Sex-Specific Placental Responses in Fetal Development

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Changes in the Perinatal Sphere

- Maternal Diet, Famine
- Maternal Stress
- Underlying maternal diseases, e.g. asthma, diabetes
- EDC, such as bisphenol A, vinclozolin, DES, other toxicants.

Developmental Origin of Adult Health and Diseases (DOHAD), Fetal Origin Adult Disease (FOAD), or Barker Hypothesis.

Examples of such diseases include:

- Stroke
- Coronary Heart Disease
- Type II Diabetes
- Hypertension
- Behavioral Disorders
The Placenta: Guardian of the Fetus

The placenta is an appropriate choice of organ to begin to monitor how *in utero* environmental changes are sensed by the developing offspring.
Placental Responses Can Affect Brain and Cardiovascular Function
Placental Types

Adapted from Flexner and Gellhorn, 1943 with modification
Pre-implantational Embryonic Development

(a) Overlap of CDX2 and OCT3/4 expression in different stages of development:
- 8-cell stage (E2.5)
- 16-cell stage (E3.0)
- Blastocyst stage (E3.5)

(b) Expression of GATA6 and Nanog in stages of development:
- Early blastocyst (E3.5)
- Late blastocyst (E4.5)

ICM, TE, Epiblast, Primitive endoderm, Trophectoderm

Nature Reviews | Molecular Cell Biology
Distinguishing TE Versus ICM Cells & Male Versus Female Embryos

Effect of Glucose Concentration on Embryo Cell Number According to Sex

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>Sex</th>
<th>Total Cells Mean ± SEM</th>
<th>TE Cells Mean ± SEM</th>
<th>ICM Cells Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>Male</td>
<td>76.3 ± 4.6a</td>
<td>60.8 ± 4c</td>
<td>15.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>76.3 ± 4a</td>
<td>61.9 ± 4.8c</td>
<td>14.4 ± 1.4</td>
</tr>
<tr>
<td>28</td>
<td>Male</td>
<td>61.1 ± 3.8b</td>
<td>45.8 ± 3.1d</td>
<td>15.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>54.8 ± 3.9b</td>
<td>38.6 ± 3.6d</td>
<td>16.1 ± 1.3</td>
</tr>
</tbody>
</table>

Major Conclusions:
- No sex differences were observed in embryonic cell numbers due to in vitro changes in glucose concentrations.
- Elevated in vitro glucose concentrations that approximate those of diabetic maternal serum decreases total cell and TE cell numbers in male and female blastocysts,

Previous Studies on Effects of Maternal Diet on Placental Gene Expression

• Prior to our study, only one published study to date examined how maternal diet governs global placental gene expression (Gheorghe et al., Placenta 2009).

• This study revealed that in mice a short withdrawal of protein for four days in mid-pregnancy has deleterious consequences on placental gene expression.

• The study, however, did not consider the possibility that male and female conceptuses might show different responses to the imposed diet.
Overall Goal

We sought to examine how maternal diet might influence the full range of placental gene expression in male and female conceptuses at around mid-pregnancy in the mouse when the morphological development of the placenta is complete but the gonads are not fully formed (i.e. minimal steroid production).
Experimental Approach

- We employed NIH Swiss dams maintained for an extended period (35 to 40wks) on a low fat (LF) (Research Diets), a very high fat (VHF) (Research Diets), and a Purina 5015 chow (C) diet (Test Diets), the latter approximating standard husbandry conditions for experimental mice during pregnancy.
Experimental Approach

We chose to collect the conceptuses at d 12.5 of pregnancy for several reasons:
* The morphological development of the placenta is complete at this point
* However, the gonads are just beginning to form and serum testosterone concentrations are minimal at this time period
* The fetal discoid placenta can easily be dissected from the maternal placenta at this stage
* This period of gestation is characterized by marked up-regulation of “rodent specific” placental genes that encode such products as prolactin-related proteins, carcinoembryonic antigen-related cell adhesion molecules (CEACAM), pregnancy-specific glycoproteins (PSG), and various cathepsins.
Experimental Approach

Discoid Placenta

• RNA from the placenta was isolated and reverse transcribed for hybridization to Agilent Whole Murine Genome 4x44K arrays (Agilent Technologies, Santa Clara, CA) and QRT-PCR.

• As intrauterine implantation position is important, female and male placentae were pair-matched to the same mid-uterine horn region, which was on the right side for all but one VHF dam, where the pair was selected from the left mid-uterine horn.

Results: Maternal Diet Alters Placental Gene Expression in Mice

Heat map based on maternal diet effects on placental gene expression. Gene tree clustering on 1,972 genes, whose expression was changed more than 2-fold with P<0.05.

Mao et al., PNAS, 2010; 107(12):5557-5562
Results: The Murine Placenta Displays Strikingly Sexually Dimorphic Differences in Placental Gene Expression Patterns

Heat map based on sex effects on placental gene expression. The placentae gene expression patterns of male conceptuses clearly clusters separately from the placentae of females, when data on the total regulated genes (with 2-fold differences) across all dietary groups are compared (P<0.05).

Mao et al., PNAS, 2010; 107(12):5557-62
Examples of Sexually Dimorphic Expressed Placental Genes Confirmed by Quantitative Real-Time PCR Analysis

- Aquaporin 9
- Chemokine (C-C motif) receptor 3
- CEA-related cell adhesion molecule 1 (mouse placental specific gene)
- Estrogen receptor-\(\alpha\)
- Hydroxy-delta-5-steroid dehydrogenase, 3\(\beta\)-and steroid delta-isomerase 5
- Olfactory receptor 1381
- Olfactory receptor 154
- Olfactory receptor 433
- Olfactory receptor 520
- Renin1
- Renin2
How do Sexually Dimorphic Differences Originate?

- **Sex Steroids** - Unlikely at 12.5 dpc
- **X-chromosome dose** - Unlikely due to X-chromosome dosage, unless the paternal X chromosome is incompletely silenced in the female placentae.
- **Epigenetics** - Likely mechanisms. After our study was published, it was demonstrated that fetal sex and maternal diet can alter DNA methylation patterns in the murine placenta (Gallou-Kabani et al., PLoS One. 2010; 5:e14398) and gene expression of histone demethylase paralogues (Kdm5c and Kdm5d, Gabory et al., Plos One 2012; e47988).
Need to Examine How *In Utero* Environmental Changes Affect in a Sex Dependent Manner Specific Placental Regions and Cells

In the spiny mouse (*Acomys cahirinus*):

- The female placenta has less spongy zone and more labyrinth region than males.
- There are sex-dependent and regional differences in placental gene expression.

O’Connell et al. Placenta 2013; 34: 119-126