SRF VACATION SCHOLARSHIP REPORT 2017

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Degree Title and year of study:	BSc Biological sciences		3/4
Supervisor's Name:	Dr Adam Watkins	**Supervisor's Department and Institution:**	School of Life and Health Sciences; Aston University
Project Title:	Defining the impact of paternal diet on offspring testicular epigenetic status		

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

The aim of this project was to determine the impact of paternal diet on offspring testicular epigenetic status in a mouse model. In addition, we wanted to establish whether offspring epigenetic status was programmed via a sperm- or seminal fluid-specific mechanism. These studies would help define our understanding of how a poor paternal diet might affect the health of his offspring across multiple generations and the mechanisms underlying any transgenerational programming.

Prior to the start of this project, male C57BL6 mice were fed either a normal protein diet (NPD) or low protein diet (LPD). Using these males, four groups of offspring were generated in which either both the sperm and seminal fluid came from NPD males (termed NN offspring), both came from LPD males (termed LL) or just the sperm (LN) or seminal fluid (NL) came from LPD fed males (in each case the first and second letter denotes the dietary background of the sperm and seminal fluid respectively).

We isolated RNA (RNeasy; Qiagen) from adult male offspring testes for the expression analysis of central regulators of DNA methylation (*Dnmt1, 3a, 3b, 3L)*, histone modifications (*Hdac1, Hdac2, Kdm3a)* and RNA methylation (*Alkbh5, Fto, Mettl3, Mettl14, Wtap, Ythdf1, Ythdc1*) by RT-qPCR.

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

Overall, the experiment remained the same as proposed within the original application. However, due to shortage of time, we were unable to determine telomere length within our samples. Apart from this, the experiment went as planned.

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

We observed that paternal low protein diet modified offspring expression of central epigenetic regulators in a sperm or seminal fluid specific manner. Interestingly, we observed that the most significant changes in expression levels were observed when the dietary origin of the sperm and seminal fluid did not match (NL and LN groups).

Analysis of the relative testicular expression levels of DNA methyltransferases in F1 offspring revealed NL and LN offspring displayed significantly reduced expression of *Dnmt3a, 3b* (figure 1) and *3L* when

compared to NN and LL offspring (P< 0.05). *Dnmt3a, 3b* and *3L* are all involved in de-novo DNA demethylation and genomic imprinting, especially in germ cells and during development.

We also observed significant changes in the expression profiles of the histone modifiers *Hdac2* and *Kdm3a* between groups. Here, while the expression of *Hdac2* was reduced in NL and LN groups, the expression of *Kdm3a* (figure 1) was increased. The deacetylation of histones by *Hdac2* results in the repression of gene expression while histone demethylation by *Kdm3a* results in gene activation. *Kdm3a* is specifically involved in the packaging and condensation of sperm DNA during spermatogenesis.

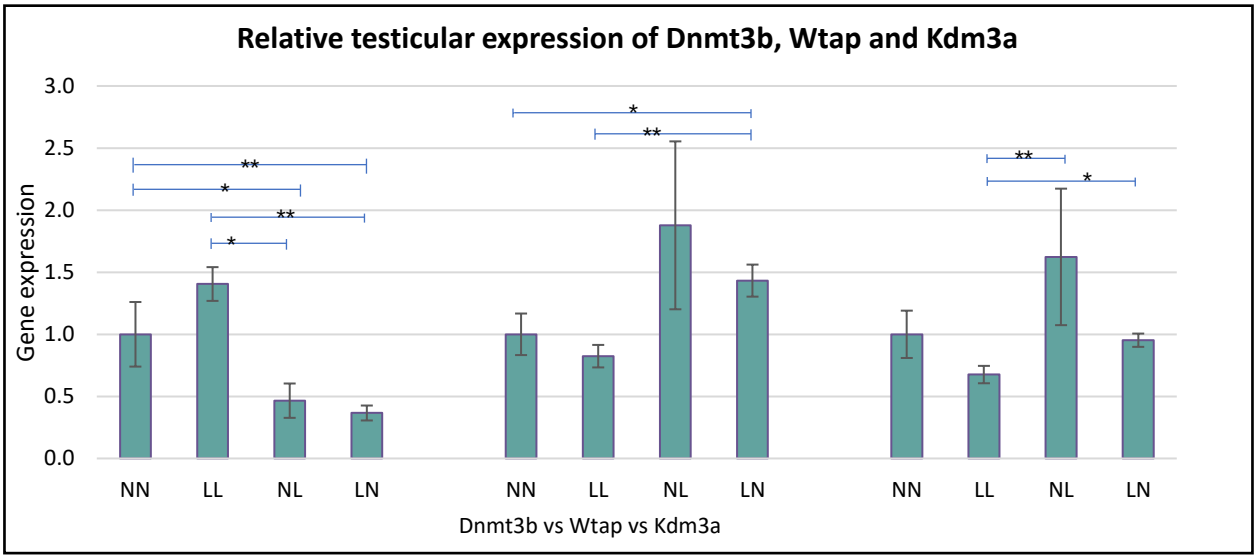

Figure 1: This graph displays the alteration of gene expression between Dnmt3b, Wtap and Kdm3a; based on transgenerational paternal inheritance via sperm and seminal fluid, to the F1 progeny. N = 8 males per treatment group. * P<0.05; **P<0.01.

Finally, we analysed the testicular expression of several genes involved in the methylation of RNA. Here, we observed reduced expression of *Fto, Mettl13,* and *Ythdf1* in NL and LN offspring when compared to NN and LN offspring. In contrast, the expression of *Mettl14* and *Wtap* (figure 1) were increased in LN and NL offspring These findings suggest differential expression profile of central RNA methyltransferases and demethylases on offspring testes in relation of paternal diet which may impact on factors such as RNA translation, stability and splice variants.

Together, these data show that poor paternal diet affect offspring epigenetic status via a sperm and seminal fluid specific mechanism providing a mechanism to affect offspring health across multiple generations.

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

These results show that paternal diet does impact on expression profiles of multiple epigenetic regulators within the testis of F1 adult offspring males. These results also indicate that multiple epigenetic mechanisms including DNA/RNA methylation and histone modifications are involved. These results indicate that poor paternal diet may influence offspring development across multiple generations and provide novel insight into the underlying mechanisms.

Interestingly, we observed the biggest differences in our NL and LN groups, where the sperm and seminal fluid were from different dietary backgrounds. These observations suggest a novel paternal semen 'mismatch hypothesis'. We propose that a mismatch between the genomic programming of the

embryo (via the sperm) and the programming of the uterine environment (via the seminal fluid) has the largest impact on offspring health, greater than if both the sperm and seminal fluid are from a poor nutritional origin.

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

The project was a great opportunity to contemplate whether I would like to go into research. I specifically learnt a lot about techniques in molecular genetics relating to reproductive biology. Undertaking a research project within this field has sparked an interest for me to further study in this field. Working towards a target within a timeframe, analyzing data and doing hands on laboratory work has given me a new insight into what is involved in biomedical research. This has re-affirmed my wish to pursue this type of research as a career and has helped me decide that I will definitely want to continue to study further. While I have yet to decide which field of biology I would like to specialise in, this project has meant that reproductive biology is an option I would consider seriously.

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

Student: The SRF scholarship has been a wonderful opportunity to expand my knowledge and understanding on a specific topic in reproductive biology. In addition, it has allowed me gain valuable insight into how current research is carried out, what is required in the preparation for a project, how it is carried out and how the data are collected and analysed. It has been a great way to gain valuable practical experience and learn about the industry as whole, helping me to confirm whether I wish to pursue a career in research or not.

Supervisor: The SRF vacation scholarships are a fantastic opportunity for students to gain additional insight into how academic research is conducted, gain essential laboratory skills and strengthen decisions about future career pathways. Sofia conducted an ambitious project within my laboratory that has helped to shed new light into the mechanisms linking paternal diet with offspring health. These studies would not have been possible without Sofia's hard work and a generous award from the SRF