SRF VACATION SCHOLARSHIP REPORT 2017

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Degree Title and year of study:	BSc Biomedical Science Year 2		
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Project Title:	The regulation of stanniocalcin-1 in human pregnancy		

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

Stanniocalcin- 1 (STC-1) is a glycoprotein that was first found to regulate calcium and phosphate homeostasis in bony fish. In mammals, STC-1 has both paracrine and autocrine functions and it has been found to be involved in many physiological as well as pathophysiological processes like pre-eclampsia. Pre-eclampsia is a disorder in pregnancy that affects up to 8% of pregnant women. It is characterised by high blood pressure, proteinuria and may result from poor placental perfusion. Studies have shown STC-1 to be present in circulation during pregnancy and its expression upregulated in pre-eclampsia.

The regulation of STC-1 secretion is still poorly understood and so the hypothesis was that this might be regulated by fluctuations in oxygen. The aims of the project were to 1) identify which cells within the placenta express STC-1, 2) determine whether the secretion of STC-1 by trophoblasts is regulated by the concentration of oxygen, 3) to look into the regulation of STC-1 secretion by progesterone and cAMP, 4) to determine which pathways progesterone acts on to regulate STC-1 secretion and 5) to determine whether serum STC-1 is elevated in the first trimester of women who go on to develop pre-eclampsia.

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

No major changes were made to the project except we were unable to investigate the effects of oxygen tension on the secretion of STC-1 by first trimester chorionic villus tissue. This was due to the unavailability of tissue samples.

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

1. **Cells in the placenta that express STC-1**

Results from immunohistochemistry showed the expression of STC-1 by syncytiotrophoblasts, cytotrophoblasts, placental endothelial cells and hofbauer cells.

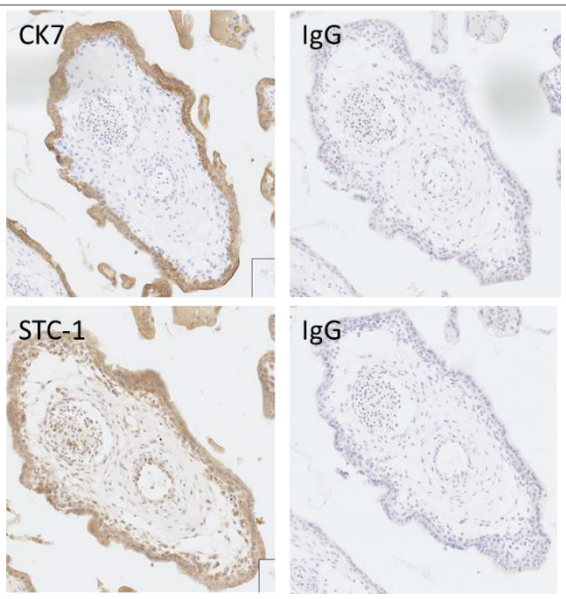

Figure: Immuno-histochemical staining of first trimester chorionic villi for cytokeratin-7 a marker of trophoblasts (top left) and the appropriate IgG control (top right) and STC-1 (bottom left) and the appropriate IgG control (bottom right).

2. Determining whether secretion of STC-1 by trophoblasts is regulated by oxygen concentration

Cells from a well characterized cytotrophoblast cell line (BeWo) were treated with cAMP and incubated for 48 hours at both 21% and 1% oxygen. The STC-1 concentrations in the supernatant were determined by ELISA. STC-1 concentrations from cells stimulated with cAMP and incubated at 1% oxygen were found to be significantly higher than those incubated at 21% oxygen.

3. Regulation of STC-1 secretion by progesterone and cAMP

We also stimulated the BeWo cells with cAMP and two different concentrations of progesterone. They were incubated at 1% oxygen as this was the concentration that produced a greater response from the previous experiment. We determined the STC-1 concentration in the supernatant by ELISA and results showed a decrease in STC-1 levels when progesterone was introduced. This decrease in STC-1 was even greater with a higher concentration of progesterone.

4. Mechanisms by which progesterone acts to regulate STC-1 secretion

We also looked at the effect of progesterone and cAMP on trophoblast cells after 24 and 48 hours. Using western blot analysis, we probed for the molecule Serum-and-glucocorticoid-regulated kinase 1 (SGK-1) as well as its phosphorylated substrate N-myc downstream-regulated gene 1 (pNDRG-1). The results showed the presence of both SGK-1 and pNDRG-1 in cells stimulated with cAMP and 1% oxygen.

5. Determining whether STC-1 is elevated in women who later develop pre-eclampsia

The concentration of STC-1 in first trimester serum from pregnancies with a known outcome was assessed. There was no significant difference in the concentration of STC-1 in first trimester serum from normal (n=18) patients and those that later develop pre-eclampsia (n=18).

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

Results from the experiments suggest that STC-1 secretion by trophoblasts significantly increases in hypoxic conditions. This could potentially explain the increased STC-1 secretion seen in preeclampsia which could be a result of low placental perfusion. Results also suggest that progesterone may be involved in inhibiting this response. However, further investigations need to be carried out to determine the mechanisms by which progesterone inhibits this response.

The results also revealed that SGK-1 and PNDRG1 could potentially be involved in the CAMP mediated pathway that leads to the secretion of STC-1 in hypoxic conditions. This will need to be investigated further.

Immunohistochemistry results showed that STC-1 is secreted by different types of cells in the placenta including trophoblasts. However, more research needs to be carried out to determine the physiological role of STC-1 on these cells.

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

This project was my very first experience working in a research environment and the experience has been invaluable. I have had the opportunity to learn new techniques like ELISA and Western Blotting and I have also been able to use my scientific knowledge to be able to analyse and evaluate results. This has made me very prepared and excited to tackle my final year research project. This experience has highlighted some of my strengths and weaknesses and it has made me a lot more confident when it comes to working in the lab. It has improved my planning and time management skills as well as my critical thinking skills. I have really enjoyed being part of the team and learning more about how research works. This project has helped me to reaffirm my decision to pursue a career in research hopefully combined with clinical practice.

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

Student: This project has provided an amazing opportunity for me to get firsthand experience in a research lab and I have been able to gain a lot of skills and knowledge that will be useful for me in the future. I would recommend this project to anyone looking to pursue a career in research.

Supervisor: Joan was an excellent student, efficient hard working and punctual. She was able to pick up the various techniques very easily. Within a short time she was planning her day and with a little help from me could devised her own experiments. Her lab book was well maintained and the results obtained were reproducible and reliable. I hope we will be able to build on these results and submit the data for publication in the not too distant future.