SRF VACATION SCHOLARSHIP REPORT 2016

Student's Name:	Laura Warwick	**Student's Institution/University:**	University of Leeds
Degree Title and year of study:	BSc Genetics		Second Year
Supervisor's Name:	Professor Helen Picton	**Supervisor's Department and Institution:**	Division of Reproduction and Early Development, University of Leeds
Project Title:	Intracellular Oxygen Profiling of Bovine Oocytes and Embryos using the MM2 Probe		

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

Oxygen consumption rate (OCR) can be used as a direct indication of metabolic activity (Lopes et al, 2005), as it reflects levels of aerobic respiration within mitochondria. OCR increases during embryo development to meet the increasing energy demand by the growing embryo (Trimarchi et al, 2000) and may identify embryo quality throughout development. During oocyte maturation and embryogenesis the quantity of mtDNA increases and higher numbers of mitochondria within an oocyte predict a fertile gamete that is fully able to support embryogenesis (Cotterill et al, 2013). Therefore a high OCR may indicate a higher quality egg/embryo.

The MM2 probe is a FRET donor, quenched in the presence of oxygen (Kondrashina et al, 2012) and therefore a low fluorescent output in the oxygen-sensitive channel indicates a high OCR. We used ratiometric measurements to represent this by dividing oxygen-sensitive by reference channel emission. Our aim was to validate the effectiveness of the MM2 probe within oocytes and embryos using Fluorescent Lifetime Imaging Microscopy (FLIM) and use this to measure oxygen consumption. Additionally we aimed to differentiate metabolism within the Trophectoderm (TE) and Inner Cell Mass (ICM) of blastocysts, as these are believed to have different metabolic phenotypes (Houghton, 2006).

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

The focus of the project was as expected. However as the project aimed to develop and validate a new method of measuring OCR in eggs and embryos, protocol changes were expected from the offset. Different types of microscopy were tested. FLIM could not be used to image the MM2 probe as the detectors needed were not available. An upright multiphoton microscope proved successful but presented challenges when imaging live tissue before and after interventions.

Also, TE and ICM metabolism were accurately quantified according to cell counts after staining with Hoechst 3342 and Propidium iodide, rather than by eye (Thouas, 2001).

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

Assisted reproduction technologies were use to mature bovine oocytes to metaphase II (MII) and to generate blastocysts *in vitro*. A total of 14 blastocysts and 7 MII oocytes were used for oxygen profiling. The MM2 probe can be successfully used to quantify OCR in bovine embryos and probe uptake was visualized throughout the embryos to the inner most cells (Figure 1A and B). Multiphoton microscopy was found to be most effective method of probe detection using a laser power of 10-15%. We also found that the MM2 probe had no effect on embryo viability even after incubating embryos overnight. We were able to confirm that the MM2 probe was sensitive to oxygen by controlling and manipulating the oxygen levels and measuring the resulting fluorescence. The data points generated were used to create a calibration curve. A fixative solution consisting of 10mM sodium dithionite, 2% formaldehyde and 2% glutaraldehyde was used to deplete oxygen to 0%, whilst fixing in ethanol gave an ambient control of 21% oxygen. As expected the embryos in 0% demonstrated the brightest fluorescence as there was no

oxygen present to quench whereas the embryos exposed to 21% had lower fluorescence.

Whilst blastocysts worked well with the MM2 probe and gave consistent results, OCR measurements in oocytes were inconsistent (Figure 1C). This was due to the auto-fluorescence of oocytes which made it difficult to visualize the MM2 probe; the latter was possibly due to the presence of lipids and in future research this could perhaps be overcome by bleaching the oocyte.

After performing cell counts on the blastocysts we found a weak correlation between the TE:ICM ratio with the basal oxygen consumption rate suggesting that the higher the TE:ICM ratio the lower the basal oxygen level seen in that embryo (Figure 1G).

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

This pilot data shows that the MM2 probe can be used to quantify blastocyst oxygen consumption rate. However its use in oocytes presents more problems and needs to be investigated and validated further to try and find a more consistent method for its uptake and use. In further studies it will be possible for the probe to be used to determine the localization of intracellular metabolism as well as levels of mitochondrial and non-mitochondrial metabolism. Correlating basal oxygen levels to TE:ICM ratios suggested that blastocysts with more TE tended to have higher oxygen consumption, though further replicates are required to confirm this.

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

This project has given me a completely different opinion of research. Prior to starting I never thought that I would enjoy research and had not considered it as a career path. After thoroughly enjoying this project and the work I did during it, I am now considering research as one of my options-particularly completing a PhD. This experience has been invaluable and has taught me the importance of research, but also that it is fun. It has given me a wider academic knowledge as well as a range of skills from the laboratory that I can carry on in the future. Overall it has confirmed that a career in reproductive science is for me.

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

Student: It is an amazing opportunity that I hope students continue to apply to, it means that students can complete projects and gain experience in areas that they may otherwise struggle to find. I would recommend it to anyone considering a career in embryology or research.

Supervisor: This SRF scholarship was a great success. Our scholar was highly motivated to learn and rapidly integrated into our research team. She received training in a range of assisted reproduction technologies and bright field and fluorescent microscopy. She significantly extended her understanding of reproductive biology and made a valuable contribution to our research.

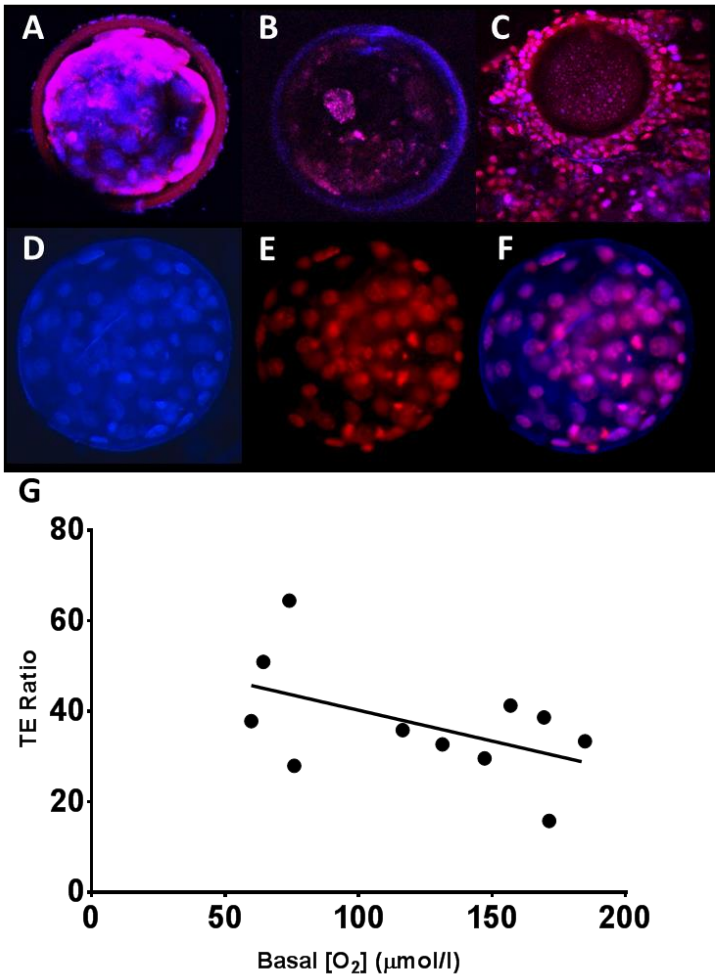

Figure 1. Demonstrates the main findings and aspects of the project. **A+B)** MM2 stained blastocysts imaged using the multiphoton microscope, the reference fluorescence emitted by the probe is that seen in blue, oxygen-sensitive fluorescence seen in red. **C)** A cumulus oocyte complex stained with MM2 **D)** Hoescht stain- labeling all cells present within the blastocyst for total cell count. **E)** Propidium iodide showing cells of the TE only **F)** Combined cell count image **G)** Suggests a trend that higher TE ratios have lower oxygen concentration implying higher consumption rate (n=11 blastocysts)

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