SRF VACATION SCHOLARSHIP REPORT 2017

Student's Name:	Jessica McLachlan	**Student's Institution/University:**	The University of Edinburgh
Degree Title and year of study:	Bachelor of Science with Honours Biomedical Science (Reproductive Biology)		3rd year
Supervisor's Name:	Dr. Rupsha Fraser	**Supervisor's Department and Institution:**	MRC Centre for Reproductive Health, The University of Edinburgh
Project Title:	The effect of the ovulation induction drugs, clomiphene citrate and letrozole, on placental differentiation		

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

Anovulation – where ovulation does not occur - is a common cause of infertility, and is largely due to hormonal imbalances. Clomiphene citrate (CC) and letrozole (L) are common anovulation treatments. CC is a hypothalamic estrogen receptor inhibitor, causing inhibition of estrogenic negative feedback on the hypothalamus and upregulation of the HPG axis. The subsequent increase in FSH promotes ovarian follicle growth, leading to ovulation. L is an aromatase inhibitor and blocks the conversion of androgens into estrogens, again reducing estrogenic negative feedback on the hypothalamus and upregulating the HPG axis.

Preparation of the endometrium, which includes decidualisation and induction of a receptive epithelial phenotype, is highly dependent on estrogen and progesterone and is critical for embryo implantation. Despite high rates of ovulation following CC or L administration, pregnancy rates remain low, which may be attributed to anti-estrogenic effects on the endometrium. It is therefore possible that by altering hormonal dynamics, CC and L could interfere with endometrial development and sufficiency for successful embryo implantation.

Using the trophoblast differentiation markers Cdx2 and human chorionic gonadotrophin (hCG), this project aims to determine how CC and L, through their influences on endometrial development, may affect placental differentiation.

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

Embryoid bodies (EBs) were created with the WA09 (H9) cell line (WiCell®, Madison, WI), via the hanging drop method or free-form formation from cell clusters, followed by transfer to gelatin-coated plates. These did not grow when cultured in conditioned medium, and became infected. Therefore no results were obtained. hCG was intended to be used as a marker, rather than Stra13 as initially proposed, as hCG is produced early and throughout the differentiation process. Additionally, the St-T1b cells were treated with or without CC or L for 5 or 7 days (rather than all for 7 days), to account for the 5-7 day and 2 day half-lifes of CC and L, respectively.

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

Images of the 5 and 7 day CC or L-treated St-T1b cells were obtained using a Zeiss Axiovert 200 inverted microscope (Figure 1). For the 7-day cultures, it is clear that the treated cells are of a lower cell density than the EtOH control, and are of a rounder morphology – similar to the decidualisation stimulus control. The images and any differences between cell cultures are much less clear for the 5-day cultures, which may in part be due to the shorter culture time.

In addition to the original experiments outlined in the application, enzyme-linked immunosorbent assay analysis was carried out on remaining 5- and 7-day conditioned medium. However, irregular readings were recorded, likely due to user error.

RNA extraction from the drug-treated St-T1b cells was attempted but gave extremely low yields, likely due to insufficient trypsinisation of the cells when removing them from their initial flask. It was intended to complete an RT-PCR with the RNA, but it was not attempted – instead, I observed and learnt how to do RT-PCR with another group.

The EBs were intended to be cultured with the conditioned media and hCG and Cdx2 visualised by immunofluorescence. Instead, to learn how to do immunocytochemistry, murine blastocysts were collected from IVF procedures, which I observed, and I learnt the technique of immunocytochemical labelling of the resulting blastocysts.

Unfortunately this project was time-restricted and would have benefitted from having more time allocated to it. However, a I learnt a great deal, particularly that several of the above techniques require further practise and refinement.

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

It was expected that CC or L treatment would indirectly influence the expression of the trophoblast differentiation markers Cdx2 and hCG. Such a result would suggest that CC and L have anti-estrogenic effects on the endometrium that interfere with successful embryo implantation. Any differences in cell density and morphology between the 5-day culture St-T1b drug treated cells and the controls are not particularly clear. However, the 7-day culture St-T1b drug treated cells are of a lower cell density than the EtOH control and are similar in morphology (round) and density to the decidualisation stimulus control – this suggests that CC and L do not interfere with decidualisation. Nonetheless these observations are purely morphological and require further support, such as consideration of differentiation markers and secreted factors.

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

I have thoroughly enjoyed my experience in the laboratory, and am sure I will find my new experience and knowledge invaluable to my future studies. As a result of my time at the laboratory, I have decided I would like to apply for a laboratory based Honours project, and am excited to further develop my skills and techniques. I have found that I very much enjoy laboratory work and the satisfaction when something goes well, and will look into laboratory-based career options after finishing my Bachelor's degree.

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

Student: I have learnt the following:

- Cell culture methods
- Use of the fume hood and associated techniques
- RNA extraction and RT-PCR
- Microscopy techniques
- EB formation protocols
- Immunocytochemistry
- The process of designing experiments and troubleshooting

This has been an extremely valuable experience and I have learnt a great deal, all of which will help me in completing my lab based Honours project and in my future career which I hope to be in industry lab work.

Supervisor: Jessica showed great enthusiasm and grasped concepts quickly, and I believe she would benefit from having more extensive laboratory experience.

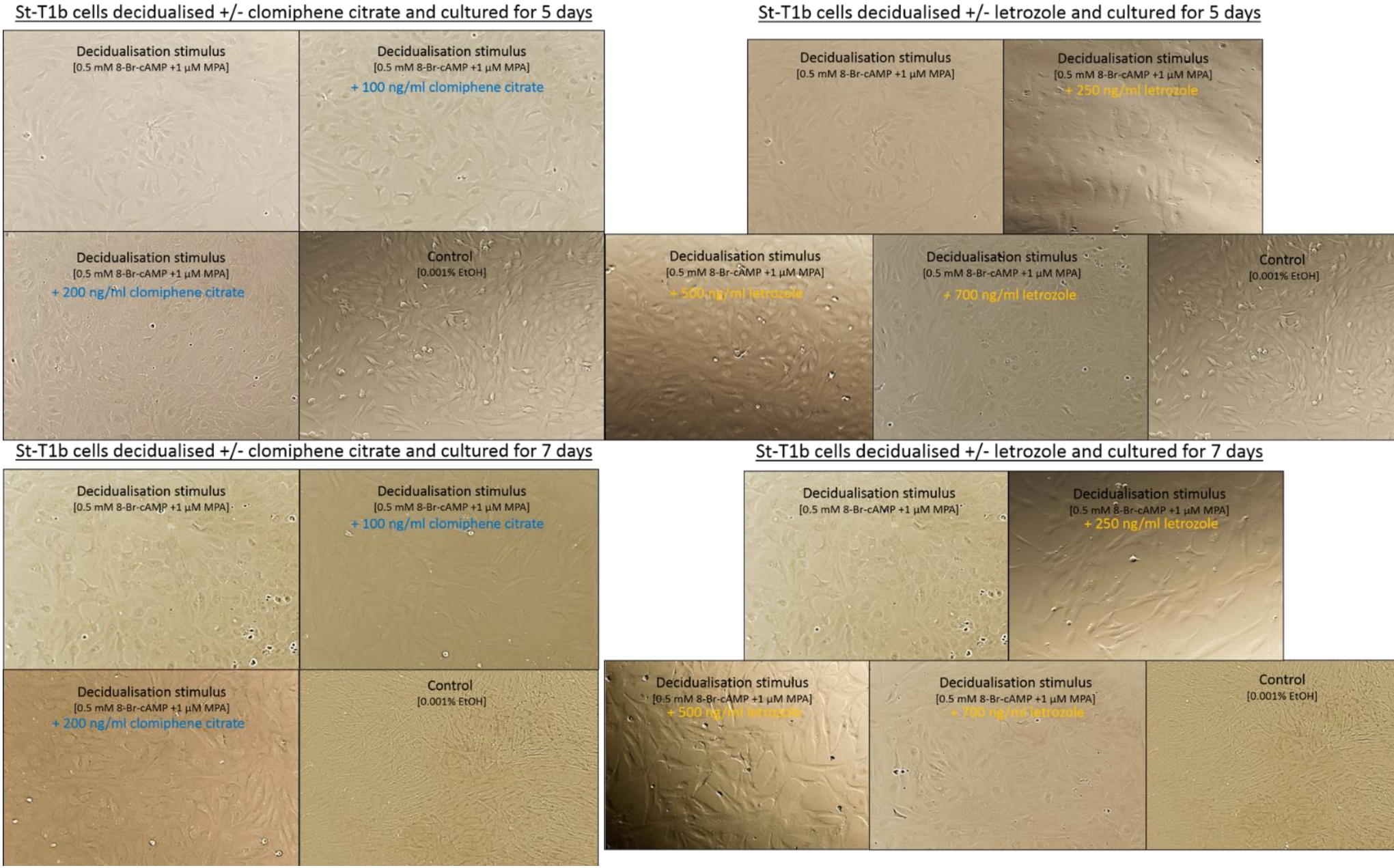

Figure 1: St-T1b cells decidualised with or without CC or L, for 5 or 7 days.

Images obtained using microscopy of the St-T1b cells treated with varying concentrations of CC and L for 5 or 7 days. The conditioned media from the St-T1b cells was intended to be used as culture medium for the EBs, and RNA extraction and PCR was attempted on the ST-T1b cells. Remaining conditioned media was also used in an ELISA – as such, they represent three experiments within the project.