SRF VACATION SCHOLARSHIP REPORT 2018

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 Word document.

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:	Mabel Vos	**Student's Institution/University:**	Inholland University of Applied Sciences, Amsterdam
Degree Title and year of study:	BSc Biomedical Laboratory Research, specialisation in Bioresearch. Graduated July 2018. Now 1st year MSc Biomolecular Sciences.		
Supervisor's Name:	Dr. Sari Pennings	**Supervisor's Department and Institution:**	Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh
Project Title:	The interplay between repressive epigenetic marks and development in mouse preimplantation embryo culture.		

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

Trimethylation of lysine 27 on histone H3 (H3K27me3) is a histone post-translational modification established by the methyltransferase EZH2 of the PRC2 complex. H3K27me3 is associated with repression of developmental gene expression and known to maintain the pluripotent state. DNA methylation is also known to repress (developmental) genes. In mouse embryonic stem cells, it is suggested that DNA methylation and H3K27me3 are counterparts of each other and together regulate correct silencing of genes. In an effort to elucidate how DNA methylation is affected by reduced H3K27me3 expression, I investigated whether there is an interplay between these two repressive marks in early mouse embryogenesis. The project aimed to reduce H3K27me3 levels in preimplantation mouse embryos by inhibiting the catalytic subunit EZH2 with the small molecule inhibitor GSK126 in vitro. Mouse preimplantation embryos were cultured in the presence of 1 µM GSK126 and collected at different stages ranging from 4 to 40-cell. Thereafter the effect of EZH2 inhibition on 5mC and 5hmC expression was assessed by immunocytochemistry, imaging by fluorescent microscopy and followed by analysis with ImageJ. In addition, qRT-PCR was carried out to assess EZH2 and JMJD3 (demethyltransferase of H3K27me3) expression in 8-cell, mid morula, early blastocyst and late blastocyst stage embryos.

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

The project did not change, but qRT-PCR analysis was added to assess gene expression of the H3K27me3 specific methyl- and demethyltransferases, EZH2 and JMJD3, respectively. I did this because there were indications that there was a feedback mechanism of H3K27me3 maintenance at play.

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

Inhibition of EZH2 in the preimplantation embryo resulted in a dynamic change of 5mC and 5hmC.
The preimplantation mouse embryos were grouped by their size (3 to 4 cell, 5 to 9 cell, 10 to 16 cell and >16-cell). 5mC was hypothesised to be upregulated in the treatment group if 5mC would take over the repressive role of H3K27me3. However, at the global nuclear level we only observed a small and non-significant upregulation in embryos that were of 3 to 9 cell size, whereas 5mC was significantly reduced in the >16-cell embryo. 5hmC is the first oxidative product of 5mC by TET enzymes and it appears to be involved in active demethylation. Therefore,

5hmC was expected to be reduced in the treatment group. We observed a non-significant increase of 5hmC with embryo size in the treatment group. I compared results of H3K27me3 expression that were already available in the lab with these findings. I did not observe a relationship between H3K27me3 expression and expression of 5mC. We then assessed the gene expression of EZH2 and JMJD3 in the 8-cell, mid morula, early blastocyst and late blastocyst embryos with quantitative Real-Time PCR. We found that EZH2 as well as JMJD3 was upregulated in the treatment group. This upregulation was significant (P<0.05) in the 8-cell (E2.5) and late blastocyst stage (E4.5).

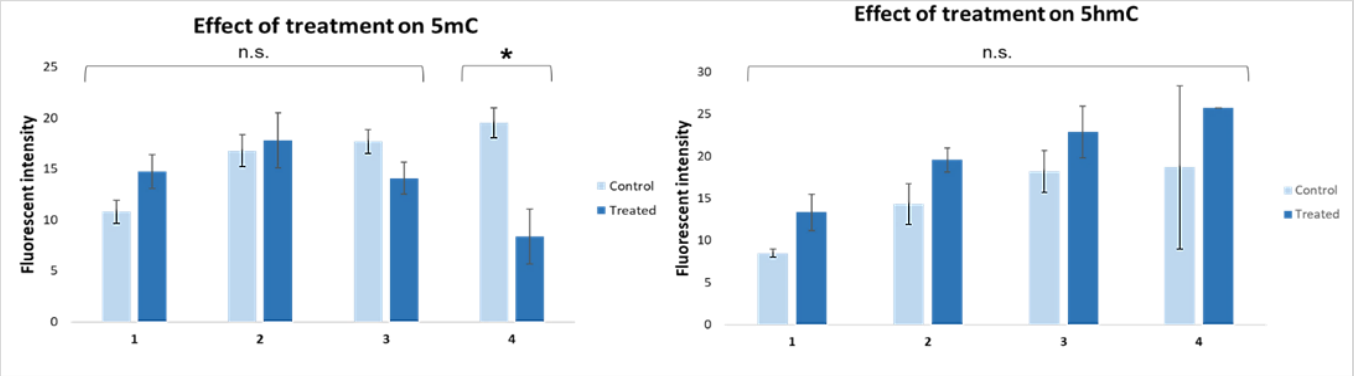

Figure 1: ***The effect of 1 µM GSK126 on 5mC and 5hmC expression in the preimplantation embryo****. Effect of treatment on 5mC* ***(1):*** *3-4 cell embryo, n= 7 control, n=13 treated. P=0.066.* ***(2)****: 5-9 cell embryo, n= 14 control, n=10 treated. P=0.747.* ***(3)****: 10-16 cell embryo, n= 11 control, n=9 treated. P=0.085.* ***(4)****>16-cell embryo, n=4 control, n= 4 treated. P=0.017. Effect of treatment on 5hmC . (1) 3-4 cell embryo, n= 4 control, n=9 treated. P=0.056.* ***(2)****: 5-9 cell embryo, n= 10 control, n=7 treated. P=0.081.* ***(3)****: 10-16 cell embryo, n= 9 control, n=7 treated. P=0.310.* ***(4)****>16-cell embryo, n=2 control, n= 1 treated. P=NO.*

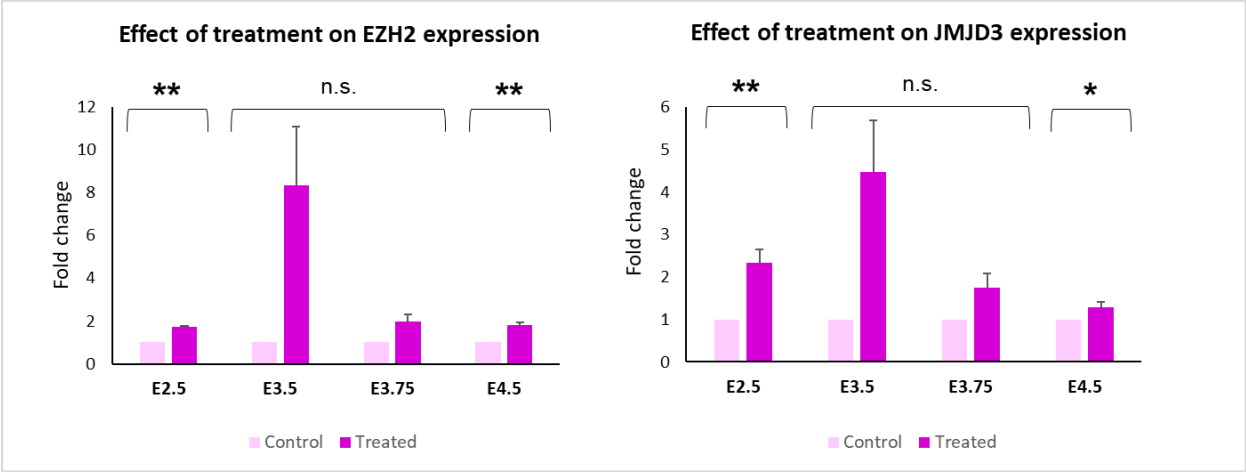

Figure 3: ***The effect of 1 µM GSK126 on EZH2 and JMJD3 expression.*** *qPCR was used to assess mRNA transcripts in 8-cell, mid morula, early blastocyst and late blastocyst stage embryos (E2.5, E3.5, E3.75 and E4.5). N=2 for the 8-cell, mid morula and early blastocyst. N=3 for the late blastocyst.*

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

In the experiment following EZH2 inhibition, the 5mC and 5hmC levels seem to change dynamically. Because the N numbers were low particularly for the larger embryo sizes, these results combined from 3 replicates (>16-cell one replicate) were not as yet conclusive but need to be repeated. A late 5mC decrease and a trend for increased 5hmC, if confirmed, did not appear to be connected with H3K27me3 levels. Because EZH2 and JMJD3 were both upregulated in the GSK126 treatment group in two replicates so far, this indicates that inhibition of EZH2 disrupts normal expression of these genes. Importantly, it may suggest that histone methylation and demethylation of H3K27 are tightly controlled and a feedback mechanism potentially exists in embryos, which was not reported for cells.

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

This experience broadened my horizon and I enjoyed this research project a lot. I became even more enthusiastic about working in the research field and I see myself working here in the future. What was especially helpful is that my interest in embryology and developmental biology was confirmed by this project. After the summer I will continue in a taught masters. I am sure I want to continue my career in research by applying for a PhD afterwards. I am confident this project boosted my chances of getting a PhD position because I improved my hands-on experience in the lab.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*
Student: *The SRF Vacation Scholarship opportunity was very valuable to me, as it allowed me to explore my interest in embryology and developmental biology. It also helped to broaden my view on research and boosted my professional skills and confidence. The research experience I gained will help me to achieve my PhD study aims. I hope you will be able to keep offering these Vacation Scholarships to future students.* ***Supervisor:*** *I thank the SRF for supporting Mabel Vos during her Vacation Scholarship research. Thanks to her project, hypotheses could be tested, which revealed a new clue for further investigation. Mabel was a valued member of the lab who enjoyed doing reproductive biology research, which confirmed her interests in embryology and epigenetics. Mabel's PhD study prospects will greatly benefit from her summer research experience.*