SRF VACATION SCHOLARSHIP REPORT 2016

Student's Name:	Haya Khan	**Student's Institution/University:**	University of the West of Scotland
Degree Title and year of study:	3rd Year BSc Hons Biomedical Science		
Supervisor's Name:	Dr Fiona Menzies	**Supervisor's Department and Institution:**	School of Science & Sport, University of the West of Scotland
Project Title:	An investigation into the effect of IL-33 on nitric oxide production by placental endothelial cells		

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

Pre-eclampsia (PE) is estimated to affect ~6% of pregnancies in the UK and is considered to be due to endothelial dysfunction as a result of inappropriate placental inflammation. The placenta is a unique vascular organ by having two separate vascular systems. Work by the supervisor's group shows systemic levels of the cytokine IL-33 and its receptor (ST2) change throughout pregnancy. Also, ST2 levels are higher in the blood and placenta of PE women, with IL-33 lower in PE placentas compared to healthy placentas (*manuscript in preparation*). The role that IL-33 has on specific placental cell types is of great interest. The aim of this project is to investigate the effect of IL-33 on endothelial cells. In order to determine altered endothelial function, nitric oxide production will be measured. Endothelial cells can produce nitric oxide from the amino acid L-arginine using the vasoregulatory endothelial nitric oxide synthase (eNOS) or the inflammation-associated inducible nitric oxide synthase (iNOS).
More specifically, the following objectives were identified:
(1) What is the effect of IL-33 on nitric oxide production by human umbilical cord endothelial cells (HUVECs)?
(2) Do HUVECs alter the balance of eNOS and iNOS in response to IL-33?

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

N/A

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

Effect of LPS on nitrite production by HUVECS

In the first experiment we wanted to determine if a known inflammatory stimulator (*E.coli* lipopolysaccharide, LPS) would induce nitrite production by HUVECs. To do this, we exposed HUVECs to LPS (0, 0.1, 1, 10ng/ml) from two different *E. coli* strains, 055.B5 and 011B4, for 24 or 48 hours, collected supernatants and Greiss assay performed (n=5). At all concentrations tested, no significant production of nitrite was detected with either strain of LPS.

Effect of IL-33 on nitrite production by HUVECs

We next wanted to determine if IL-33 would induce nitrite production by HUVECs. To do this, we exposed HUVECs to recombinant human IL-33 (0, 1, 10, 100ng/ml) for 24 or 48 hours, collected supernatants and Greiss assay performed (n=5). Levels of nitrite in supernatants from IL-33 treated cells were comparable to the basal levels from unstimulated cells, with results similar for two separate experimental runs.

Effect of IL-33 on iNOS and eNOS mRNA expression by HUVECs

Although we did not detect any nitrite in the supernatant, we wanted to determine if (a) HUVECs expressed mRNA for iNOS and eNOS, and (b) if expression was modulated by stimulation with rIL-33. HUVECs were exposed to rIL-33 (0, 1, 10, 100ng/ml) for 18 hours, RNA extracted and cDNA synthesized. PCR was performed to determine

expression of the housekeeping gene TOP1, along with iNOS and eNOS. Primers for iNOS and eNOS were taken from a previous publication (Wheeler *et al.* (1997). *J. Clin. Invest*. 99:110-116) and bioinformatical analysis performed to check specificity. PCR showed that TOP1 and iNOS were similarly expressed across all samples. eNOS was also similarly expressed across all sample, although the negative control showed a weak band, meaning this PCR will need repeated to confirm results. Future studies will involve QPCR to quantify results.

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

The cause or cellular mechanisms of pre-eclampsia have not been fully established. It was hypothesised that altered IL-33 levels in the placenta contribute to PE by modulation of endothelial cell function, and this project focussed specifically on nitric oxide production by these cells. It can be concluded that LPS and IL-33, at the concentrations and time points examined, do not have an effect on nitric oxide production by HUVECS nor do HUVECs alter the balance of eNOS and iNOS in response to IL-33. Although the results did not match the hypothesis of this study, it does not reflect that there is no link between IL-33 and nitric oxide production by placental endothelial cells. Future studies will include experiments such as real-time PCR, which would allow quantifying the level of gene expression. Also, isolation of placental endothelial cells from healthy and pre-eclamptic placentas would allow for definitive comparison.

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

This experience has allowed me to develop key laboratory skills that would not be possible during my normal degree programme. The first step was to to undertake appropriate laboratory health and safety training. This experience has allowed me to plan and design my own experiments, manage my time, and develop group-working skills within a busy research team, allowing me to put into practice what I have been learning in my degree programme. I have leanrned cell culture and molecular techniques that will be invaluable to my development as a researcher in the biomedical field. The experience has been helpful for me to reflect on the following regarding my chosen career:

1. The pace of the work
2. The variety of the work and what the research has to offer
3. The future potential of the research

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

Foundations such as 'Society for Reproduction and Fertility' help students like me to explore fields of reproduction research that have vaguely been investigated. The investigation of the effect of IL-33 on endothelial cells in the context of pregnancy caught my interest when I fathomed its depths and by receiving funding from SRF I could explore this phenomenon and gain a better understanding of the effects it has on pregnancy or cardiovascular disease.

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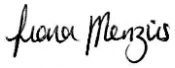
Supervisor:  09/09/2016