



SRF VACATION SCHOLARSHIP REPORT 2018

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Degree Title and year of study:	BSc Biomedical Science Year 3		
Supervisor's Name:	Dr Suman Rice	Supervisor's Department and Institution:	Cell Biology & Genetics Research Centre, St George's University of London
Project Title:	Regulation of anti-Müllerian hormone (AMH) expression: correlation with vitamin D and oestrogen receptor		

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

Vitamin D has a fundamental role in reproduction and its actions are mediated exclusively by its receptor (VDR). Anti-Müllerian hormone (AMH) also has an important function in normal and disordered ovarian folliculogenesis associated with polycystic ovary syndrome (PCOS). The discovery of a VD response element on the AMH promoter indicates that VD regulates AMH expression and there is growing evidence to support the down-regulation of AMH expression by oestradiol (E2), primarily via alteration of the ratio of oestrogen receptors (ER) α and β .

Aims: To investigate the relationship between the expression of AMH, VDR and ERs in mouse ovarian follicles and using the human KGN granulosa cell-line.

Methods: KGN cells were treated with testosterone (500nM) \pm forskolin (25 μ M) \pm PHTPP -- a selective ER β antagonist (at 10⁻⁶, 10⁻⁷ & 10⁻⁸M) for 48h. The mRNA expression of ER α , ER β and AMH was measured using qPCR with RPL7 as the normalizer (n=4-5). Using immunohistochemistry, the expression of AMH, VDR and ER β in mouse ovaries (n=3) were analysed.

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

No human corpus luteum samples were investigated as stated in the research proposal, other than this there were no project changes.

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

The results of the qPCR experiments were as follows, forskolin was found to increase ER α :ER β expression 150-fold in the human KGN granulosa cell-line although no increase in AMH mRNA expression was seen.

PHTPP (a selective ER β antagonist) increased ER α :ER β expression (40-fold at 10⁻⁶M) in a dose dependent manner, with a corresponding (7-fold) increase in AMH mRNA expression.

The immunohistochemistry experiments found that AMH protein levels were highest during the late pre-antral and early antral phases of folliculogenesis; matching conclusions made by previous studies.

VDR expression was found to be strongest in pre-ovulatory follicles however no clear relationship between VDR and AMH expression was found.

Some evidence showed that there was an inverse relationship between AMH and ER β expression, with later stage follicles expressing less AMH but more ER β as seen in fig. 1-3 below.

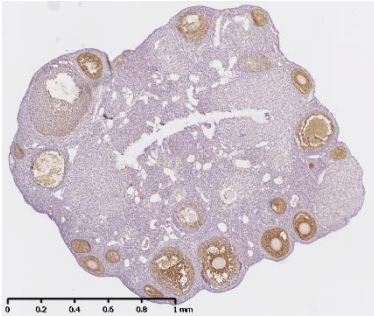


Fig.1
AMH expression in mouse ovary 2B
1° 1:50 2° 1:500
Intense staining seen in late pre-antral and early antral follicles.
Weaker staining seen in pre-ovulatory follicle indicated by full arrow.

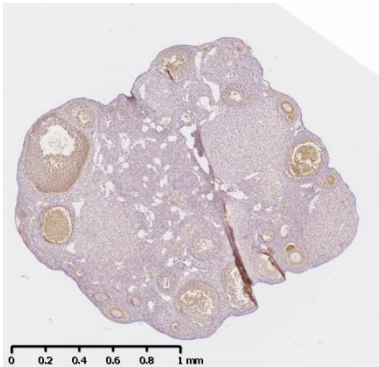


Fig.2
Er β expression in mouse ovary 2B
1° 1:50 2° 1:1000
Non-specific staining seen in antrum of antral follicles.
Strong staining seen in pre-ovulatory follicle indicated by empty arrow.

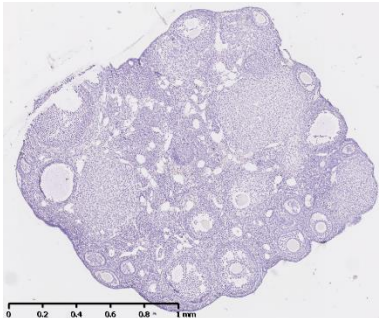


Fig.3
Negative control in mouse ovary 2B

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

In conclusion, forskolin had no effect upon the expression of AMH mRNA via increase of the ER α :ER β expression whereas treatment with the selective Er β antagonist, PHTPP did cause an increase in AMH mRNA expression.

One possible explanation for these results could be cross-talk from other pathways that are activated by a forskolin-induced increase in cAMP, which could inhibit AMH expression.

Although more research would be required to confirm this theory, there appears to be an inverse relationship between ER β expression and AMH expression within follicles of the ovary. This could mean that ER β , and ultimately the hormone oestrogen, has an effect upon the expression of AMH within follicles. Contextually, AMH is found to be elevated in women who suffer with PCOS, the underlying physiology of which is still to be determined.

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

The Summer Vacation Scholarship was an invaluable experience that enabled me to practice laboratory techniques and fine tune transferable skills such as organisation and time-management. It was an incredibly interesting project for me as I was able to research a topic that is particularly close to my heart as well as learn more about the inner workings of a lab and build my confidence. I especially enjoyed learning new techniques such as qPCR and cell culture and hope to use what I have learnt this summer throughout my future career. In regard to future studies, the scholarship has lead me to consider a masters project and has spurred me to look for work within a laboratory setting.

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

I would recommend the scholarship to anyone who is eager to learn about research and practice skills in organising and analysing experiments. The generous stipend allows for total focus upon the project and I would like to personally thank the Society for Reproduction and Fertility for the experience and opportunities that the scholarship has given me.