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:	Arvis Tam	**Student's Institution/University:**	University of Edinburgh
Degree Title and year of study:	Year 3, Bsc Biomedical Sciences		
Supervisor's Name:	Rod Mitchell	**Supervisor's Department and Institution:**	Centre for Reproductive Health, Queen's Medical Research Institute
Project Title:	The effets of vincristine cancer therapy on prepubertal testicular tissue		

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

Currently, the most common forms of cancer treatment include chemotherapy and radiotherapy. However, many chemotherapeutic agents also have the potential to damage healthy tissues (gonadotoxicity) resulting in long-term morbidity. The impacts on fertility after such cancer treatments result from damage to the germ cell populations within the testis. A decline in germ cell population as a result of treatment during childhood can affect future spermatogenesis and fertility in adulthood. However, the impact of many chemotherapy agents has not been determined in human-relevant models. Vincristine is a chemotherapy agent used as part of the regimen in Acute Lymphoblastic Leukaemia (ALL), the most common form of childhood cancer. Vincristine has traditionally been considered to be 'low risk 'for gonadotoxicity. However, recent data from mouse studies suggest that vincristine may result in germ cell loss in the testis.

This project aims to identify the direct effects of vincristine exposure on germ and somatic cell populations in the prepubertal human testis. The project will specifically aim to investigate and compare the gonadotoxicity of vincristine with another agent (cisplatin), known to carry a high-risk of gonadotoxicity.

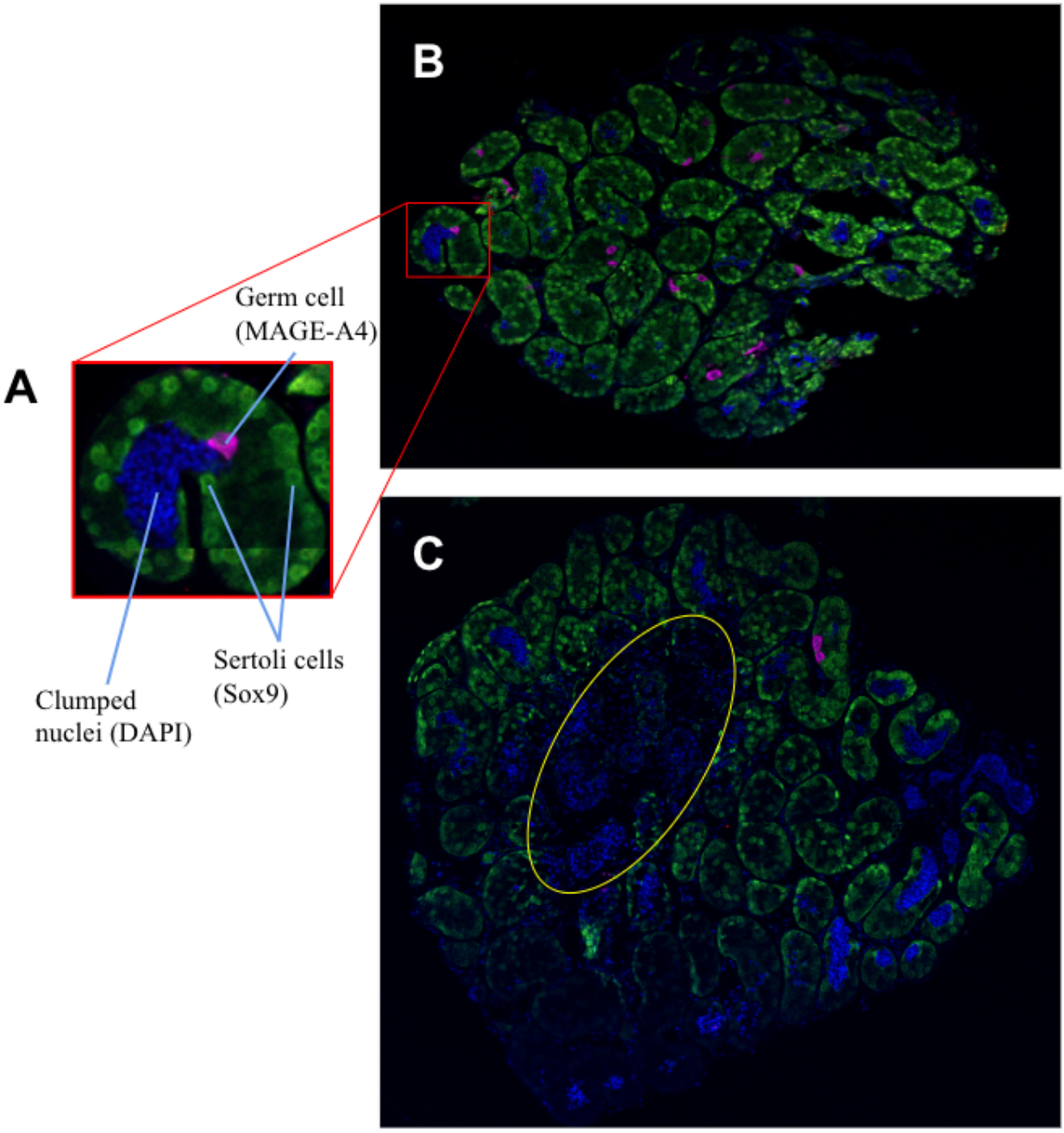
Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

No changes were made to the experimental method. However, due to time constraints, I was not able to carry out a complete quantitative analysis of the stained tissue (but I received training on how the remaining analysis would have been carried out). Instead, a semi-qualitative analysis was performed on a portion of the sample tissue, which involved quantification of germ cells as well as ranking the severity of the observed tissue damage which will be further explained below.

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

The analysis of the experiment was performed on immunofluorescence stained tissues using Sox9, MAGE-A4 and Hoechst antibodies that stain Sertoli cells, Germ cells and nuclei respectively. Figure A

highlights the different coloured staining imaged using a confocal microscope: Sox9 with Cy3 fluroflore staining (green), MAGE-A4 with Cy5 fluroflore staining (pink) and Hoeschst with DAPI staining (blue).



In order to quantify the severity of the tissue damage, the images were ranked on a scale of 0 (largely healthy tissue), 1 (moderate tissue damage with few small patches of clumped nuclei) and 2 (large areas of cell death).

Figure B represents a healthy vehicle control tissue. Notable features that indicate healthy tissue include defined tubules, correctly arranged Sertoli cells around the lumen, and numerous germ cells present on the basement membranes. This tissue would therefore be catergorised as 0 on our tissue damage scale.

Figure C is an image of a Vincristine (10ug) treated testicular tissue and demonstrates significant tissue damage; a reduced number of germ cells and large areas of clumped nuclei (depicted by the yellow oval) indicate damage to the tubules and considerable cell death. This tissue, as well as other tissues that exhibit similar charateristics, would be catergorised as 2 on the tissue damage scale.

This semi-quantitative analysis was performed on the remaining tissue images and yielded the following results. The number of germ cells (MAGE-A4 stained cells) were counted by hand, and on average 11.5 germ cells were present in vehicle control cells. Vincristine 1ug treatment saw a decrease in germ cell

count to an average of 10.8 cells, while the 10ug treatment saw a larger decrease to 8.5 cells. Regarding the severity of tissue damage, the vehicle control tissues averaged 0.41 on our scale, but remained at 0.62 for both 1ug and 10ug treatments indicating little to moderate damage.

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

These results demonstrate a negative correlation between the concentration of Vincristine treatment and the germ cell count, as well as a positive correlation between the concentration of Vincristine treatment and the severity of the tissue damage. This would therefore support the hypothesis that increasing Vincristine concentration in treatments are increasingly gonadotoxic, indicating a positive correlation, as it induces a varying degrees of cell death and tubule damage.

However, this conclusion is solely based on the preliminary semi-quantitative analysis and is therefore not indicative of what the complete analysis of the experiment would suggest. Further analysis that quantified the area of cell death and took into account other cell types would be required to reach a definitive conclusion.

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

Due to the pandemic I had limited opportunities to conduct practical research and gain laboratory experience, but this project has given me invaluable experience and insight on what a career in research would involve. On top of new laboratory techniques, this experience has also taught me how to develop experimental design and analyses, as well as how to read and write scientific papers more effectively which will definitely be useful in my future studies.

This experience has also provided me with an opportunity to interact with some of the scientific community here through lectures, seminars and lab discussions. As a result, I am now considering pursuing a research Masters degree or any potential future studies in medical biology.

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

Student: I am grateful to have been selected as a recipient for the SRF Vacation Scholarshuip this year, as this made my research project even more fulfilling. It was a pleasure meeting my colleagues in the lab, and they have given me lots of guidance and support throughout my journey. I look forward to returning to the lab in the future, and I hope other students will also be able to benefit from this scholarship.