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

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Abstract

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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Publications arising from thesis to date

Referred journal articles

Zander DL, Thompson JG, Lane M. Perturbations in Mouse Embryo Development and Viability Caused by Ammonium are More Severe after Exposure at the Cleavage Stages. *Biol Reprod* 2006 Feb;74(2):288-94. Epub 2005 Oct 12.

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Zander DL, Kind, KL. Thompson JG, Lane M. Exposure of Preimplantation Mouse Embryos to Ammonium Alters Resultant Placental Gene Expression 2005 *Hum Reprod Suppl* 1 Volume 20 pg 112

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Zander-Fox DL, Mitchell M, Thompson JG, Lane M. Repercussions of a transient decrease in pH on embryo viability and subsequent fetal development. *Reprod Fertil Dev* 2007; Special Issue pg 112, Abstract 404

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'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***

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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	*In vitro* culture
IVF	*In vitro* fertilisation
IVM	*In vitro* 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