SRF VACATION SCHOLARSHIP REPORT 2016

Student's Name:	Adam Andreani	**Student's Institution/University:**	University of Cambridge
Degree Title and year of study:	BA and MB BChir (year 2)		
Supervisor's Name:	Dr Amanda Sferruzzi-Perri	**Supervisor's Department and Institution:**	Department of Physiology, Development and Neuroscience, University of Cambridge
Project Title:	Understanding the molecular mechanisms by which the placenta adapts to support fetal growth during pregnancy		

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

Functional and morphological changes in the murine placenta have been reported following undernutrition, hypoxia, and consumption of a high sugar and fat diet[1–3]. Placental adaptations have an effect on the absolute and relative amounts of metabolic substrates transferred to the fetus, which is important for the developmental programming of fetal organs systems, with implications for the risk of developing diseases later in life[4].

Several differentially expressed genes have been identified in genetically modified mouse placentas that failed to adapt during pregnancy (unpublished data from supervisor). However, the specific roles of these genes in the placenta have not been identified. The aims of this project were 1) to assess the expression of these genes in the placental exchange labyrinthine (Lz) and endocrine junctional zones (Jz) in relation to morphology, and 2) to determine whether their expression altered in response to maternal environmental manipulation. The mouse placenta is used as a model for investigating human placental function as both show significant similarities in morphology and genetic regulation. Furthermore, data on the effects of maternal environmental manipulations on murine placental structure and function already exists[1–3] and provide the means for investigating the molecular mechanisms underlying placental adaptations in this project.

References

1. Sferruzzi-Perri, A. N. *et al.* Placental-specific Igf2 deficiency alters developmental adaptations to undernutrition in mice. *Endocrinology* **152,** 3202–3212 (2011).
2. Higgins, J. S., Vaughan, O. R., Fernandez de Liger, E., Fowden, A. L. & Sferruzzi-Perri, A. N. Placental phenotype and resource allocation to fetal growth are modified by the timing and degree of hypoxia during mouse pregnancy. *J. Physiol.* **00,** n/a–n/a (2015).
3. Sferruzzi-Perri, A. N. *et al.* An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB J.* **27,** 3928–3937 (2013).
4. Sferruzzi-Perri, A. N. & Camm, E. J. The programming power of the placenta. *Frontiers in Physiology* **7,** (2016).

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

Immunohistochemistry and in situ hybridization assessments were not carried out to localize gene expression due limitations in sourcing antibodies and in situ probes. However, we were instead able to stain a subset of the samples collected during the project with haematoxylin and eosin to assess changes in placental morphology during gestation and correlate such changes with our gene expression data.

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

Nov and Dio2 were more abundantly expressed by the Lz relative to the junctional zone Jz at all ages. Sult1E1, Selenbp1, Mgst1, Cdx2 and Cited2 were also more highly expressed by the Lz compared to the Jz, at specific gestational ages. Only the expression of Dio3 was greater in the Jz relative to the Lz and this was observed at all three ages. In the Lz, Cdx2 and Dio3 increased and Mgst1, Sult1e1 and Cited2 decreased with age. In the Jz, Selenbp1 and Dio3 increased, Cited2, Sult1e1 and and Dio2 decreased with age. There was a peak in Nov expression in the Jz at D16, relative to day 13 of gestation (D13) and day 19 of gestation (D19), with no effect of gestational age in the Lz.

UN increased expression of Dio3 in the placenta on D16, with levels returning to control levels by D19. UN did not affect the expression of other genes analysed at D16 or D19. On D16, Nov, Mgst1, and Dio3 were increased by a HSHF diet relative to the control group, although there was no longer an effect of diet seen on D19. The HSHF diet did not affect the expression of other genes analysed at D16 or D19. On D19, expression of Sult1E1, Cited2, and Dio3 was increased in response to 10% hypoxia, relative to the normoxic control group, although only Dio3 showed a significant difference relative to the respective pair-fed normoxia control. On the other hand, Selenbp1 expression was decreased in the pair-fed group relative to the normoxic group, and increased in the 10% hypoxic relative to the pair-fed group, although there was no statistically significant difference between the hypoxic group and the normoxic control group. There was no effect of 13% hypoxia on the expression of any genes analysed at D19.

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

The qualitative changes in the dimensions and density of the different placental zones with gestational age are consistent with previous work. Zone-specific changes in gene expression with increasing gestational age show that certain genes, such as Cited2, Sult1E1, and Dio3, show similar trends in each zone between D13 and D19, which may correlate a common function and temporal significance. Others show zone-specific changes in expression, which suggest either that the genes have differing functions in the Lz or Jz, or that changes in gene expression correlate to an increasing divergence in the functional significance of these genes in these regions. This work also suggests that expression of several genes assessed in the placenta were altered with maternal undernutrition, hypoxia and/or a diet high in sugar and fat and may play a role in adapting placental phenotype.

A full description of the project and its results can be found in the full project report, which could not be included in this report form due to space limitations.

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

This project represents my first experience in conducting research and therefore has provided me with my first opportunity to form an informed opinion of my future career path. I have found the project illuminating, in terms of its interest, as well as, in identifying my strengths and weaknesses and my likes and dislikes of research. Following these 8 weeks, I am still very interested in the possibility of a career in pure research or combining research with clinical practice. In the shorter term, it has increased my enthusiasm for my third year research project and improved my skills and confidence in the laboratory, which will be invaluable in making the most of this next project. Furthermore, the wealth of experience from these projects will be very useful when deciding whether to apply for the MB PhD program offered by the university and pursue a career involving research.

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

Student: The generous scholarship funding enabled me to fully pursue the aims of the project without worrying about the financial cost to my supervisor's lab.

Supervisor: Adam has generated novel data on the developmental and environmental modulation of newly identified genes in the mouse placenta. Data generated will contribute to paper/s being prepared for publication.

Appendix

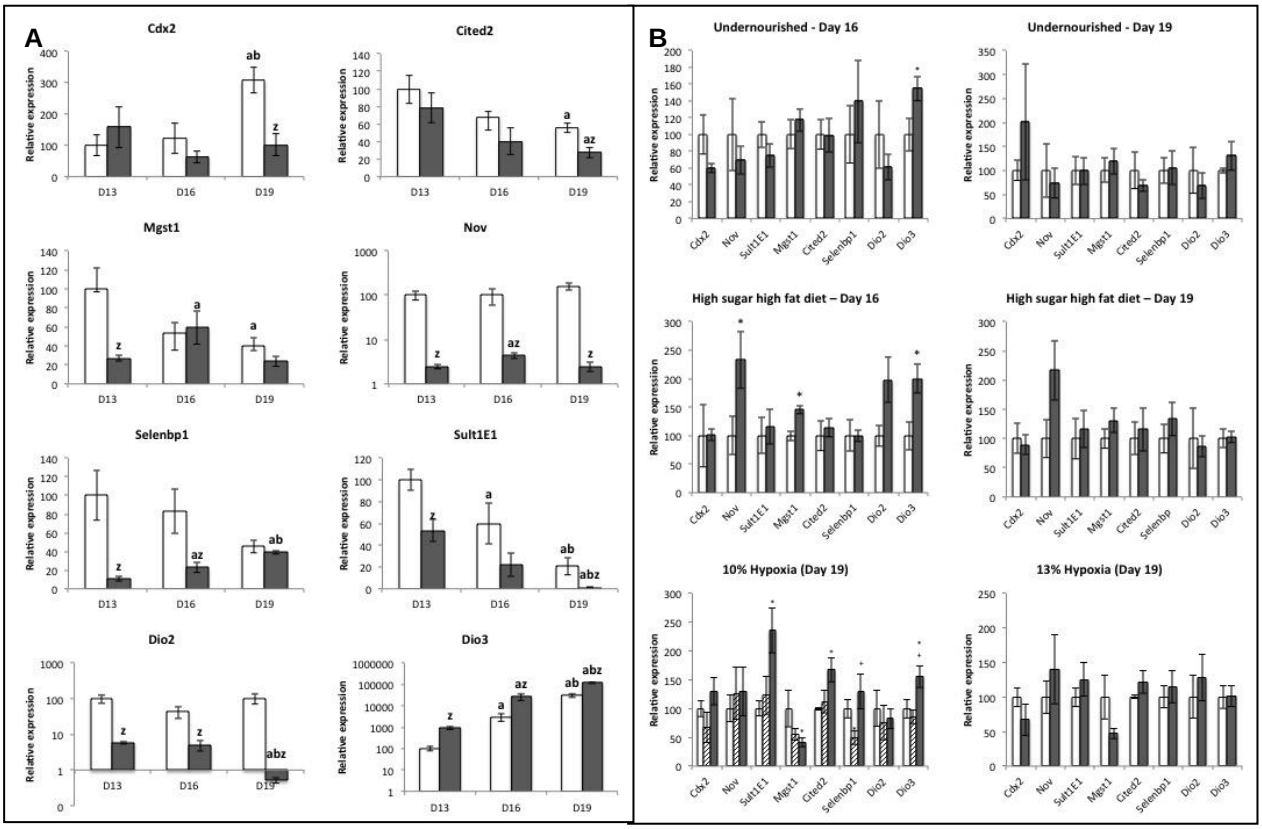

Figure 1. Temporal and environment-dependent changes in gene expression in the Lz and Jz. A - The mean ± SEM expression of each gene at D13, D16, and D19 was normalised to the expression of Gapdh in the same sample and shown relative to the expression of the same gene in the Lz at D13. Differences in expression relative to the Lz at D13 (z), relative to the same zone at D13 (a), and relative to the same zone at D16 (b) were assessed by student t-tests ($P < 0.05$). White bars – Lz; grey bars – Jz. **B** - The mean ± SEM expression of each gene were normalised to the expression of Hprt in the same sample and shown relative to the control group on the same day. Differences in expression relative to same age controls (*) and relative to same age pair-fed normoxic controls (+) were assessed by student t-tests ($P < 0.05$). White bars – controls; hashed bars – normoxia pair-fed relative to 10% hypoxia group intake; grey – environmental manipulation.