SRF VACATION SCHOLARSHIP REPORT 2017

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Student's Name:	Daniel Stark	**Student's Institution/University:**	Imperial College London
Degree Title and year of study:	Biochemistry with a Year in Research (BSc 4YFT) Year 3		
Supervisor's Name:	Dr Mark Fenwick	**Supervisor's Department and Institution:**	Department of Oncology & Metabolism Sheffield University
Project Title:	Quantification of c-Jun expression in oocytes provides evidence of spatial regulation of primordial follicle activation in the mouse ovary		

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

Female mammals are born with a limited supply of immature primordial follicles that are capable of developing into fertilisable eggs. Once these primordial follicles are activated to begin developing, the process cannot be reversed; thus it is important that follicle activation is carefully regulated. Various signalling pathways have been linked to maintaining follicles in a quiescent state or regulating activation; however, the source or the nature of the signal is not known. The immediate early gene *c-Jun* is a transcription factor that is activated by a range of extracellular growth factors and cytokines. Cellular expression of *c-Jun* is therefore indicative of a response to external stimuli. Using immunofluorescence, we analysed the expression and localization of c-Jun protein in sections of mouse ovary. Using this information, we aimed to determine the relationship between *c-Jun* expression in oocytes and follicle activation. A corollary aim was to discover whether *c-Jun* expression in primordial oocytes was linked to the number of neighbouring follicles.

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

The initial proposal was to evaluate the expression of three immediate early genes: *c-Jun, c-Myc* and *c-Fos.* After the initial immunohistochemistry experiment, c-Myc and c-Fos expression was limited in small follicles. By comparison, *c-Jun* looked like the most promising candidate for having an effect on primordial follicle maintenance; in particular, we saw substantial variation in oocyte expression in primordial follicles within each ovary. This prompted us to then investigate the potential cause of this variation, by analyzing in detail the association with follicle size and number of granulosa cells, as well as the number of neighbouring follicles.

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

Using immunofluorescence on twelve juvenile (day 4 and 8) mouse ovary sections densely populated with small follicles, we found that c-Jun protein was localised with variable intensity in the oocyte cytoplasm of primordial follicles and with increased intensity in oocytes of early growing follicles. Based on this, we hypothesised that primordial oocytes with higher *c-Jun* expression may be poised to initiate growth. In order to determine the nature and the source of the activation signal, the % positive pixels of c-Jun in 2298 oocytes across all twelve sections was measured and the location (XY coordinates) of each oocyte mapped. c-*Jun* expression in primordial oocytes was positively associated with the number of growing oocytes within a 30µm radius. The proportion of primordial oocytes with strong c-Jun positivity (>50%) increased from 49% where there were no growing neighbours to 81% where there were 3 or more growing neighbours.

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

c-Jun is a transcription factor that controls numerous cell processes such as growth and differentiation, and is itself activated by a wide range of growth factors. We have shown that levels of c-Jun in primordial follicles are higher in those follicles that have growing follicles nearby, suggesting that

growing follicles may secrete a stimulatory signal (such as GDF9), and hence possibly cause those primordials to activate and initiate growth. In order to gain a better understanding of this process, the exact stimulatory signal that is being produced by growing follicles will need to be identified. Furthermore, more information about the genes that c-Jun regulates in the oocyte will be required.

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

Although I was already interested in pursuing a career in research, my experience undertaking a research project in a university laboratory has made me sure that I want to go on to do an MSc and/or PhD. I feel as though I've gained vital experience, not just in terms of learning experimental techniques, but in what it involves to perform a research project and how to deal with things when they don't go to plan. I am delighted to have the opportunity to present my own data at Fertility 2018. I am also hoping to be able to publish my results, which will be an excellent stand-out point on my CV and will enable me to take the next step in my career.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*

Student: *I have found the whole experience of this research project massively enjoyable and rewarding, and would like to thank SRF again for offering me this scholarship. The only issue I had was with the payment of the stipend. As I was paid through Sheffield University as a casual worker, I was taxed on my stipend, and it is proving to be a very long process to get the tax refunded.*

Supervisor: *This summer scholarship scheme has been an excellent opportunity for both Daniel and myself. Although Daniel had some time in my lab as a placement student beforehand, the summer studentship allowed him to focus on working to achieve the aims of a project within a relatively short time frame - and by reporting to the SRF in some ways adds a level of accountability and ownership of his project. Although other summer studentship schemes are available, the SRF scheme is more appealing as it offers 8 weeks, with a little more money and the incentive to engage with the society by presenting at Fertility is a huge benefit. Having said that, I would also urge SRF to consider paying the stipend directly to the student to avoid problems where the student is not registered with the institution. As a consequence of Daniel being taxed from his stipend, the University of Sheffield payed the employer contribution from the £750 lab consumable budget, so I was left with around £300 to support his project.*