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:	Cara Robb	**Student's Institution/University:**	The University of Edinburgh
Degree Title and year of study:	BSc Reproductive Biology, 3rd Year (Deanery of Biomedical Sciences)		
Supervisor's Name:	Dr. Jacqueline Maybin	**Supervisor's Department and Institution:**	Centre for Reproductive Health, Queen's Medical Research Institute
Project Title:	A pilot study to examine ovarian hormones in women with long COVID across the menstrual cycle		

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

Long COVID is defined as signs and symptoms of COVID-19 that continue or develop 4 weeks after acute COVID-19 infection, including ongoing symptomatic COVID-19 (4-12 weeks) and post-COVID-19 syndrome (>12 weeks)[1]. Approximately 1 in 20 people with symptomatic COVID-19 continue to experience symptoms 8 weeks after onset[2]. Many women with long COVID have reported disturbance to their menstrual duration, frequency, regularity and/or volume[3].

Menstruation is regulated by ovarian hormones. It is triggered by the withdrawal of progesterone (and estradiol) at the late secretory phase of the cycle, resulting in endometrial inflammation. This leads to the shedding of the functional endometrium at menstruation[4]. Testosterone can interfere with menstruation if levels are abnormal[5].

The effect of COVID-19 on ovarian hormone levels and the endometrial response to these hormones remains unknown[3].

We hypothesised that women experiencing long COVID symptoms have altered ovarian hormone levels that may contribute to menstrual disturbance.

The aims of the project were to determine if women with long COVID have (1) altered serum ovarian hormone levels and (2) altered endometrial ovarian hormone receptors during the proliferative, secretory and/or menstrual phase of the cycle when compared to women who have never had COVID-19.

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

Due to time limitations, this project focused solely on ovarian hormones. I will examine inflammatory mediators in the future.

We aimed to perform immunohistochemistry on endometrial tissue samples to detect the presence and location of endometrial hormone receptor levels. However, some participants declined endometrial sampling, resulting in lower n-numbers.

Therefore, we performed quantitative RT-PCR on endometrial samples, rather than semi-quantitative immunohistochemical staining in low n-number groups. Optimising these immunohistochemistry protocols enhanced my laboratory training and facilitated timely analysis of endometrial ovarian hormone receptor localisation after recruitment.

I prioritised analysis of peripheral blood serum samples to detect ovarian hormone protein levels.

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

With ethical approval and consent, women with long COVID provided peripheral blood samples during the proliferative (n=4), secretory (n=4) and menstrual (n=5) phases of the cycle. Three women also provided endometrial biopsies at the proliferative (n=3), secretory (n=2) and menstrual (n=2) phases. All women were aged 18-55, had regular menstrual cycles (24-38 days), and were not taking exogenous hormones.

Controls were sourced from our bio-resource, collected pre-COVID-19 pandemic/pre-Dec 2020, and matched for age, parity and BMI. There were two control samples for every long COVID sample (N=26 serum and N=14 endometrial tissue).

Protein levels of ovarian hormones oestradiol (E2), progesterone (P4) and testosterone (T) were measured by ELISA in blood serum samples. Endometrial hormone receptor (PR-A, PR-B, ER-α, ER-β, AR) mRNA was quantified using qRT-PCR in whole endometrial biopsies. Differences in control and long COVID patients during each stage of the cycle were analysed using unpaired t-tests.

When comparing serum hormone levels in those with long COVID and controls, we found E2 and P4 protein were consistently lower in those with long COVID across all phases of the cycle, although differences were not statistically significant. T was significantly lower during the menstrual phase in those with long COVID versus controls (P=0.038).

Relative ovarian hormone receptor mRNA (ESR1, PGR, PGR-B and AR) concentrations in proliferative endometrium revealed no significant differences in those with long COVID versus controls.

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

The results show statistically lower menstrual serum T in women with long COVID, which could result in endometrial effects contributing to the reported menstrual disturbance. In addition, E2 and P4 levels were consistently lower in women with long COVID. Although not statistically significant, these findings may result in clinically significant endometrial effects that could also contribute to menstrual disturbance.

This pilot study suggested that lower testosterone levels during the menstrual phase in patients with long COVID may affect endometrial function but this work requires replication in a larger cohort of patients. Study of other factors important in menstrual physiology (e.g. inflammation, coagulation, hypoxia) is required to understand the mechanisms underpinning menstrual disturbance in those with long COVID.

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

Laboratory skills: I gained experience in PCR, immunohistochemistry and ELISA techniques that I wouldn't have had the opportunity to in my undergraduate programme, especially considering that I haven't had proper in-person university labs since first year.

Scientific skills:

- I attended weekly lab meetings, where I contributed to discussions and presented my project.
- My scientific writing has improved as I wrote an abstract which was accepted at the SRF Satellite meeting where I am set to present my results next year.
- I performed data analysis on the ELISA serum hormone level results and the endometrial hormone receptor PCR results.

Career development: As my current plans are to study medicine as a post-graduate, this internship has opened my eyes to the value of research and the possibility of working in a combined clinical and scientific field in the future.

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

Student: I am incredibly grateful for this opportunity which has been invaluable in furthering my interest in reproductive biology. I have experienced first-hand the reality of working in research, which I wouldn't have necessarily been able to in my undergraduate programme. I would like to thank the SRF and the QMRI for facilitating this project – in particular, Jackie Maybin, Kate Walker, Moira Nicol and Rocío Martínez Aguilar for their supervision and support!

Supervisor: Cara was a delight to have in the laboratory during her Summer Scholarship. She gained practical laboratory skills in ELISA, PCR and immunohistochemistry as well as data analysis, statistics and literature review and attended departmental seminars. She presented her project at our laboratory meetings, developing her scientific presentation skills. She submitted an abstract, which has been accepted for presentation at the SRF Satellite meeting, Fertility 2023.

References

1. National Institute for Health and Care Excellence [Internet]. COVID-19 rapid guideline: managing the long-term effects of COVID-19. 2020 Dec 18. Available from: https://www.nice.org.uk/guidance/ng188

2. Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, et al. Attributes and predictors of Long-COVID: analysis of COVID cases and their symptoms collected by the Covid Symptoms Study App. medRxiv. 2020 Dec 19.

3. Sharp GC, Fraser A, Sawyer G, Kountourides G, Easey KE, Ford G, Olszewska Z, Howe LD, Lawlor DA, Alvergne A, Maybin JA. The COVID-19 pandemic and the menstrual cycle: research gaps and opportunities. Int J Epidemiol. 2021 Dec 2; 51(3): 691-700.

4. Critchley HOD, Maybin JA, Armstrong GM, Williams ARW. Physiology of the Endometrium and Regulation of Menstruation. Physiol Rev. 2020 Apr 29; 100(3): 1149-1179.

5. Bui HN, Sluss PM, Blincko S, Knol DL, Blankenstein MA, Heijboer AC. Dynamics of serum testosterone during the menstrual cycle evaluated by daily measurements with an ID-LC-MS/MS method and a 2nd generation automated immunoassay. Steroids. 2013 Jan; 78(1): 96-101.