



The impact of *in vitro* stress on pre-implantation embryo development, viability and mitochondrial homeostasis.

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A thesis submitted to the University of Adelaide in total fulfilment of the requirements for the degree of Doctor of Philosophy.

January 2010

Contents

1	Literature review	23
1.1	Introduction	25
1.2	Oocyte maturation, fertilisation and pre-implantation embryo development	26
1.2.1	Oocyte maturation and ovulation	26
1.2.2	Fertilisation	27
1.2.3	Embryo development	28
1.3	The <i>in vivo</i> embryo environment	32
1.4	Metabolism of the pre-implantation embryo	35
1.5	Energy sources	35
1.5.1	Pyruvate and lactate	35
1.5.2	Glucose	37
1.5.3	Oxygen consumption	37
1.5.4	Amino acids	37
1.6	pH	38
1.7	Embryo culture	40
1.7.1	Amino acids	44
1.7.2	Ammonia and ammonium	46
1.7.3	Ammonium in culture	47
1.7.4	Ammonia/ammonium and urea in livestock	50
1.8	Mitochondria	51
1.8.1	Origin	51
1.8.2	Structure and purpose	52
1.8.3	Mitochondrial involvement in disease	55
1.9	Mitochondria in the oocyte and embryo	56
1.9.1	Mitochondrial numbers	56
1.9.2	Mitochondrial structure and function	59
1.9.3	Mitochondria and Ca ²⁺ homeostasis	60
1.9.4	Mitochondrial distribution, $\Delta\Psi_m$ and stress response	61
1.10	Epigenetics and DNA methylation	63
1.10.1	Imprinting	64
1.10.2	Methylation in fertilisation and embryo development	64
1.10.3	Epigenetics, the Environment and Embryo Culture	65
1.11	Conclusions and Hypotheses	68
1.12	Specific Aims	71
2	Materials and Methods	73
2.1	Media preparation	75
2.1.1	Preparation of glassware	75
2.1.2	Embryo culture and handling media	75
2.1.3	Media preparation	75
2.1.4	Preparation of hyaluronidase	76
2.2	Mouse embryo bioassay	76
2.3	Animals and ovulation induction	79
2.3.1	Animals	79
2.3.2	Induction of ovulation	79

2.3.3	Collection of pre-implantation embryos	80
2.4	Surgical procedures	81
2.4.1	Vasectomy	81
2.4.2	Embryo transfers	81
2.4.3	Isolation of fetuses and placenta	82
2.5	Embryo culture	82
2.5.1	Manipulation of embryos	82
2.5.2	Preparation of culture dishes	83
2.5.3	Embryo culture	83
2.6	Assessment of embryo development <i>in vitro</i>	84
2.6.1	Assessment of embryo morphology	84
2.7	Selection of ammonium and DMO concentrations	95
2.8	Assessment of cell parameters	96
2.8.1	Simple cell number staining	96
2.8.2	Differential cell number staining	96
2.8.3	Apoptosis level analysis	101
2.9	Glucose Uptake by blastocyst	105
2.10	Assessment of mitochondrial homeostasis	105
2.10.1	Determination of mitochondrial distribution	105
2.10.2	Determination of mitochondrial calcium levels	107
2.10.3	Determination of mitochondrial membrane potential ($\Delta\psi_m$)	108
2.10.4	Assessment of reactive oxygen species levels	108
2.11	Assessment of metabolic parameters	109
2.11.1	Assessment of malate aspartate shuttle activity	109
2.12	Assessment of ATP and ADP levels	112
2.13	Immunohistochemistry	114
2.13.1	5-Methylcytosine antibody staining	114
2.14	Measuring intracellular pH	115
2.15	PCR	116
2.15.1	Extraction of cDNA from blastocysts	116
2.15.2	Extraction of cDNA from placental tissue	117
2.15.3	Real-time reverse transcription PCR	118
2.16	Placental transport of Methyl-D-Glucose, 3-0-[Methyl- ¹⁴ C)	123
2.16.1	Injection of radio-labelled substrate	123
2.16.2	Scintillation counting	123
3	Impact of ammonium exposure on blastocyst viability, glucose uptake and fetal and placental growth	125
3.1	Introduction	127
3.2	Experimental design	128
3.2.1	Culture conditions	128
3.3	Statistics	130
3.4	Results	131
3.4.1	Effect of ammonium on morula cell number	131
3.4.2	Effect of ammonium on glucose uptake	133
3.4.3	Effect of ammonium exposure on blastocyst viability after transfer to pseudopregnant recipients	137
3.5	Discussion	141
4	The effect of temporal intracellular pH decrease on blastocyst viability and fetal and placental outcomes	145
4.1	Introduction	147
4.2	Experimental design	149
4.2.1	Culture conditions	149
4.3	Statistics	151

4.4	Results	152
4.4.1	Measurement of intracellular pH	152
4.4.2	Effect of DMO exposure on embryo development.....	153
4.4.3	The Effect of DMO on morula cell number	158
4.4.4	Effect of DMO on blastocyst cell allocation	160
4.4.5	Effect of DMO on blastocyst apoptosis.....	161
4.4.6	Effect of DMO on blastocyst viability.....	162
4.5	Discussion	165
5	The effect of ammonium and DMO exposure, during the first cleavage division, on mitochondrial and metabolic parameters	171
5.1	Introduction	173
5.2	Experimental design.....	174
5.2.1	Culture conditions.....	174
5.3	Statistics	175
5.4	Results	176
5.4.1	The effect of ammonium and DMO on mitochondrial distribution.....	176
5.4.2	The effect of ammonium and DMO on mitochondrial calcium levels	178
5.4.3	The effect of ammonium and DMO on mitochondrial membrane potential	180
5.4.4	The effect of ammonium and DMO on reactive oxygen species level.....	182
5.4.5	The effect of ammonium and DMO exposure on early cell division	183
5.4.6	The effect of ammonium and DMO on lactate uptake and malate-aspartate shuttle activity.....	184
5.4.7	The effect of ammonium and DMO on ADP and ATP levels and ratio.....	185
5.4.8	185
5.5	Discussion	189
6	The assessment of permanent alterations on mitochondrial homeostasis and energy production after exposure to ammonium or DMO	193
6.1	Introduction	195
6.2	Experimental design.....	195
6.2.1	Culture conditions.....	195
6.3	Statistics	196
6.4	Results	197
6.4.1	The effect of ammonium and DMO exposure on mitochondrial membrane potential.....	197
6.4.2	The effect of ammonium and DMO exposure on reactive oxygen species production at the 8-cell stage	198
6.4.3	The effect of ammonium and DMO exposure on ADP and ATP levels and ratio at the 8-cell stage	199
6.5	Discussion	203
7	The effect of ammonium or DMO exposure on DNA methylation status.....	205
7.1	Introduction	207
7.2	Experimental design.....	208
7.3	Statistics	209
7.4	Results	210
7.4.1	Effect of ammonium or DMO exposure on global DNA methylation at the 2-cell stage, after 21 hours of culture.....	210
7.4.2	Effect of ammonium or DMO exposure, on global DNA methylation at the 2-cell stage, after 16h, 19h, 22h and 25h of culture.....	211
7.4.3	Effect of ammonium and DMO exposure, during the first cleavage division, on global DNA methylation after 67h culture.	213
7.4.4	Effect of ammonium or DMO exposure, during the first cleavage division, on Dnmt Family Gene Expression at the blastocyst stage.....	215

7.5	Discussion	216
8	The effect of ammonium exposure, during embryo culture, on placental gene expression and function	219
8.1	Introduction	221
8.2	Experimental design	222
8.3	Statistics	223
8.4	Results	224
8.4.1	The effect of ammonium exposure on Day 15 placental gene expression	224
8.4.2	The effect of ammonium exposure on placental transport	229
8.5	Discussion	231
9	Concluding remarks	235
9.1	Introduction	237
9.2	Thesis discussion	238
9.3	Conclusion	245
10	Appendix	249
10.1	0.9% Saline preparation	249
10.2	1.2% Avertin preparation	249
10.2.1	Stock solution (100x)	249
10.2.2	Working solution	249
10.3	RNase buffer preparation	249
10.4	PI/RNase A mix preparation	249
10.5	Ca ²⁺ calibration solutions	250
10.5.1	Solution A (Ca ²⁺ free solution)	250
10.5.2	Solution B (Ca ²⁺ saturation solution)	250
10.6	Metabolic assays	250
10.6.1	Glycine-hydrazine buffer	250
10.6.2	NAD ⁺	250
10.6.3	Lactate standard	250
10.6.4	Lactate cocktail	251
10.6.5	ATP and ADP standard	251
10.6.6	Epps buffer	251
10.6.7	ADP Cocktail	251
10.6.8	ATP Cocktail	252
10.7	Mounting media for immunohistochemistry	252
10.8	pH measurement	252
10.8.1	Valinomycin stock solution (x2000)	252
10.8.2	Valinomycin working solution	252
10.8.3	Nigericin stock solution (x1000)	252
10.8.4	Nigericin working solution	253
10.8.5	Decrease in pH _i with increasing concentrations of DMO	253
11	Complete Bibliography	255

Figures and tables

Figure 1-1: Diagram depicting cellular divisions of a mouse embryo from fertilisation on Day 1 to the Blastocyst stage on Day 5. Zygote (Day 1): fertilised egg, the second meiotic division is complete leading to the formation of the second polar body and the male and female pro-nuclei. 2-cell (Day 2): initial cellular division has occurred and genome activation occurs in the mouse. 4-cell (Day 2-late): cleavage to 4-cells and genome activation begins to occur in the human. 8-cell (Day 3): cleavage to 8-cells. Compacted moruls (Day 3-late): after the 8-cell stage the embryo undergoes compaction, cells polarise and flatten maximising cell contacts. Early Blastocyst (Day 4-late): Fluid is secreted internally to form a blastocoelic cavity and cell differentiation occurs. Expanded Blastocyst (Day 5): the blastocoelic cavity is enlarged and cell differentiation has occurred giving rise to two cell types; trophoctoderm (TE) cells surrounding the exterior and an eccentrically located inner cell mass (ICM) cells. The TE gives rise to placenta tissue and the ICM gives rise to fetal tissue.	31
Figure 1-2: Schematic diagram depicting the changes in metabolic substrate requirements and methylation during pre-implantation embryo development.	34
Figure 1-3: A three-dimensional diagram of a mitochondrion cut longitudinally. The F_0F_1 complexes (small red spheres), which synthesise ATP, are intramembrane particles that protrude from the inner membrane into the matrix. The matrix contains the mitochondrial DNA (blue strand), ribosomes (small blue spheres), and granules (large yellow spheres). (Figure from (Lodish 2000)).....	54
Figure 1-4: Diagram of the electron transport chain used to synthesise ATP. Figure adapted from (Naviaux and McGowan 2000).	55
Figure 1-5: Diagrammatic representation of the changes in the number of mitochondria during development of the female germ line and the bottleneck concept. The estimate of the number of mitochondria per cell is indicated in the flowchart on the right. (Figure adapted from (Shoubridge and Wai 2007)).	58
Figure 1-6: Redox and energetic metabolism in the mammalian embryo. Schematic representation of the metabolic pathways producing NADH, NADPH and GSH functioning in the cytosol and mitochondria. Figure obtained from (Dumollard <i>et al.</i> 2007b).	60
Figure 1-7: DNA methylation during embryo development. The pink line depicts maternal DNA and the blue line depicts paternal DNA.	65
Figure 2-1: Mouse zygote at 24 hours post hCG (Day 1) at 20x objective.....	85
Figure 2-2: Mouse zygote at 24 hours post hCG (Day 1): a) 10x objective; b) 40x objective	85
Figure 2-3: Mouse 2-cell after 19 hours culture (Day 2): a) 10x objectives; b) 40x objective	87
Figure 2-4: Mouse 4-cell and 8-cell embryos after 43 hours culture (Day 3): a) at 10x objective; b) at 20x objective	87
Figure 2-5: Murine 4-cell embryo: a) and 8-cell embryo b) at 40x objective (Day 3).....	89
Figure 2-6: Murine morula embryos at 72 hours culture (Day 4): a) 20x objective; b) 40x objective .	89

Figure 2-7: Murine early blastocysts after 72 hours culture (Day 4): a) at 20x objective; b) 40x objective	91
Figure 2-8: Expanded and hatching mouse blastocysts after 91 hours of culture (Day 5) at 20x objective	91
Figure 2-9: Expanded and hatching mouse blastocysts after 91 hours of culture (Day 5) at 40x objective	93
Figure 2-10: Simple cell number stained morula stage embryo. Each cell nuclei is stained orange....	99
Figure 2-11: Differentially stained murine blastocyst. Pink staining indicates nuclei of TE cells and blue staining indicates nuclei of ICM cells.....	99
Figure 2-12: Images of blastocysts stained using TUNEL technique. a) TUNEL positive control; b) PI stain of positive control; c) merged positive control; d) TUNEL negative control; e) PI stain of negative control; f) merged negative control; g) TUNEL stain of ammonium treated blastocyst; h) PI stain of ammonium treated blastocyst; i) merged images of ammonium treated blastocyst	103
Figure 2-13: Schematic diagram of a mouse 2-cell embryo indicating the position of the regions measured. The black squares indicate the position of up to 24 pixel boxes which were drawn on each image using a transparent template overlay (4 in the peri-nuclear, 4 in the cytoplasm and 4 at the exterior of each cell) where the pixel intensity was measured.	106
Figure 2-14: Standard curve for lactate as well as reaction equation. Levels of lactate in a sample can be assessed by a linear increase in fluorescence with a linear increase in lactate concentration.....	111
Figure 2-15: Malate-aspartate reducing equivalent shuttle. Schematic of reactions involved in the malate-aspartate shuttle, which transfers an electron across the inner mitochondrial membrane resulting in the net transfer of NADH from the cytoplasm into the mitochondria. The shuttle consists of reactions catalysed by four enzymes: cAspAT, mAspAT, cMDH, and mMDH. (Figure obtained from (Lane and Gardner 2005a).....	111
Figure 2-16: Standard curve for ATP and ADP as well as reaction equation for measuring 1. ATP levels 2. ADP levels. Levels of ATP in a sample can be assessed by a linear increase in fluorescence with a linear increase in ATP concentration. Levels of ADP in a sample can be assessed by a linear decrease in fluorescence with a linear decrease in ADP concentration.....	113
Figure 3-1: Temporal exposure of embryos to 300µM ammonium (NH ₄ ⁺).	129
Figure 3-2: The effect of temporal ammonium exposure on morula/early blastocyst cell number after 67 hours of culture.....	132
Figure 3-3: The effect of temporal ammonium exposure on glucose uptake in the resultant blastocysts.	133
Figure 3-4: Effect of culture with ammonium on glucose distribution in cultured blastocysts.....	135
Figure 4-1 : Temporal exposure of embryos to 2mM DMO.	150
Figure 4-2: Effect of incubation with 2mM DMO on intracellular pH.	152
Figure 4-3: Murine blastocysts after 91 hours of culture (Day 5) at 40x objective.....	155
Figure 4-4: The effect of temporal DMO exposure on cell number after 67 hours of culture.....	159
Figure 4-5: Percentage of apoptosis in blastocysts after varying stages of DMO exposure.....	161

Figure 5-1: Experimental design for 2-cell stress exposure.....	174
Figure 5-2: Schematic diagram indicating the position and name of the three different regions measured: nuclear, cytoplasmic, cortical, overlaid on an image of a mouse 2-cell embryo stained with JC-1. The reddish staining seen around the cortical region is indicative of higher mitochondrial membrane potential than in intermediate and nuclear region.	175
Figure 5-3: Representative images of 2-cell embryos after staining with Mitotracker Green	176
Figure 5-4: The effect of incubation from the zygote to the 2-cell stage, with either ammonium or DMO, on mitochondrial distribution.....	177
Figure 5-5: Representative images of 2-cell embryos after staining with Rhod-2-AM a) control b) ammonium c) DMO	178
Figure 5-6: The effect of incubation with ammonium or DMO from the zygote to the 2-cell stage on mitochondrial calcium levels	179
Figure 5-7: Representative images of 2-cell embryos after staining with JC-1 a) green channel b) red channel c) merged image d) control merged image e) ammonium merged image f) DMO merged image.....	180
Figure 5-8: The effect of incubation with ammonium or DMO from the zygote- 2-cell on mitochondrial membrane potential.	181
Figure 5-9: The effect of incubation with ammonium or DMO from the zygote- 2-cell on intracellular reactive oxygen species levels.	182
Figure 5-10: The effect of incubation with ammonium or DMO on division of embryos from the 2-cell to the 4-cell over time.....	183
Figure 5-11: The effect of incubation with ammonium or DMO from the zygote- 2-cell on lactate uptake.	184
Figure 5-12: The effect of incubation with ammonium or DMO from the zygote- 2-cell on ADP levels	185
Figure 5-13: The effect of incubation with ammonium or DMO from the zygote- 2-cell on ATP levels	186
Figure 5-14: The effect of incubation with ammonium or DMO from the zygote- 2-cell on ATP:ADP ratio	187
Figure 6-1: Experimental design for reversibility assessment. Assessments on cellular parameters were conducted at the 8-cell stage after control conditions; exposure to the stress throughout development; or exposure during the first cleavage division followed by control conditions for the next 24 hours to the 8-cell stage.....	196
Figure 6-2: Effect of ammonium and DMO exposure for either 19 hours or 43 hours on whole embryo mitochondrial membrane potential at the 8-cell stage.	197
Figure 6-3: Effect of ammonium and DMO exposure for either 19 hours or 43 hours on intracellular reactive oxygen species levels at the 8-cell stage.	198

Figure 6-4: Effect of ammonium and DMO exposure for 19 hours on ADP levels at the 8-cell stage.	199
Figure 6-5: Effect of ammonium and DMO exposure for 19 hours on ATP levels at the 8-cell stage.	200
Figure 6-6: Effect of ammonium and DMO exposure for 19 hours on ADP:ATP ratio at the 8-cell stage.	201
Figure 7-1: The effect of ammonium or DMO exposure after 21 hours culture on relative global DNA methylation	210
Figure 7-2: The effect of ammonium or DMO exposure on 2-cell global methylation over time.	212
Figure 7-3: The effect of ammonium or DMO exposure, during the first cleavage division, on relative global DNA methylation in morula/early blastocyst stage embryos after 67h culture.	214
Figure 7-4: The effect of ammonium or DMO exposure, during the first cleavage division, on <i>Dnmt</i> family gene expression at the blastocyst stage.	215
Figure 8-1: The effect of 300µM ammonium exposure at varying stages of pre-implantation embryo development on day 15 placental gene expression.	226
Figure 8-2: The effect of 300µM ammonium exposure at varying stages of pre-implantation embryo development on Day 15 placental gene expression. N=4 placentas extracted	226
Figure 8-3: The effect of 300µM ammonium exposure at varying stages of pre-implantation embryo development on Day 15 placental gene expression. N=4 placentas extracted	227
Figure 8-4: Regression analysis of <i>H19</i> placental gene expression relative to placental weight	228
Figure 8-5: Regression analysis of <i>Slc2a3</i> placental gene expression relative to placental weight ...	228
Figure 8-6: The effect of exposure to 300µM ammonium during the first cleavage division on placental glucose transport of Methyl-D-Glucose, 3-0-[Methyl-14C] on Day 15 of embryo transfer (indicative of amount of glucose transported per gram of placenta).	229
Figure 8-7: The effect of exposure to 300µM ammonium continually throughout pre-implantation embryo development on placental glucose transport of Methyl-D-Glucose, 3-0-[Methyl-14C] on Day 15 of embryo transfer (indicative of amount of glucose transported per gram of placenta).....	230
Figure 9-1: Diagram depicting the sensitivity of the pre-implantation embryo to external stress.....	240
Figure 9-2: Diagram depicting the cellular and mitochondrial perturbations which result in altered metabolism and energy production.....	243
Figure 9-3: Diagram depicting the possible mechanism behind altered fetal growth after exposure to sub-optimal condition during pre-implantation embryo development	246
Table 2-1: Media components for culture, handling and imaging media	78
Table 2-2: Media components for Simple G1.2 (Batch Test Media).....	79
Table 2-3: Composition of calibration solutions for pH.....	116
Table 2-4: Details of primers used for the analysis of gene expression in mouse blastocysts.	120
Table 2-5: Details of primers used for the analysis of gene expression in mouse Day 15 placentas.	121
Table 3-1: The effect of culture with ammonium on embryo development after 67 hours of culture	131

Table 3-2: Effect of culture with 300µM ammonium on blastocyst implantation and fetal development	138
Table 3-3: Effect of culture with 300µM ammonium on fetal and placental parameters.....	139
Table 4-1: The effect of culture with DMO on embryo development after 19, 43 and 74 hours of culture	157
Table 4-2: The effect of culture with DMO on embryo development after 91 hours of culture	157
Table 4-3: The effect of culture with DMO on embryo development after 67 hours of culture	158
Table 4-4: The effect of culture with 2mM DMO on blastocyst cell allocation after 91 hours of culture	160
Table 4-5: Effect of culture with 2mM DMO on blastocyst implantation and fetal development.....	162
Table 4-6: Effect of culture with 2mM DMO on fetal and placental parameters.....	163
Table 7-1: The effect of culture with ammonium or DMO during the first cleavage division on embryo development after 67 hours of culture	213
Table 8-1: Average Day 15 placental and corresponding average fetal weights of placentas extracted for gene expression.	225

It is recognised that the environment to which the fetus is exposed *in utero*, after implantation, can program longer term health outcomes and alter the possibility of disease onset later in life. It is becoming evident that the environment, to which the pre-implantation embryo is exposed, can also affect the ability of the embryo to form a viable pregnancy as well as altering fetal growth.

Despite this understanding, little is known about the mechanism by which the environment can 'program' the pre-implantation embryo. Using model stress systems, either ammonium or DMO in the culture medium, this thesis addressed the hypothesis that suboptimal environmental conditions may alter mitochondrial homeostasis and function and/or epigenetic parameters and these are the possible mechanisms responsible for the altered fetal outcomes seen.

While common measures of embryo quality such as on time blastocyst development were not affected by either stress, more in-depth investigations found several striking differences. Exposure to DMO significantly decreased blastocyst cell number and allocation to the inner cell mass and trophectoderm, as well as increased blastocyst apoptosis. After exposure to DMO, blastocysts were transferred to pseudopregnant recipients, and both the ability of the embryos to implant and develop into a fetus was impaired as well as fetal weights and crown rump length were significantly reduced indicative of altered growth. Similar results have also been demonstrated after pre-implantation embryos are exposed to ammonium *in vitro*.

Exposure to ammonium during pre-implantation embryo development also altered placental gene expression and function, indicating a possible mechanism of the observed reduced fetal growth parameters.

Interestingly, the pre-implantation embryo appears to be the most vulnerable to an environmental stress during the pre-compaction stage, in particular the zygote to 2-cell transition, as exposure to either stress during this stage alone shows similar perturbations to if the stress was present for the entire pre-implantation developmental period.

At this early stage of embryo development, mitochondria are the sole energy generators and are therefore critical for embryo function. This study determined that either ammonium or DMO stress exposure, during the first cleavage division, significantly perturbed mitochondrial distribution, membrane potential and ATP/ADP levels. Removal of the stress did not allow these effects to be completely reversed, implicating mitochondrial perturbations as a possible mechanism behind altered embryo programming.

During pre-implantation embryo development there are also significant epigenetic changes which are vital for re-programming the embryonic genome. Both *in vitro* stresses significantly altered DNA de-methylation at the 2-cell stage and reduced blastocyst gene expression levels of DNA methyltransferases (*Dnmt3a* and *Dnmt3b*), which are responsible for *de novo* methylation. Together these data highlight the importance of pre-implantation embryo development as a critical period of

growth in which the presence of environmental stress can have an impact on metabolic homeostasis and critical epigenetic events that may be responsible for the downstream effects seen on fetal growth. These results are not only important for assisted reproductive therapy, where the presence of an *in vitro* laboratory stress can potentially alter embryo programming, but are also important for *in vivo* embryo development where the health and wellbeing of the mother can also potentially influence the *in utero* environment and thus the long-term health outcomes of her child.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Deirdre Linda Zander and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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17th July 2009

Publications arising from thesis to date

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Acknowledgements

'Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained'. Marie Curie

To begin I would firstly like to say the biggest thank you to my primary supervisor, Dr Michelle Lane. Thank you, Michelle, for introducing me to the wonderful world of the embryo, mitochondria and the 'never boring' topic of metabolism. As a lost and undecided undergraduate student, I was helped by you to pick a career that I love, and your enthusiasm and passion for research has been a constant source of inspiration. Thank you for being there during all the moments of elation and the moments of despair. I am honoured to have had you as a supervisor and mentor.

Secondly I would like to thank my co-supervisor, Associate/Professor Jeremy Thompson, whose enthusiasm and dedication to science and knowledge is truly remarkable. I have been inspired by his perspective on life, and if I take even a small proportion of his passion for science with me when I leave, I will be a better researcher for it. Thank you, Jeremy, for all your help, support and guidance; it has been a privilege to have had you as a supervisor.

I would also like to thank my mentor, friend and fellow researcher Dr Megan Mitchell. Thank you, Megan, for being there for me during all the ups and downs in both research and life. You have helped me make important decisions about experiments, conference presentations and, most importantly, where to go visit after the conference is over. Thanks for all the great trips away in Europe, Las Vegas and New Zealand. You have been an inspiration and a true friend... 'Sweet Home Alabama'!!!

Thank you also to all the gang at the Research Centre for Reproductive Health who have assisted me with experiments and who made my PhD years a truly memorable and fun experience, and a big thank you to my editor, Nena Bierbaum, for all her help in grammatical editing and formatting.

I would also like to thank my friends and family, especially my Mum, Dad and Sister Bridgette for all their help and inspiration during my PhD. Your love and encouragement helped me get through and emerge victorious, tightly clutching a bound volume containing four years of successes, failures and many helpless moments of complete confusion and frustration.

And finally I would like to give the biggest thank you to my husband David. His love, support and encouragement has been unwavering, and I would never have survived the past 4 years if he had not been there constantly supporting me and believing in my ability to finish my PhD. Thank you for being so understanding and knowing how to make me feel better when things went wrong. I love you, Sweetie, and this thesis is dedicated to you.

This work was supported by the National Health and Medical Research Council Program Grant and The Queen Elizabeth Hospital Postgraduate Scholarship Award.

Common abbreviations

ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
BSA	Bovine Serum Albumin
DMO	5,5-Dimethyl-2,4-Oxazolidinedione
hCG	Human Chorionic Gonadotrophin
HSA	Human Serum Albumin
ICM	Inner cell mass
IVC	<i>In vitro</i> culture
IVF	<i>In vitro</i> fertilisation
IVM	<i>In vitro</i> maturation
i.p	Intraperitoneal
IU	International units
MMP/ $\Delta\Psi_m$	Mitochondrial membrane potential
PBS	Phosphate Buffered Solution
pH _i	Intracellular pH
PI	Propidium Iodide
PMSG	Pregnant Mares' Serum Gonadotrophin
PUN	Plasma urea nitrogen concentration
PVP	Polyvinal-pyrrolidone
RDP	Ruman degradable protein
ROS	Reactive oxygen species
RUP	Ruman undegradable protein
TE	Trophectoderm