Maternal diet, embryo effects and offspring health

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Mammalian Folliculogenesis and Oogenesis

The Responsibility of Motherhood
-it can be scary!

Maternal-embryonic communication:

Short-term:
- Fertilisation
- Blastocyst morphogenesis
- Coordination of implantation
- Maternal immunotolerance

Long-term:
- Developmental plasticity - 'selecting' the right phenotype to fit future environment

Implications: DOHaD, ART, IVC, maternal health at conception

Periconceptional Environment

DOHaD

In vitro culture

Developmental plasticity

Maternal obesity

Maternal sickness

Protein rich

Protein deficient

High fat
Mouse Low Protein Diet Model

<table>
<thead>
<tr>
<th>Diet Composition (g/kg)</th>
<th>18% protein</th>
<th>9% protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>180</td>
<td>90</td>
</tr>
<tr>
<td>Corn starch</td>
<td>425</td>
<td>485</td>
</tr>
<tr>
<td>Fiber</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>213</td>
<td>243</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AIN-76 mineral mix</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>AIN-76 vitamin mix</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil (gm/kl)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

- Mild challenge: 9% protein is sufficient for non-pregnant rodents – therefore not starvation but normal range
- Large study (19 mothers, 114 offspring per treatment) allows detailed associations to be identified
- No effect on gestation length, litter size or male:female ratio


Maternal Emb-LPD and Postnatal Cardiovascular Phenotype

Adult Emb-LPD offspring exhibit:
- Relative hypertension
- Smaller heart mass (females)
- Increased lung ACE activity
- Reduced arterial vasodilatation


Similar datasets:
- Rat Emb-LPD, Kwong et al, 2000, Development Mouse LDP during oocyte maturation,
- Mouse ART culture, Watkins et al, 2007, PNAS

Maternal Emb-LPD and postnatal behaviour

- Assays measure anxiety-related locomotor and exploratory activities
- Mean of tests repeated five times over weeks 8, 11, 14, 17 and 20 after acclimatization
- Emb-LPD offspring exhibit ‘hyperactive’ behaviour


with Hugh Perry and colleagues
Embryo environment and long-term health
Maternal Emb-LPD alters conceptus and postnatal growth and adiposity

Watkins et al. (2008) BOR 78:299

Maternal Emb-LPD alters conceptus and postnatal growth and adiposity

10 15 20 25 30 35 40 45

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‡‡

0.4 0.8 1.2 1.6

Birth weight (g)

Females

LPD

NPD

Embryo-LPD

5 10 2 4 6 8 1 0 1 2

Weeks of age

LPD

NPD

Embryo-LPD

Developmental Mechanisms?
Emb-LPD caused: (1) hypertension and dysfunctional CV system
(2) hyperactive behaviour
(3) increased adiposity
(3) increased gestational growth

Correlations:
> Perinatal weight following Emb-LPD or LPD correlates positively with later adult weight, hypertension and, in turn, abnormal behaviour (p<0.05)

Increased gestational growth → Adult disease

Hypothesis:
> Maternal protein undernutrition induces compensatory responses to enhance nutrient delivery to conceptus during gestation to protect and stabilise fetal growth and development

> When responses are appropriate (LPD), normal growth results, but when inappropriate (Emb-LPD), excess perinatal growth results

> Such responses, whether appropriate or not, may confer competitive fitness but also associate with adult disease

> Are embryo-mediated responses clinically relevant?

Board 1 of 3

Richard Oreffo
Stuart Lanham
Emma Lucas
Adam Watkins

Maternal Emb-LPD and fetal bone development
µCT scan d17 fetal skeleton
Emb-LPD – increased bone development

NPD

LPD

Emb-LPD
**CLINICAL RELEVANCE:**

- **Systolic and diastolic blood pressure** levels higher in IVF children (8-18 years) than controls (P < 0.001) (Ceelen M et al (2008) J Clin Endocrinol Metab. 93:1682-8)
- **Growth velocity** higher in IVF children and is predictive of later elevated blood pressure (Ceelen et al, 2009) Increased early growth disease
- **Birth weight** (adjusted for gestational age, gender) of IVF children significantly different dependent upon commercial culture medium used (Dumoulin et al 2010 Human Reproduction 25:605-612)

- **Dutch winter famine**, 5 months, 1944-45, Amsterdam. Offspring from women exposed to famine during embryo and early gestation show
  - increased prevalence of coronary heart disease; increased blood pressure
  - increased BMI and glucose intolerance Painter et al, 2006a,b; de Rooij et al, 2006; Ravelli et al, 1999.

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**When do responses to maternal diet occur?**

Increased perinatal growth in Emb-LPD conceptuses is detectable in late gestation, a response induced by the blastocyst stage independent of later maternal environment

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**How do embryos sense their mother’s nutritional status?**

A role for amino acids, insulin and mTOR signalling

Depleted maternal insulin and branch chain AA availability following Emb-LPD

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How do embryos sense their mother’s nutritional status?
A role for amino acids, insulin and mTOR signalling

Emb-LPD leads to reduced mTOR signalling via S6 in blastocysts

How do embryos respond to their mother’s nutritional status?
Blastocyst protein synthesis rate is responsive to maternal dietary protein – but is stable in LPD

How do embryos respond to their mother’s nutritional status to protect fetal growth?
A role for extra-embryonic lineages

LPD - compensatory increase in ribosomal biogenesis to stabilise protein synthesis rate

Inner cell mass → Foetus
Trophectoderm → Chorio-allantoic placenta
Primary endoderm → Yolk sac placenta
Maternal LPD induces responses in the visceral yolk sac

- LPD increases numbers of endocytic vesicles
- LPD increases rate of endocytosis

Visceral yolk sac megalin (Lrp2 gene)
- 600 kDa transmembrane multiligand (~35) endocytic receptor, LDL-R family
- Localised to apical surface of yolk sac visceral endoderm
- Major role in yolk sac endocytosis

Response by trophectoderm to maternal Emb-LPD treatment:
- Increased proliferation and cell spreading
Emb-LPD stimulates endocytosis in trophectoderm: increased ligand digestion, lysosomes and major receptor (megalin)

Maternal Emb-LPD effect on ES cell derivation and phenotype

- Reduced pool of pluripotent cells within ICM or early emerging ES cell cluster?
Emb-LPD alters ES cell survival and apoptosis

LPD increases level of apoptosis in ESCs, possibly mediated through reduced p-total ERK-1,2 survival signalling

Periconceptional Environment

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Maternal sickness at conception affects development and health into adulthood

- Induce mouse maternal sickness and inflammatory response
  - Bacterial LPS i.p. injection on Day 1 (zygote)
  - 10, 50 or 150 μg/kg Salmonella enterica enteritidis LPS or saline control

Blastocyst ICM:TE reduced
Fewer ICM cells

Blood pressure normal
Postnatal growth normal

Distinct phenotype from LPD model

Williams et al, 2011 BMC Biology 9:49
Maternal LPS injection at zygote stage – offspring effects

**CONCLUSIONS**

- **The Responsibility of Motherhood**: Maternal-embryo interactions with life-long consequences are broad, mediated through maternal nutrition, health and physiology affecting adult offspring CV, behaviour, growth, adiposity, immune responsiveness.
- **Embryo sensing mechanism**: Maternal LPS is first detected by embryos through local reduction of intra-maternal amino acid availability causing reduced blastocyst mTOR signaling via S6 pathway.
- **Embryo response mechanisms**: Compensatory responses by the blastocyst stage:
  - evidence of increased ribosomal biogenesis to protect biosynthesis rate
  - stimulate extra-embryonic lineages (trophoblast : visceral endoderm)
  - ES cells from Emb-LPD blastocysts show evidence of increased stress/apoptosis
- **From growth to disease**: Promoting growth will maintain competitive fitness but with the trade-off of disease risk in later life. Activation of growth compensation during gestation correlates positively with disease onset in adulthood.
- **Complexity of processes**: The path from maternal-embryo interaction to developmental programming is an integration of biological processes at hormonal, metabolic, signal transduction, cell cycle and epigenetic levels.

**Thanks!**