SRF VACATION SCHOLARSHIP REPORT 2022

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 PDF document and save it as: First name, Last name and 'VS.'

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:	Nathan John Parks	**Student's Institution/University:**	University of Central Lancashire (UCLan)
Degree Title and year of study:	BSc (HONS) Biomedical Sciences (Year 3)		
Supervisor's Name:	Dr Stéphane Berneau	**Supervisor's Department and Institution:**	School of Pharmacy and Biomedical Sciences, University of Central Lancashire (UCLan)
Project Title:	Investigating the role of DPP4 adhesive and enzymatic functions at implantation using an in-vitro model.		

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

In the UK, 1 out of 6 couples are infertile and 10% of them suffer from recurrent implantation failures. The clinical success rate has been stagnant at 25-30% per cycle. The knowledge of molecular events at the embryo implantation remains limited, hindering further increase in clinical pregnancy success rates. Identifying epithelial endometrial candidates from published differentially expressed genomics datasets of human endometrial biopsies sets priorities for in vitro assays using a model of embryo implantation. A transmembrane receptor, Dipeptidyl peptidase-IV (DPPIV), was one of the identified endometrial targets involved in cell-cell adhesion. Complementing the interesting function complexity, DPPIV contains an enzymatic domain which we identified to be active, in vitro, in Ishikawa cells lysates by a series of DPPIV inhibitor concentrations and aim to further investigate.

The project is designed to identify new insight into the adhesive pathway at Implantation at the embryo-maternal interface by investigating the role of DPPIV enzymatic function in relation to fibronectin using a range of inhibitors, gliptins.

Project aims:

1. Localise DPPIV in endometrial Ishikawa cells
2. Determine the impact of the inhibition of DPPIV enzymatic function on endometrial cell adhesion
3. Identify the role of DPPIV enzymatic function in an in-vitro model of implantation

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

No. The function-blocking antibody suggested in the proposal was tested prior to the project and data were elusive, therefore, this 8-week project focused on the inhibitory function of Ile-Pro-Ile, a potent DPPIV inhibitor.

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

1. DPPIV was identified in Ishikawa cells and at BeWo spheroid attachment site

Immunocytochemistry (ICC) using an anti-DPPIV antibody of Ishikawa cells showed DPPIV to be detected at the lateral and apical cell membrane (Fig. 1A). In confluent monolayer of endometrial cells, positive cells were more present but still heterogenous (not shown). IgG from mouse serum and secondary antibody alone were used as controls for ICC.

From preliminary observations, highly DPPIV-positive region displays in the endometrial cell membrane where spheroids attachment took place (Fig. 1B).

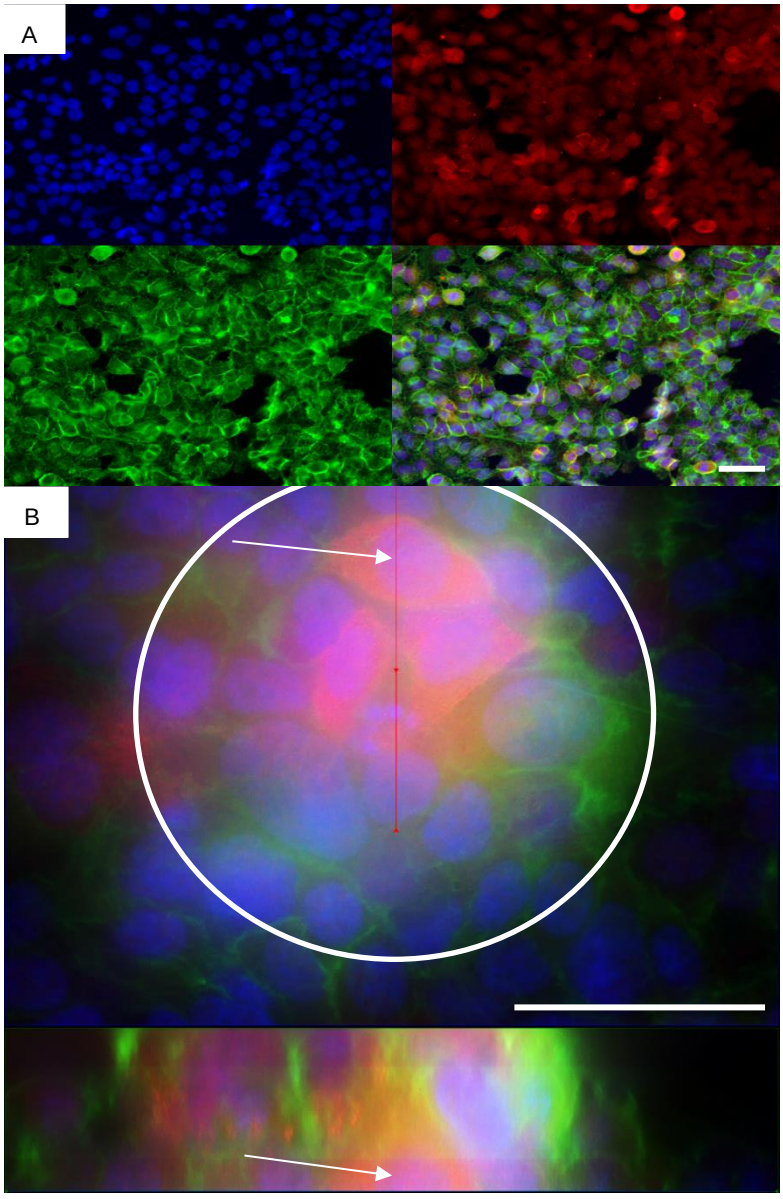

Figure 1: Representative fluorescence images of DPPIV in the endometrial cells and at trophoblast spheroid attachment site

A= Immunocytochemistry of Ishikawa monolayer using DAPI (nucleus, blue), an anti-DPPIV antibody (red) and phalloidin (actin filaments, green).

B= Z-stack image of Ishikawa monolayer and spheroid co-culture using an anti-DPPIV antibody (red), DAPI (blue) and phalloidin (green). Circled area of z-stack shows the site of attachment between spheroid and endometrial cell monolayer. Arrow show highly DPPIV-positive endometrial cells

2. **Inhibition of DPPIV using Ile-Pro-Ile significantly decreased endometrial cell attachment**

A potent DPPIV inhibitor, Ile-Pro-Ile, was used to test the link between the adhesive and enzymatic role of DPPIV in Ishikawa cells. Along with different wells coatings using fibronectin (DPPIV ligand), poly-L-lysine and BSA (negative control), prior to the attachment assay, cells were divided into three conditions: control (untreated cells), cells treated with 25ug/ml Ile-Pro-Ile for 10 minutes or cell treated with 50ug/ml Ile-Pro-Ile for 10 minutes. The presence of high concentration of Ile-Pro-Ile led to a significant decrease (approx. 48%) in the attachment of the endometrial cells equalling the attachment observed using a negative control of BSA (basal attachment).

3. **Attachment trophoblast spheroids was impaired by the inhibition of endometrial** DPPIV

Using a co-culture model of trophoblast spheroids into an endometrial monolayer, we identify that almost all BeWo spheroids attached within 1 hour. From preliminary results, the spheroid attachment was reduced by at least third onto monolayers pre-treated with Ile-Pro-Ile (DPPIV inhibitor).

Vehicle controls for each condition determined that in each concentration had a non-significant role in affecting the rate of attachment.

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

In this study, combined with its subcellular localisation at spheroids attachment sites, we demonstrated that the inhibition of enzymatic activity of DPPIV had a negative impact on the DPPIV-specific binding in Ishikawa cells, leading to the impairment of the trophoblast/endometrial attachment in-vitro.

From the preliminary data, the inhibition of DPPIV in an in-vitro co-culture model using endometrial monolayer and trophoblast spheroids triggered a decrease in spheroid attachment. DPPIV seems to play a key role in spheroid adhesion and potentially invasion in an in-vitro environment. The enzymatic function of DPPIV at the time of implantation may be crucial to embryo adhesion/ invasion in-vivo and can be further investigated to understand what effect it may have in natural/IVF cycles and for women suffering from recurrent implantation failure women.

These data set the scene for the investigation of DPPIV enzymatic activity and its prescribed inhibitors at implantation.

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

This project has provided me with greater insight into the world of research in academia. It has been incredibly valuable in informing me about conducting research / pursuing a career in research in the future. The specific techniques being used in this experience have massively increased my lab ability and confidence in the lab for future projects in my degree.

My overall experience in this lab project has encouraged me to pursue a PhD in Reproductive Sciences after my bachelor's degree in which I will be able to apply my newly gained skills and resilience in the lab as well as plead to a positive career in research.

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

Student: I would like to thank everyone involved in the funding of this project and I am immensely grateful to the SRF society for allowing me to take part in this brilliant opportunity. I hope more undergrad students like myself will get the chance to take part in an SRF VS and the benefits it provides to technical ability and career perspectives.

Supervisor: The SRF Vacation Scholarship is a great opportunity for BSc students to discover the research in academia from principal investigators who embrace sharing their skills and enthusiasm toward research. Thanks for selecting our team for the student scholarship (including lab consumables support).