Scientific Report

The aim of my project was the application of *in situ* hybridization method to localize the FSH and LH beta subunits in the pituitary gland of European hake (*M. merluccius*). I performed my STSM at the laboratory of Prof. Weltzien at the "Norwegian University of Life Sciences" in Oslo.

The first period was dedicated to cloning and sequencing of the FSH and LH beta subunits with Dr. Nourizadeh-Lillabadi.

The obtained sequences were used for the *in situ* hybridization probe synthesis. The cloning sequences were amplified and labeled with two different RNA labeling mixes; FSH β with digoxigenin (DIG), and LH β with fluorescein isothiocyanate (FITC).

The pituitary glands from two hakes in different stages were divided in several parts and used for probe testing. From the same individuals, a part of the hindbrain was used as a negative control.

Together with Dr. Sandvik, we adapted the *in situ* hybridization protocol to pituitary glands of hake. The used antibodies were anti-DIG and anti-FITC, which activated two different fluorophores, Tyramide-TAMRA and Tyramide-FITC, for FSH β and LH β respectively. After *in situ* hybridization analysis, the data were analyzed with confocal microscopy.

The preliminary results showed that the *in situ* hybridization protocol was successfully transferred to hake samples. Indeed, the presence of both subunits was observed in the pituitary, and no expression was observed in the brain samples. Cells expressing FSH β and LH β did not overlap, confirming previous data from the literature. Since the pituitaries were divided in different parts, we cannot observe the exact localization of mRNA expression. However, ongoing experiments will reveal the expression pattern of these genes in the hake pituitary.

Learning *in situ* hybridization technique, I prepared the ground to continue investigating the location of mRNA expression of these and other genes that play a role in hake reproduction.

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