**SRF VACATION SCHOLARSHIP REPORT 2018**

**Student’s**

**Name:**

 Niall Slater **Student’s**

**Institution/University:**

Institute of metabolism and Systems Research at the University of Birmingham

**Supervisor’s Department and Institution:**

University of Sheffield

**Degree Title and year of study:**

Biomedical Science BSc 3

Dr Sarah Conner, Dr. Jackson Kirkmann-Brown & Dr. Mick O’Reilly

**Supervisor’s**

**Name:**

**Project Title: Mapping steroid metabolomics within the tertiary human ovarian follicle**

**Briefly outline the background and aims of the project** *(max 200 words)*

 The aims of this project were to delineate the steroid metabolome of the tertiary ovarian follicle, by using RT-qPCR and Liquid chromatography mass spectrometry looking at key enzymes and hormones present in the different cell types within the follicle (the COV434 cell line of granulosa cells, primary cumulus cells and human follicular fluid from patients undergoing fertility treatment.)

We planned to use RT-qPCR, looking for the mRNA expression of the key steroidogenic enzymes CYP17A1, CYP19A1 and HSD3β2 in the granulosa cell line and cumulus cells to look for differences as it had been suggested that these hormones levels may differ between cells (with it been previously shown that there wasn’t CYP17A1 expression within cumulus cells retrieved from patients undergoing ICSI or IVF) within the tertiary follicle but few studies have looked at this in detail.

CYP19A1 (aromatase) is a key enzyme in the process of androgen to oestrogen conversion. CYP17A1 encodes for two different isoforms of the same enzyme,17 α hydroxylase activity, which is responsible for the conversion of pregnenolone to 17 hydroxy- pregnenolone(17OH-preg) and progesterone to 17 hydroxy-progesterone(17OHP), and 17,20 lyase activity, which is responsible for the conversion of 17OH-preg to the androgen precursor DHEA. We planned to look at activities of both isoforms using mass spectrometry to assess the levels of different steroids present.

**Did the project change from that proposed in the application? If so, what changes were made and why?** *(max 100 words)*

No major changes were made to our experiments overall.

**What were the main results/findings of the project?** *(max 300 words)*

Through the RT-qPCR, the levels of mRNA expression were measured for CYP17A1, CYP19A1 and HSD3B2 but most interestingly was the expression of CYP17A1 in the cumulus cells, because this had been shown previously to not be expressed in the cumulus cells.

However, when looking at the enzyme CYP17A1 in mass spectrometry it indicated that the CYP17A1 was non-functioning due to poor conversion of the precursor steroids.

**Figure 1**

Figure 1 shows the mRNA expression from our RT-qPCR showing the genes CYP17A1,CYP19A1 and HSD3B2. The steroidogenesis of granulosa and cumulus cells was compared. This shows there was expression of all three genes in both the COV cell line and the primary cumulus cells.

**Figure 2**

Figure 2 shows the steroid metabolome for follicular fluid, which shows that the follicular fluid contained progesterone (around 14000nmol/L.) and also showed lower levels of 17OHP nd pregnenolone.

**Figure 3**



Figure 3- This is the steroid metabolome of the COV434 cells after exposure to the precursor steroid substrates (200nM of progesterone, pregnenolone and 17OHP separately.) All of these will be converted to different steroids through the enzyme CYP17A1(pregnenolone to 17-OHPreg and then also progesterone to 17-OHP use 17a-hydroxylase enzyme, then the conversion of 17-OHPreg to DHEA requires the 17,20-lyase enzyme.)

 However, the metabolism of pregnenolone and 17OHP was very minimal. And there wasn’t much conversion of the progesterone either, which suggests that the CYP17A1 may be less or non-functional.

**What do you conclude from your findings?** *(max 150 words)*

The results from the RT-qPCR didn’t show the expected result of there being different steroidogenesis in the COV granulosa cells and the cumulus cells, as there was expression of CYP17A1 mRNA in the cumulus cells in our experiment which wasn’t expected. However, the mass spectrometry results suggested that the CYP17A1 gene was non-functioning as there was little to no conversion of steroids in the COV434 cell lines. Therefore, this will need future investigation to determine the difference between these cell types.

This further research would include repeats and an increased sample size as this may be beneficial to ensure reliability in determining differences. Also, this could be done with other follicular cell types such as the theca cells just to see if there were any differences with the steroidogenesis of this.

 **How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice?** *(max 150 words)*

This experience has provided me with a great head start on understanding how a research lab is run, which will be extremely helpful in my lab project for third year as I’ve learnt some key techniques in the lab, but additionally will be of great value through my literature review, and presentation of data, as throughout the placement I was reading different papers around the topic and presenting to peers and staff there.

This placement has also increased my passion for this area of science (fertility and reproduction) that wasn’t taught much at my university, so it’s been a great learning experience.

In respect to my future studies, this has influenced my decision into considering applying for the NHS Scientific Training Programme for 2019 along with other lab based graduate roles.

**Please use the space below to add any other comments/thoughts about the SRF Vacation**

**Scholarship** *(max 100 words)*

***Student:*** The SRF vacation Scholarship was a very enjoyable experience and one which I learnt a great deal from, that I wouldn’t have done through university alone. In particular, seeing and working in a research lab was very beneficial, as university only gives a limited lab experience in comparison.

It will also help greatly with my last year of my degree and I would highly encourage any student thinking about taking part in this scholarship to definitely apply as it would be of huge value to them.

***Supervisor:*** Niall was an enthusiastic and dilligent summer student over the course of his 8 week studentship at the Institute of Metabolism and Systems Research. He developed competence in a number of molecular biological techniques, and approached problems in a rationale and scientific manner. I have no doubt that he would be well-suited to a career in science and academia and would be delighted to work with him in the future.