SRF VACATION SCHOLARSHIP REPORT 2016

Student's Name:	Leow Hui Wei	**Student's Institution/University:**	University of Edinburgh
Degree Title and year of study:	Bachelor of Medicine, Bachelor of Surgery (MBChB) Year 2		
Supervisor's Name:	Professor Hilary OD Critchley	**Supervisor's Department and Institution:**	MRC Centre of Reproductive Health, Queen's Medical Institute of Research, University of Edinburgh
Project Title:	A study to examine the effect of Selective Progesterone Receptor Modulators (SPRMs) on the endometrial fibrinolytic system in women with heavy menstrual bleeding (HMB).		

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

Heavy menstrual bleeding (HMB) is defined as excessive menstrual blood loss which interferes with the woman's physical, emotional, social and material quality of life [1]. It can occur alone or with other symptoms [1]. Women with fibroids have HMB [2]. Current medical treatments are often ineffective. There is an unmet need for alternative treatments [3]. Haemostasis, vasoconstriction and epithelial repair are required to reduce menstrual blood loss [4].

Progesterone regulates uterine function. Progesterone receptors (PR) are an attractive therapeutic target. Selective Progesterone Receptor Modulators (SPRMs) are ligands for PR. SPRM, ulipristal acetate rapidly controls HMB; it's mechanism of action upon endometrial bleeding is unknown [5]. Fibrinolysis induces the degradation of fibrin clot. It is enhanced by urokinase plasminogen activator (u-PA) and tissue plasminogen activator (t-PA), but inhibited by plasminogen activator inhibitor 1 (PAI-1) [6]

An overactive fibrinolytic system interferes with haemostasis and contributes to HMB. Women with HMB have raised t-PA activity on day two of menstruation [7]. The levonorgestrel-releasing intrauterine system (LNG-IUS), a PR ligand, reduces HMB by inhibiting t-PA secretion and promoting PAI-1 [8, 9]. This project investigates whether SPRM (ulipristal acetate) affects haemostasis by modulating components of the endometrial fibrinolytic pathway, (u-PA, t-PA, PAI-1).

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

No changes from original application.

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

mRNA quantification using real time quantitative polymerase chain reaction (RT-qPCR)

Results from RT-qPCR quantification of u-PA revealed that the relative expression of u-PA mRNA was significantly greater in proliferative phase compared to secretory phase endometrium and endometrium post SPRM, UPA, administration. Progesterone significantly increases u-PA release in endometrial cells [10]. UPA may act as a powerful progesterone antagonist [5]. This may result in lower u-PA mRNA levels post SPRM, UPA, administration due to decreased progesterone action.

RT-qPCR quantification of t-PA and PAI-1 revealed no significant differences between the study groups. Endometrial tissue extracted was a mixture of epithelial, stromal, immune and endothelial/ vascular cells. It is possible, as in other reports on location: mRNA for t-PA was located in blood vessel walls [11].

Plasmin activates TGFB1 and TGFB3 from their latent forms, and alters progesterone receptors in human endometrial stromal cells [12, 13]. The expression of TGFB1R mRNA was significantly greater in proliferative compared to secretory phase endometrium, and expression of TGFB2R mRNA was significantly greater in secretory phase endometrium compared to endometrium post SPRM, UPA, administration. These changes may

be due to mechanisms in endometrium to regulate PR expression [14]. The reason for low mRNA expression of TGFB1 and TGFB3 following SPRM, UPA, administration requires further study.

Protein localisation using immunohistochemistry (IHC)
CD-41 is a platelet cell-surface marker. Platelets play an important role in primary haemostasis. There was no detectable immunostaining in the negative control and the positive control gave expected immunostaining pattern. Preliminary data indicated weak staining of stromal and glandular epithelial cells in proliferative phase endometrium. Staining was evident in stromal cells, glandular cells and blood vessels and more pronounced in secretory phase endometrium. Post-UPA administration endometrium revealed weak staining of stromal cell and glandular epithelial cells. Platelets may be implicated in haemostasis post UPA administration. More extensive studies are required to validate these results.

A preliminary examination of immunohistochemical localization of t-PA indicated that this protein was absent in proliferative phase endometrium. Secretory phase endometrium showed weak staining of stromal cells and glandular epithelial cells. Similar staining patterns were observed in endometrium post-UPA administration. Further studies are required to establish the effect of UPA administration on t-PA localization.

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

SPRM (UPA) administration appears to impact upon the endometrial fibrinolytic pathway and may modify menstrual bleeding. UPA may also act on inflammatory pathways or on the vasculature of the endometrium to reduce heavy menstrual bleeding. UPA may act via Hypothalamic-Pituitary-Ovarian (HPO) to control Luteinizing Hormone and prevent ovulation. Many women administered UPA report amenorrhoea. SPRM administration may reduce HMB via anovulation. The mode of action of SPRM on human endometrium and how MBL is reduced requires further study. Immunohistochemical analysis of all validated genes would be desirable in order to elucidate their localization within the endometrium during proliferative phase, secretory phase and post UPA administration. Western blotting would allow the identification and quantification of protein present within endometrial cells, and further complement the data derived from RT-qPCR.

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

I found the experience of working in a clinical research laboratory invaluable. I enjoyed working in a team that practices translational medicine. I witnessed the interface of a research project from the bedside to the bench in the field of women's health. I acquired an insight into life as an academic clinician. It provided me with scientific training and enhanced my oral and written scientific communication. I obtained general bench-side skills such as pipetting accurately and use of basic laboratory equipment. In the operating theatre, I observed the culture of teamwork within medicine - everyone's input was respected and everyone taught each other at different levels. This project enabled me to reaffirm my ambition to become an obstetrician and gynaecologist and deepened my passion for women's health. I am motivated to become an academic clinician so that I may contribute to future research in the field of reproductive biology/ medicine.

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

Student: *Working in a research environment enabled me to acquire many transferable skills such as time-management, presentation and record keeping. I gained an appreciation of the demands of research and time-frames involved in producing a publication. All this has provided me with a firm foundation to build upon during my forthcoming intercalated Honours year (University of Edinburgh, BSc in Reproductive Biology).*

Supervisor: *Amanda worked diligently during her short vacation scholarship. She acquired several laboratory techniques: commendable given her lack of previous "bench" experience. Amanda read beyond the project and availed herself of additional opportunities. She proved herself an excellent team member.*

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