SRF VACATION SCHOLARSHIP REPORT 2019

The form below should be completed by the student, then forwarded to the supervisor for approval and submission to srf@conferencecollective.co.uk within 8 weeks of completing the project. Please submit the form as a Word document.

A maximum of one figure (with legend of less than 100 words) may be appended if required.

Please note: excerpts from this form may be published on the SRF website, so please ensure content is suitable for website publication, and does not compromise future dissemination of data in peer-reviewed papers etc. The SRF reserves the right to edit responses to ensure suitability for publication on the website, newsletter or in promotional material.

Student's Name:	Eva Shelmerdine	**Student's Institution/University:**	University of Manchester
Degree Title and year of study:	MSci Developmental Biology (3rd year)		
Supervisor's Name:	John Aplin	**Supervisor's Department and Institution:**	Maternal and Fetal Health University of Manchester
Project Title:	Tight junction decoupling from actomyosin in receptive endometrial epithelium is regulated by protein O-GlcNAcylation		

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

Understanding the mechanisms behind altered cell function that lead to endometrial receptivity in the uterus during the receptive phase of the menstrual cycle is vital for improving the outcomes of assisted reproductive technology (ART). One proposed mechanism through which the uterine endometrium becomes receptive is a loss of apical-basal polarity due to a reorganization of the tight junctions. Epithelial tight junction organization in primary endometrial organoids was examined in response to hormone-induced receptivity. Using an endometrial cell line and primary epithelial organoids, junctional organization in response to different glucose concentrations and subsequently different downstream protein O-GlcNAcylation was investigated.

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

My proposed project was to address how altering the nutrient environment affects GlcNAcylation of histone deacetylases (HDACs) in endometrial cells.

Responding to recent developments in the lab, I focused instead on how altering the nutrient environment affects localisation of ser19-phosphorylated myosin light chain (pMLC) to tight junctions.

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

In endometrial organoids, active ser19-phosphorylated myosin light chain (pMLC) was found to co-localise with zona occuldins (ZO-1) at apicolateral tight junctions. Treatment of organoids with hormones to mimic the receptive phase abolished pMLC junctional localisation. In Ishikawa cells grown to confluence in 5mM glucose, pMLC also localised to tight junctions, however in 17mM glucose this localisation was lost. pMLC localisation was not glucose-responsive in organoids. In Ishikawa cells, treatment with the O-linked GlcNac transferase inhibitor OSMI-1 rescued pMLC co-

localisation with ZO-1 in 17mM glucose, while treatment with the ROCK inhibitor Y27632 led to loss of pMLC and ZO-1 from junctions.

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

Endometrial epithelial tight junction coupling to contractile actomyosin through MLC may be crucial for receptivity, and diabetes could affect receptivity through protein O-GlcNAcylation downstream of dysregulated glucose levels. Decreased pMLC likely loosens tight junctions, allowing apical localisation of cell-cell adhesion proteins required for embryo attachment, while also permitting epithelial breaching by invasive trophoblast during implantation. MYPT1 is a highly O-GlcNAcylated protein that is regulated by ROCK and targets MLC for dephosphorylation, and thus may serve as a glucose-sensitive molecular switch for endometrial epithelial receptivity.

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

This studentship has proven to be invaluable to me as a student. It has given me the opportunity to develop my skills and confidence in the lab (something I am certain will be useful in my future studies and career) and also given me a chance to learn what life as an academic researcher is truly like. This experience will certainly help inform the decisions I must make regarding my next steps following the completion of my degree.

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

Student: The SRF summer Vacation Scholarship has given me an opportunity to really experience what life is like as an academic researcher – something I do not truly get an experience of in my normal studies. Without this scholarship I would not have had this opportunity so I would like to thank the SRF and the Maternal and Fetal Research Centre for facilitating this scholarship!

Supervisor: Eva made the best of her studentship, and we are grateful for the flexibility of the SRF in agreeing to provide very useful lab consumables, with the Biochemical Society supplying living expense support. With advice and lab supervision from Dr Peter Ruane she changed direction from the original proposal, picking up on a pathway that had just emerged from a differential proteomics screen. She generated some nice data and is scheduled to present a poster at the Fertility 2020 meeting in Edinburgh in January.