**UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2022/23**

***NB: This whole report will be posted on the Society’s website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.***

**Name of student:**

Ms. Lauren Barrett

Twitter Handle\*: lauren99barrett

*(\*optional)*

**Name of supervisor(s):**

Professor Gerard O’Keeffe, Department of Anatomy & Neuroscience, University College Cork, Ireland.

**Project Title: (no more than 220 characters)**

|  |
| --- |
| To determine the effects of sFlt-1 on neuronal differentiation, migration and morphology using an *in vitro* neuronal model of relevance to pre-eclampsia. |

**Project aims: (no more than 700 words)**

There is now an extensive body of evidence that the *in-utero* experience is a critical determinant of the long-term risk of adverse neurodevelopment outcomes in the offspring [1, 2]. Hypertensive disorders of pregnancy (HDP) are estimated to affect approximately 5-15% of all pregnancies, therefore are among the most common prenatal complications [3]. HDPs are classified into four categories, as recommended by the International Society for the Study of Hypertension in Pregnancy and the most common of these is pre-eclampsia [4]. Pre-eclampsia is recognised as the leading cause of maternal death and maternal and fetal morbidity. Previously thought to be simply due to impaired trophoblast invasion followed by the development of the clinical manifestations of the disease, it is now known that the underlying aetiology of pre-eclampsia is far more complex and includes a state of systemic maternal alterations in circulating factors which may alter fetal developmental trajectories, which in turn may increase the risk of long-term vascular, cognitive, and psychiatric sequelae in the offspring across the life-span [5-8]. In support of this, we have recently published a systematic review and meta-analysis examining the association between pre-eclampsia and neurodevelopmental outcomes in the offspring [2]. Pooled results from this study showed that exposure to HDP (including pre-eclampsia, gestational hypertension, and chronic hypertension) were associated with a 35% increase in the odds of ASD when compared to those unexposed to HDP (OR=1.35; 95% CI: 1.11-1.64) [2]. Subgroup analysis examining pre-eclampsia alone and ASD increased the odds ratio to 1.50 (95% CI: 1.26-1.78), whereas all other HDP (which may include pre-eclampsia) were associated with a non-significant increase in the odds of ASD (OR: 1.25, 95% CI: 0.90-1.73). Despite this, the molecular basis of this is known but a leading candidate for a mediator of this association may neuronal exposure to elevated levels of placental soluble fms-like tyrosine kinase 1 (sFLT1) which is well known to be elevated in pre-eclampsia, but its effects of developing neurons are largely unknown. The aim of this work was to determine whether exposure to increasing concentrations of sFLT1 altered the differentiation, migration, and the growth of neuronal processes in neurons. To do this, we differentiated the SH-SY5Y cells and the human neural progenitor cell line ReNcell® VM cells (a type of neural progenitor cells) into a mixed culture of mature neurons and glia and exposed them to sFlt-1 at varying times during their development. The outcomes measured were neurite growth, cytotoxicity, and differentiation.

**References**

1. Ursini, G., et al. Nat Med, 2018. 24(6): p. 792-801.

2. Maher, G.M., et al. JAMA Psychiatry, 2018. 75(8): p. 809-819.

3. Brown, M.A., et al. Pregnancy Hypertension, 2018.

4. Tranquilli, A.L., et al. Pregnancy Hypertension 2014. 4(2): p. 97-104.

5. Hakim, J., M.K. Senterman, and A.M. Hakim. International Journal of Pediatrics, 2013. 2013.

6. Davis, E.F., et al. Pediatrics, 2012.

7. Nomura, Y., et al. Arch Gynecol Obstet, 2017. 295(6): p. 1319-1329.

8. Pinheiro, T.V., et al., J Dev Orig Health Dis, 2016. 7(4): p. 391-407.

**Project Outcomes and Experience Gained by the Student (no more than 700 words)**

The main conclusion from this project is increasing concentrations of sFlt-1 alters the growth and development of cells in these experimental systems. Overall, these results offer a novel mechanism by exposure to the circulating factors elevated in pre-eclampsia may later neurodevelopmental process occurring at the time of exposure. This work is currently being prepared for publication as part of a larger study from the host laboratory.

In terms of experiment, I gained invaluable experience carrying out this project over the summer. I completed a full lab induction which included general lab rules and safety protocols involving PPE. A full introduction to cell culture was provided where I learned about aseptic techniques, storage areas and the fume hoods. I was introduced to the protocol followed for ordering reagents using requisition forms. Hazards and risk assessment documents were explained including MSDS & SOPs, as well as correct waste disposal procedures. I completed safety courses on BioRaft and Epigeum. I was given access to the online booking system for the equipment which gave me the opportunity to plan my own experiments and work independently as a researcher. I carried out full microscopy training in my time at the lab also for both live and fixed samples.

I have learned transferrable lab skills during my time working on this project. I independently grew and split SHSY5Y cells as well as plating them for treatment. I created and maintained stock solutions of media and reagents for my experiments, all while avoiding contamination issues. When issues arose with our microscope, I was able to adapt and use PFA to fix my cell plates to preserve my experiments and then image at a later date. This allowed me to learn the protocol for PFA fixing and collecting the media samples to carry out LDH assays. Once the plates were imaged using the inverted microscope, I learned how to carry out image analysis using software such as ImageJ to measure neuronal growth and differentiation and organised this data neatly into labelled excel sheets. I then carried out the appropriate data analysis tests such as one-way ANOVA and t-tests on the data collected from my images and visualisation using GraphPad prism. All these techniques are vital and will continue to be used as I continue my career in research. As a part of the lab group, I attended weekly meetings, in which I presented research updates to both the principal investigators and the other students.

Additionally, I joined the Anatomical Society as an Early Career member. This has provided me with a supportive community of fellow young researchers as well as offering academic and social connections. Also, as an Early Career member and recipient of the summer undergraduate scholarship, I have the exciting opportunity of attending the upcoming winter conference to present a poster of the work I have carried out thus far.

Following on from my final year project, investigating the same topic, I believed that a career in research is what I would like to pursue. Working on this project for the summer confirmed my interest in research and gave me the confidence to apply for a PhD programme. I interviewed for a PhD position funded by the Anatomical Society and was delighted to be offered the position. The work I had completed during the summer aided me in this process as it gave me much more research experience, most of which was carried out independently, and gave me the confidence in my ability to succeed at this role.

**Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:**

Winter Meeting in Liverpool Jan 3rd-5th 2024

**Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)**

I attended the Winter Meeting of the Society in Liverpool from January 3rd-5th 2024 and presented my poster on January 4th. My poster showcased my results from the summer internship. There are 3 experiments each of which have an experimental timeline showing the methods used. The p value was set at p > 0.05 for all statistical tests. The results section includes the results from each of the 3 experiments, along with a representative image. The results for experiment 1 & 2 were found using a one-way ANOVA and include a graph for normalised neurite length and a graph for the LDH assay, with the absorbance readings also being normalised. For experiment 3, a student’s t-test was used to compare the sFlt-1 group against the control for each of the 3 groups: Stage 1 only, Stage 2 only and Stage 1 & 2.

The conclusions that can be drawn from these experiments are listed. Experiment 1 & 2 show that the highest level (100ng/mL) of sFlt-1 reduced neurite growth. The LDH assay for both experiments showed no significant effect, meaning that these effects occurred without impacting cytotoxicity. The last experiment showed that this high level of sFlt-1 also impacted neuronal differentiation. The overall conclusion of this study was that there is a direct effect of sFlt-1 on neurite growth in *in vitro* models of neuronal development. We suggest that developing fetal neurons that are exposed to PE, and thus elevated levels of sFlt-1, may have altered patterns of neurite growth during development.

**Brief Resume of your Project’s outcomes**: **(no more than 200-250 words)**.

*The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.*

**To determine the effects of sFlt-1 on neuronal differentiation, migration and morphology using an in vitro neuronal model of relevance to pre-eclampsia.**

Pre-eclampsia is the most common of hypertensive disorder of pregnancy alter the developing brain. Pre-eclampsia has been shown leads to a increase in the risk of neurodevelopmental disorders in the affected children, however what causes this increased risk is unknown. It is well known that there are elevated levels of a protein called placental soluble fms-like tyrosine kinase 1 (sFLT1) present in pregnancies complicated by pre-eclampsia. The main aim of this work was to study whether sFLT1 alters neuronal development in cell models.

***Image: Migrating and differentiating neurons from neurospheres.*** *The image shown is a neurosphere generated from RenVM human neural progenitor cells. These cells were grown as neural progenitor cells for one week and then plated to induce neuronal differentiation for a subsequent week. As can be seen from the image, cells rapidly migrate out from the neurosphere during this time. The green staining is a marker called beta-III tubulin to label neurons. The red staining is a marker called GFAP to label astrocytes, which the blue staining stains cell nuclei.**This model system was used to examine how treatment sFLT1 affects these processes.*

The results show that increasing concentrations of sFlt-1 alters the growth and development of these cells which may be a novel way that pre-eclampsia may affect the developing brain of the offspring.

**Other comments: (no more than 300 words)**

No.

|  |
| --- |
| **Data Protection/GDPR**: I consent to the data included in this submission being collected, processed and stored by the Anatomical Society. Answer YES or NO in the Box below |
| YES |
| **Graphical Images**: If you include graphical images you must obtain consent from people appearing in any photos and confirm that you have consent. A consent statement from you must accompany each report if relevant. A short narrative should accompany the image. Answer N/A not applicable, YES or NO in the box below |
| YES |
| **Copyright**: If you submit images you must either own the copyright to the image or have gained the explicit permission of the copyright holder for the image to be submitted as part of the report for upload to the Society’s website, Newsletter, social media and so forth. A copyright statement must accompany each report if relevant. Answer N/A not applicable, YES or NO in the box below |
| YES |

 *Signature of student Lauren Barrett.. ...Date…10/10/23………..*

 *Signature of supervisor………Gerard O’Keeffe.......... Date……10/10/23…….…*

END OF FORM

----------------------------------------------------------------------------------------------------------------------------------------

*File: USVRS-Award Letter 2023\_ v3FINAL\_ 120523 – Professor Gerard O’Keeffe*

*File: USVRS Final Outcome Report O’Keeffe and Barrett Amended version uploaded 100624*