SRF VACATION SCHOLARSHIP REPORT 2021

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:	Lisa Abraham	**Student's Institution/University:**	University of Edinburgh
Degree Title and year of study:	Medicine MBChB		Year 4
Supervisor's Name:	Agnes Stefansdottir	**Supervisor's Department and Institution:**	Biomedical Sciences
Project Title:	The effect of doxorubicin on ex vivo fetal mouse ovaries		

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

Around 1 in 1000 pregnant women are diagnosed with cancer. Chemotherapy is generally considered safe after the first trimester, with no severe congenital effects on the foetus. However, follow up studies have not investigated the long-term effects of chemotherapy drugs on the reproductive systems of children exposed *in utero.* This is concerning, given that crucial events in in ovarian development occur during the second and third trimesters, including germ cell proliferation, follicle assembly and recruitment resulting in the formation of the so-called ovarian reserve. Given the well-documented and damaging effects of many chemotherapy agents on fertility in children and adults, it is possible that chemotherapy treatment may also affect fetal ovary development, potentially producing subtle, late-onset and long-term effects on fertility. Doxorubicin is an anthracycline-based chemotherapy regimen that is used during pregnancy in the treatment of malignant tumours including breast cancer, lymphoma and leukaemia. To date, there have been very few, if any, studies on the impact of doxorubicin exposure on the reproductive development of fetuses. This novel project aimed to investigate the effect of doxorubicin on germ cell number and DNA double strand breaks in *in vitro* fetal mouse ovaries.

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

The original proposed in vivo work was started, and mouse embryos exposed to either saline or cisplatin in utero were sectioned. H&E analysis and immunohistochemistry for germ cell markers were carried out on this tissue. However, due to time constraints on my training and the complexity of this work (which required sectioning of whole embryos instead of single gonads), it was decided to concentrate the remainder of the project on analysing germ cell number and health in embryonic day 13.5 (E13.5) foetal mouse ovaries that had been exposed to the chemotherapy agent, doxorubicin, *in vitro*.

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

Doxorubicin exposure resulted in a dose-dependent decrease in germ cell number, with a 92% and 95% reduction in germ cell number at the 0.1 and 0.5 ug/ml concentrations respectively ($p<0.01$). The changes in germ cell number are show in the bar graph in Figure 1. There was no significant change in yH2AX

expression in doxorubicin-exposed ovaries at the end of culture, however, a trend for increasing yH2AX intensity at the 0.05 and 0.1 ug/ml doxorubicin concentrations was observed (Figure 1). Figure 1 also shows immunofluorescent images ovarian sections treated with saline (control) and 0.1 ug/ml doxorubicin, respectively.

H&E staining and immunohistochemistry were carried out on sections of embryos treated with saline or cisplatin *in vivo*. However, there was not time to analyse the germ cell number or health of these images.

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

Our results show that fetal ovarian exposure to doxorubicin between E13.5-E19.5 can have adverse effects on the developing ovary, drastically reducing the number of germ cells. Although trends were observed for increasing levels of double strand breaks (as measured by yH2AX intensity), these were not significant, therefore the mechanism behind germ cell loss remains unclear. The study had an n=4, therefore increasing this sample size is important in confirming the effects of doxorubicin on foetal ovaries. Previous studies have shown that many chemotherapy agents can cross the placenta, however little data are available about the ability of doxorubicin to cross the placenta. Nevertheless, results here indicate that further work in this area is required.

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

Having had little previous lab experience, it was useful to learn how to use a microtome, carry out immunostaining and observe fluorescent microscopy. It was also helpful to learn how to analyse images using ImageJ and get more familiar with compiling results, carrying out statistical analysis and making conclusions. The experience has therefore given me skills that I might use in future research projects. Furthermore, I was able to better understand lab techniques and methodology that I had previously only read about in papers. Having learned a lot from this project, I am inspired to look for more opportunities where I might be able to do research, especially with a longer timeframe than eight weeks. Furthermore, learning more about what can influence ovarian development has piqued my interest in reproductive health and fertility, and I am more interested in this as a potential specialty for my future medical career.

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

Appendix 1:

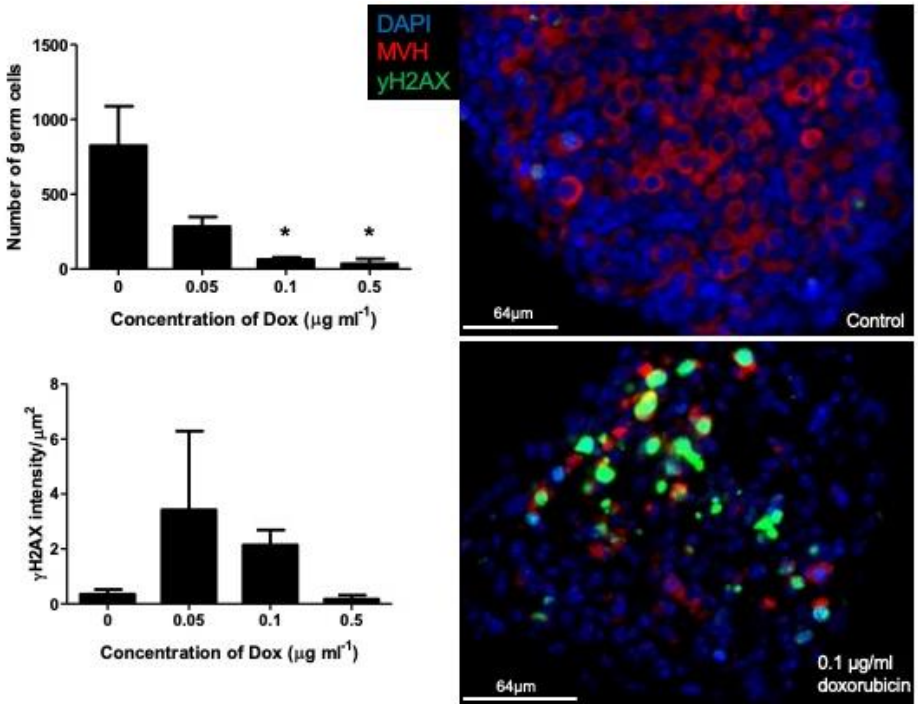

Figure 1: Top left: Bar graph showing the effect of doxorubicin concentrations on number of germ cells. Bottom left: Bar graph showing the effect of doxorubicin on yH2AX intensity. Right: Immunofluorescent images of control (top right) and 0.1 ug/ml doxorubicin-treated (bottom right) ovaries stained for DAPI, MVH and yH2AX.