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.
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

Referred journal articles


Zander-Fox DL, Mitchell M, Thompson JG, Lane M. Alterations in Mouse Embryo Intracellular pH by DMO During Pre-implantation Development Impairs Pregnancy Establishment and Perturbs Fetal Growth. RBMOnline 2009 (In Press)

Conference abstracts

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


Zander DL, Thompson JG, Lane M. Ammonium impairs mitochondrial function and homeostasis in murine 2-cell embryos. 2006 BOR, Special Issue pg 125 Abstract 240


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<td>Adenosine Triphosphate</td>
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<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<tr>
<td>DMO</td>
<td>5,5-Dimethyl-2,4-Oxazolidinedione</td>
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<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>MMP/ΔΨm</td>
<td>Mitochondrial membrane potential</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Solution</td>
</tr>
<tr>
<td>pH&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Intracellular pH</td>
</tr>
<tr>
<td>PI</td>
<td>Propidium Iodide</td>
</tr>
<tr>
<td>PMSG</td>
<td>Pregnant Mares’ Serum Gonadotrophin</td>
</tr>
<tr>
<td>PUN</td>
<td>Plasma urea nitrogen concentration</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinyl-pyrrolidone</td>
</tr>
<tr>
<td>RDP</td>
<td>Ruman degradable protein</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RUP</td>
<td>Ruman undegradable protein</td>
</tr>
<tr>
<td>TE</td>
<td>Trophectoderm</td>
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