STSM Report

REFERENCE: Short Term Scientific Mission, Cost Action FA1205

Code: COST-STSM-FA0601-5312

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Period: from 02/03/14 to 29/03/14

1. Purpose of the visit

The aim of the visit was to practise molecular methods and analyse eel oocyte/egg samples obtained from experiments performed as part of a PhD project. The host institution, National Center for Scientific Research (CNRS) unit, "Biology of Aquatic Organisms and Ecosystems" (BOREA) has expertise in application of molecular tools, hormone assays, etc. and extensive knowledge of eel reproductive endocrinology). Thus, the research group could provide the equipment and technology needed as well as the training/supervision necessary to perform the molecular analysis and interpret the results. The main objectives of this Short-Term Scientific Mission (STSM) were:

1. To receive training on molecular methods with main focus on real time PCR (RT-PCR);
2. To quantify expression levels of five estradiol receptors (ERα, ERβ1, ERβ2, GPER1, GPER2), three gonadotropin receptors (FSHR, LHR1 and LHR2) and progesterone receptors (PR) by qPCR on eel oocyte/egg samples;
3. To learn how to interpret and analyze the resulting data;
4. To write the protocol and the results as part of a scientific manuscript.

2. Description of the work carried out

Introduction to qPCR

The first thing that was done was learning the methodology of the qPCR protocol, understanding the concepts and familiarizing with the equipment and software. Together with Dr Anne-Gaelle Lafont, a strategy for the analysis of the oocyte/egg samples was defined, while taking in consideration the limited cDNA available in the samples, the way the samples would be compared and the number of genes to analyze.

Standard curves

During the following days, calculations were made to prepare standard dilutions and test each primer and optimal primer concentration. A serial dilution of the pool of the samples was prepared and used to determine the best dilution to use in analysis of the samples.

The standard curves were prepared based on existing PCR product solutions, doing serial dilutions starting with a known dilution ratio of the stock solution, except for PR, for which the standard curve was serial dilutions of the pool solution. The work with preparation of the standard curves for all ten genes continued until the end of the second week.

Reference genes and sample analysis

During the last two weeks, the samples were analyzed by qPCR for each target and reference gene. Two reference genes were used, Actin and 18S. All target genes (FSHR, LHR1, LHR2, PR, ERα, ERβ2, GPER1, GPER2) were analyzed too.

Data handling

During the last week, at the same time as qPCR analysis continued, the resulting data (CT values) was initiated, i.e. confirming that each measurement had a correct melting curve and that the whole process developed correctly. Then, each standard curve was imported into the corresponding data set and the resulting values (gene relative concentration) were exported to an excel sheet, using the software (LightCycler). In excel, results were organized according to the three sampling moments: samples (ovarian biopsies) taken just before SPE priming injection (SPE), samples taken before DHP injection (DHP) and egg samples taken after ovulation (OV). The concentration of the target genes on each sample was divided by the concentration values of the reference gene on the same sample.

3. Description of the main results obtained

Reference genes

The expression level (concentration) of 18S showed no significant variation among the three sampling moments (SPE, DHP and OV) making it a good candidate for reference gene. On the other hand, the relative concentration of actin decreased significantly in ovulated egg samples (OV) compared to the ovarian biopsies. The concentration of target genes was then divided by the expression values of 18S. The significant decrease of actin after ovulation suggests that the gene is highly expressed in the follicle cells which, normally, are lost during ovulation.

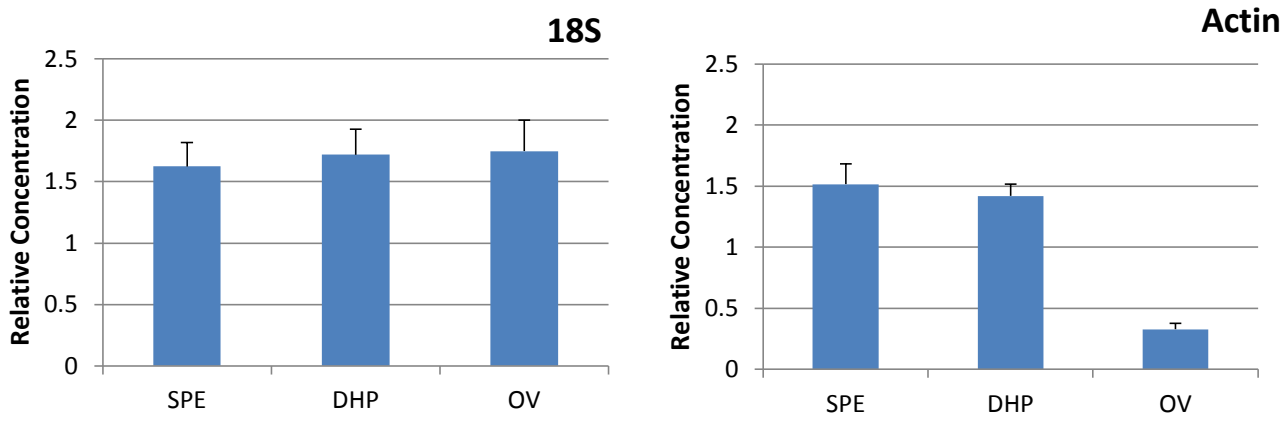

Figure 1. Expression levels obtained for 18S (left) and actin (right).

Target genes

In all estradiol receptors (ERα, ERβ2, GPER1 and GPER2) expression decreases after ovulation, especially GPER2 where the concentration was lower than the limit for detection in most ovulated egg samples. ERβ1 concentration was too low on all samples and thereby impossible to quantify. The concentration level varied little between SPE and DHP, except the expression of GPER2, which seems to increase after the SPE primer injection (Figure 2). Low gene expression of ERα, ERβ2, GPER2 on the ovulated egg samples reveals that these receptors are mainly expressed by the follicular cells that surround the oocyte. In contrast, the moderate decrease in GPER1 expression in ovulated eggs, indicates that this receptor is not only expressed by the follicular cells but also by the oocyte itself (Figure 2).

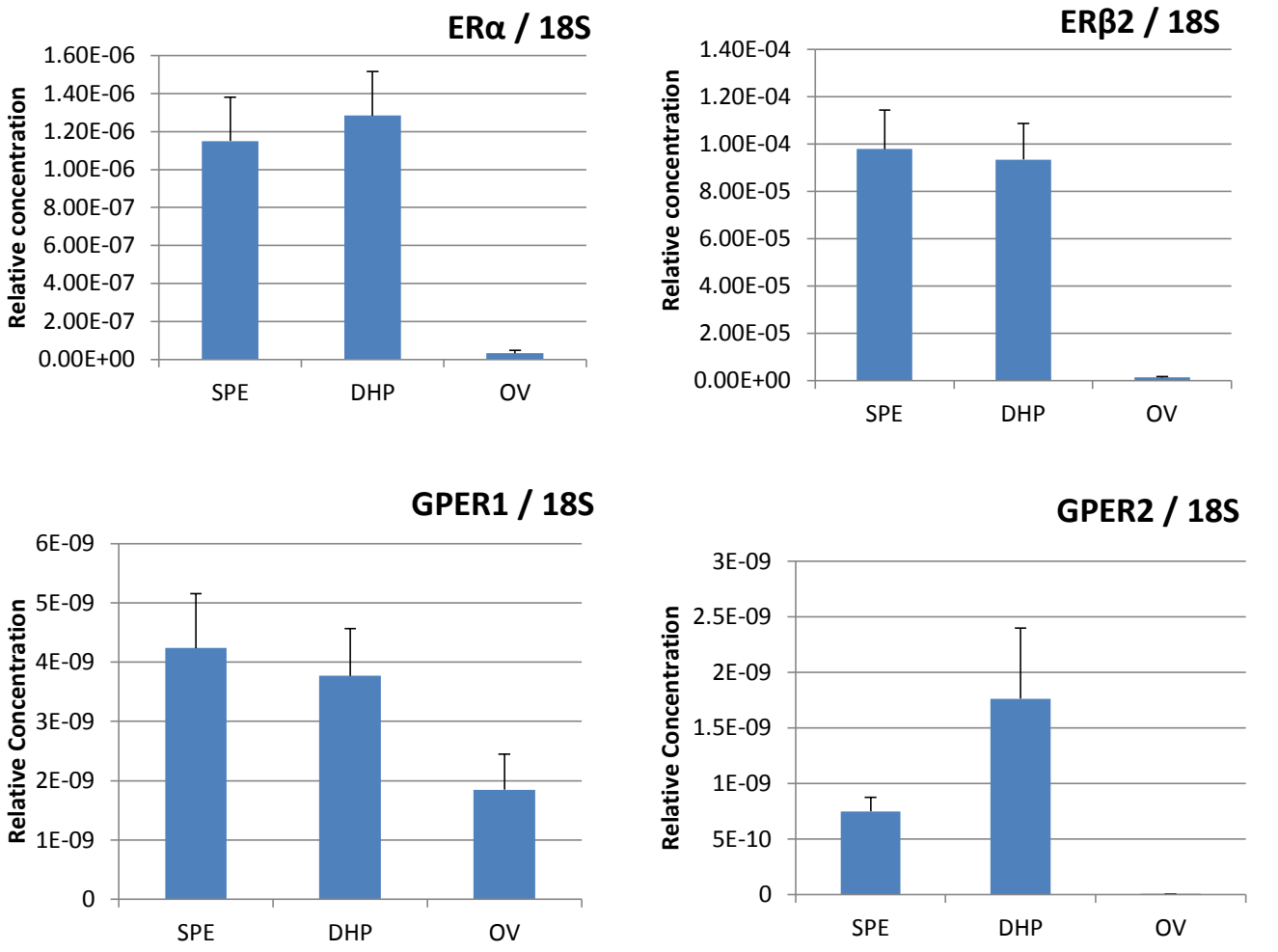

Figure 2. Expression levels of four estradiol receptors present on the ovarian tissue just before SPE priming, DHP injection and after ovulation (OV).

The expression level of the gonadotropin receptors (FSHR, LHR1 and LHR2) seems to decrease with oocyte maturation (from the moment SPE is injected, to just before DHP and close to zero on the ovulated eggs). Low gene expression on the ovulated egg samples confirms that these gonadotropin receptors are mainly expressed on the follicles that surround the oocyte and that are lost during ovulation. However, LHR1 seems somehow to be expressed in ovulated eggs, suggesting that this receptor is not only expressed on the follicles but in the oocyte as well (Figure 3).

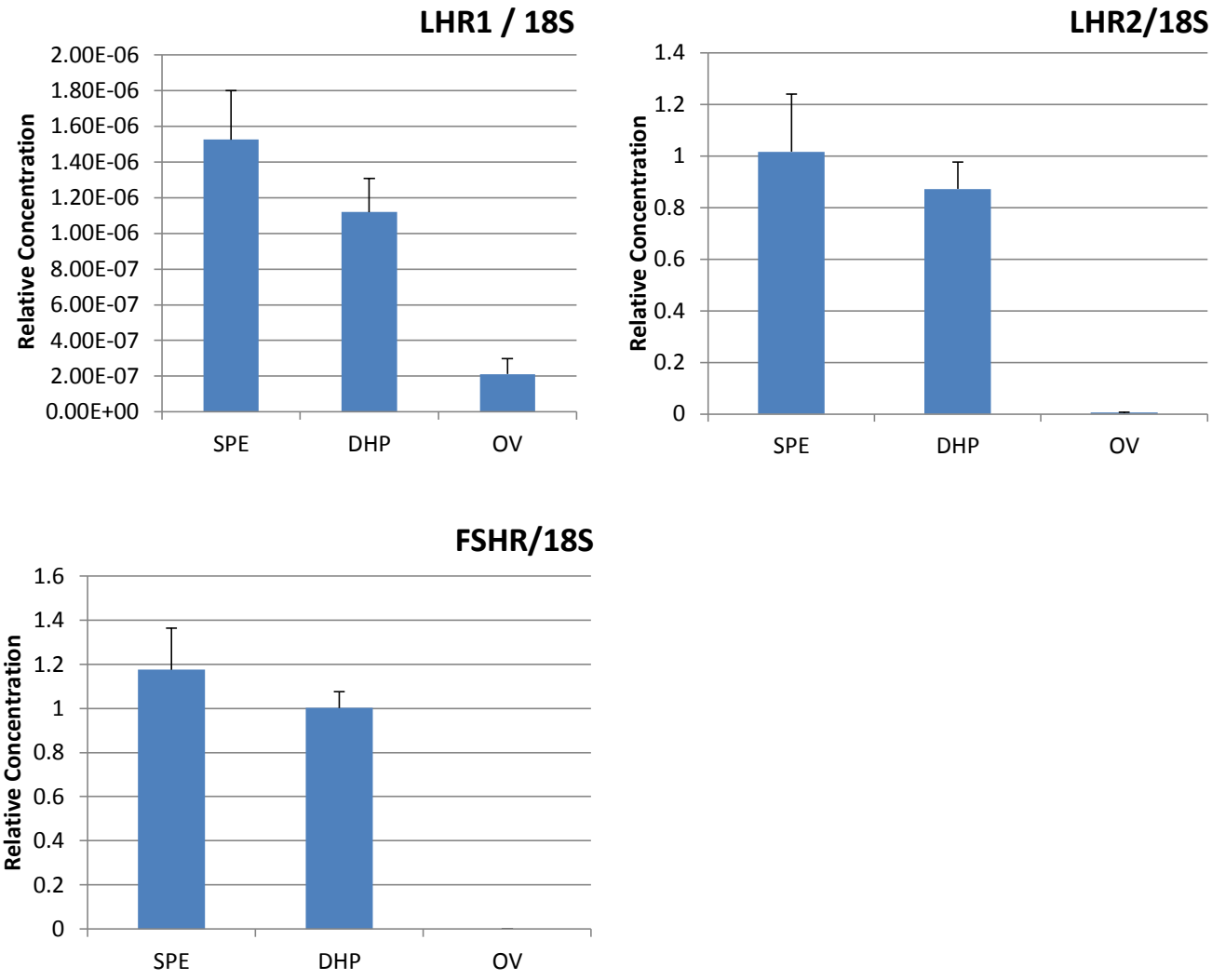

Figure 3. Expression levels of three gonadotropin receptors present in the ovarian tissue just before SPE priming, DHP injection and after ovulation (OV).

The expression level of progesterone receptors (PR) does not seem to change much from with oocyte maturation but there was no gene expression detected in any of the ovulated egg samples (Figure 4).

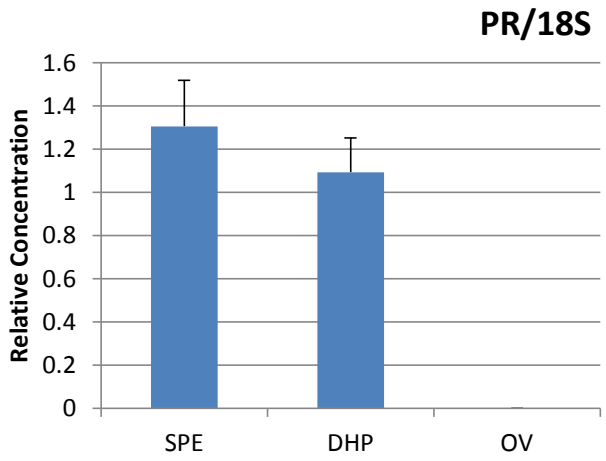

Figure 4. Expression levels of three gonadotropin receptors present on the ovarian tissue just before SPE priming, DHP injection and after ovulation (OV).

4. Future collaboration with host institution

Both parties are interested in continuing the collaboration. There will be a follow-up on the analysis of the results and collaboration will continue during the preparation process of the manuscript for publication.

5. Projected publications/articles resulting or to result from the STSM

The performed analyses and results are part of a manuscript "Follicle maturation of hormonally treated wild European eel" (provisional title) that will be submitted to a scientific journal and it will also be part of the PhD thesis.

6. Confirmation by the host of the successful execution of the mission

Filipa Fernandes's visit to our laboratory has been extremely successful. She has learned qPCR methods and has been able to perform a large number of high quality qPCR assays. Novel data have already been obtained, which will lead to further analyses and continuation of the collaboration between our both laboratories.

7. Other comments

Many thanks go to the COST AQUAGAMETE Action for financing this STSM, allowing me to learn new molecular tools, analyze my project samples and giving me the opportunity to visit the BOREA research unit at the Muséum National d'Histoire Naturelle in Paris. The PhD, experiments and research stay relates to research collaboration between Sylvie Dufour, CNRS BOREA, and Jonna Tomkiewicz, DTU Aqua, in the EU project PRO-EEL – Reproduction of European Eel: Towards a Self-sustained Aquaculture. I am grateful to Sylvie Dufour, Anne-Gaëlle Lafont, Gersende Maugars and Sylvie Baloche for receiving me in their laboratory, teaching and helping me during my stay.

Paris, April 16, 2014

Sylvie DUFOUR, Director BOREA

Filipa SILVA, PhD student STSM beneficiary