepared the ground to continue investigating the localization of mRNA expression of these and other genes that play a role in hake reproduction.

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