Learning Objectives

- Define the developmental origins theory of adult disease.
- State one epigenetic mechanism of cellular plasticity.
- Name an IVF factor that alters fetal development.
- Describe an environmental factor with adverse trans-generational effects.
The Barker Hypothesis

- Fetal undernutrition causes disproportional fetal growth and programs later coronary heart disease.
- Death from CHD rose in individuals small at birth (<2500 gm) due to growth failure rather than prematurity.
- Trends in CHD by birth weight are paralleled by similar trends in diabetes and hypertension.
- Highest prevalence of diabetes occurs in people who are small at birth and become obese as adults.
- Highest blood pressures occur in people who at birth are small for gestational age and have large placentas.
- Fetal undernutrition slows cell division during critical time intervals in various target tissues by altering cell number, function or distribution.

Barker 1995

Developmental Origins of Health and Disease (DO-HaD)

- Altered homeostasis during critical periods of development predispose individuals to adult-onset chronic diseases.
- Environmental stress in utero favors survival of offspring with the greatest capacity for energy storage to endure prolonged episodes of privation as adults (the “thrifty phenotype”).
Epigenetics: "heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence"

- Modulate environmental effects on the adult phenotype via transcriptional regulation via
  - **Cytosine methylation**: methylation of CpG islands in DNA
  - **Chromatin packaging**: post-translational modification of histone proteins (i.e., acetylation, methylation, phosphorylation ubiquitination)
  - **RNAs**: Long noncoding RNAs: short RNAs (<40 nt) siRNA, microRNAs, small fragments of rRNAs and tRNAs
- Impart cellular plasticity to intrauterine events that program the fetal genome for survival after birth through
  - energy metabolism
  - adipogenesis

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Dutch Famine 1944-1945

People exposed to famine

- in **early gestation** have a more atherogenic lipid profile, and a higher BMI & waist size at 50 yrs (women),
- in **mid/late gestation** have reduced glucose tolerance, and
- in **late gestation** with decreased protein have hypertension.

Famine during the first trimester alters DNA methylation of CpG dinucleotides in genes of adult offspring that involve
- cell growth/differentiation,
- nutrient balance, and
- intracellular cholesterol transport.

Gestational Age and Birth Weight by IVF
Nordic cohort of singletons born after FET (N=6647), fresh IVF/ICSI (N=42,242) and spontaneous conception (N=288,542)

PTB, preterm birth; LBW, low birth weight; SGA, small for gestational age; LGA, large for gestational age. *, P < 0.05; ***, P < 0.001 versus FET

Absolute increased risks: 1-3%, all outcomes

Mean Birth Weight of Term IVF Singletons (N=25,777 births)

Mean difference, 90.9 grams
Serum E2 during COH-IVF & Abnormal Placentation
(292 Singletons by initial US)

<table>
<thead>
<tr>
<th>Peak serum E2 &gt;3450 pg/mL (&gt;90th percentile)</th>
<th>OR*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>4.8</td>
<td>1.6-14.8</td>
</tr>
<tr>
<td>SGA</td>
<td>9.4</td>
<td>3.2-27.5</td>
</tr>
</tbody>
</table>

*, adjusted for patient age, BMI, parity, number of embryos transferred, day of embryo transfer and/or gonadotropin dose.

Elevated E2 during COH-IVF is associated with greater odds of developing pre-eclampsia and delivery of an SGA singleton, perhaps from abnormal spiral artery remodeling and trophoblast invasion. Imudia 2012

Elevated E2 levels in vitro impair growth of human first trimester cytotrophoblast, suggesting abnormal spiral artery remodeling due to impaired trophoblast survival. Skafar 2012

Fetal Growth and Placentation

- Transferring murine blastocysts from a natural mating to a female surrogate receiving gonadotropins impairs fetal growth and trophoblast differentiation and alters placental gene expression.
- DNA methylation and gene expression patterns vary in cord blood and placental tissue of children conceived in vitro versus natural conception.
  - Some of these genes affect:
    - adipocyte differentiation, insulin signaling and/or obesity
    - trophoblast stem cell development

Mainigi 2014, Katari 2009, Turan 2010
### Cardiovascular Remodeling and Assisted Reproduction

(100 singletons conceived by ART; 100 control pregnancies*)¶)

<table>
<thead>
<tr>
<th>3rd Trimester</th>
<th>Neonatal life</th>
<th>6 Months of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Bilaterally enlarged atria</td>
<td>↑ Diastolic BP</td>
<td>↑ Right atrial size &amp; myocardial wall thickness</td>
</tr>
<tr>
<td>↑ Right myocardial wall thickness</td>
<td>↑ Carotid/aortic intima-media thickness</td>
<td>↑ Heart rate</td>
</tr>
<tr>
<td>↓ Left ejection fraction</td>
<td></td>
<td>↑ Systolic &amp; diastolic dysfunction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Systolic BP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Aortic IMT</td>
</tr>
</tbody>
</table>

* vs. spontaneous conception
¶ Adjusting for gestational age, birth weight, preeclampsia

Valenzuela-Alcaraz 2013

### CVD Risk Factors in Children conceived by ART

<table>
<thead>
<tr>
<th>Author</th>
<th>Ages 3-18 yrs</th>
<th>BMI</th>
<th>SBP</th>
<th>DBP</th>
<th>TG</th>
<th>FPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceelen 2007</td>
<td>225 IVF; 225 non-ART singletons</td>
<td>NA</td>
<td>↑</td>
<td>↑</td>
<td>NA</td>
<td>↑</td>
</tr>
<tr>
<td>Belva 2007</td>
<td>150 ICSI; 147 non-ART singletons</td>
<td>NA</td>
<td>↑</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Belva 2012</td>
<td>217 ICSI; 223 non-ART singletons</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Miles 2007</td>
<td>69 IVF/ICSI; 71 non-ART singletons</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Sakka 2010*</td>
<td>106 IVF; 68 age-matched untreated</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Scherrer 2012¶</td>
<td>65 IVF/ICSI; 57 non-ART singletons</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Green 2013</td>
<td>61 IVF; 91 non-ART singletons</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

*, No differences in insulin resistance, serum adipokines and inflammatory markers
¶, pulmonary and systemic endothelial dysfunction

Yeung 2013
Thioredoxin-interacting protein (TXNIP): integrates cellular nutrition with redox state to regulate mitochondrial function.
Mitochondrial Function and Embryogenesis

Chemical uncoupling of oxidative phosphorylation in mouse zygotes reduces ATP production, decreases blastocyst growth, and reduces offspring birth weight followed by postnatal obesity.

<table>
<thead>
<tr>
<th>Change in fat mass between 4 of 14 weeks of age</th>
<th>Controls</th>
<th>FCCP-treated</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grams</td>
<td>1.4±0.4</td>
<td>2.0±0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Percent</td>
<td>1.8±0.9</td>
<td>3.9±0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma metabolites at 14 weeks of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.6±0.8</td>
<td>9.2±0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>3.3±0.4</td>
<td>2.4±0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.6±0.1</td>
<td>0.7±0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Oocytes from overweight women are smaller and less likely to complete embryogenesis. Post-fertilization, they reach the morula stage faster and, as blastocysts contain fewer cells, show reduced glucose consumption and elevated endogenous triglyceride levels
### Percentage of Children with MBS (6-11 years of age [N=175])

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGA</td>
<td>2.2</td>
<td>0.006</td>
<td>1.2-3.8</td>
</tr>
<tr>
<td>Maternal Obesity</td>
<td>1.8</td>
<td>0.04</td>
<td>1.0-3.2</td>
</tr>
</tbody>
</table>

Boney 2005

### All Cause Mortality in Adult Offspring by Maternal BMI
(37,709 people with birth records from 1950 to date)

<table>
<thead>
<tr>
<th>Maternal BMI</th>
<th>Hazard ratio</th>
<th>95 CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5</td>
<td>1.0</td>
<td>0.8-1.1</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>25.0-29.9</td>
<td>1.1</td>
<td>1.0-1.2</td>
</tr>
<tr>
<td>&gt;30</td>
<td>1.3</td>
<td>1.2-1.5</td>
</tr>
</tbody>
</table>

Adjusted for maternal age, social class, gestational age, infant sex and birth weight

Reynolds 2013
Maternal Obesity Programs Obesity in Offspring

- Altered DNA methylation of important genes
- Increased expression of adipogenic genes
- Greater stem cell differentiation to adipocytes
- Upregulation of lipogenic pathways

Can we alter developmental programming in nonhuman primates during intrauterine life?

Shankar 2008, Borengasser 2013
A maternal high-fat diet

- changes fetal hepatic chromatin structure and postnatal gene expression
- alters fetal protein translation via DNA methylation and histone and chromatin modifications

Increased maternal adiposity from anabolic steroids

- alters SC adipogenesis and modifies DNA methylation of visceral fat in offspring.
- induces a metabolic-like syndrome in adult offspring.


Children (F2) of Fathers From the Dutch Famine Birth Cohort (F1) Who Were Undernourished in Utero

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Unexposed</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>52</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>34</td>
<td>36</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight (gms)</td>
<td>3351</td>
<td>3342</td>
<td>NS</td>
</tr>
<tr>
<td>Adult weight (kg)*</td>
<td>78.8</td>
<td>73.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>25.2</td>
<td>23.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*, after correction for age and sex of F2

Offspring from in utero undernourished fathers were heavier and more obese as adults than those from fathers and mothers not undernourished in utero

Veenendaal 2013
Avon Longitudinal Study of Parents and Children (303 probands & 1818 parents/grandparents born in northern Sweden)

Paternal grandfather's food supply is linked to the mortality rate of grandsons alone, suggesting that sex-specific, male-line trans-generational responses exist in humans

Pembrey 2006

Intergenerational Epigenetic Information in Sperm

Rando 2016
**Transgenerational Amplification of Obesity**

- **Male**
  - HDF for 10 weeks
  - 22% ↑ adiposity, NI GTT

- **Normal Female**

- **Male**
  - 6% weight gain
  - GT

- **Female**
  - 53% ↑ Adiposity
  - GT

- **Male**

- **Female**
  - 79% ↑ Adiposity
  - GT

Fullston 2013

**Transgenerational Amplification of Impaired Reproductive Health**

- **Male**
  - HDF for 10 weeks
  - 22% ↑ adiposity, NI GTT

- **Normal Female**

- **Male**
  - ↑ sperm ROS & DNA damage
  - ↓ Motility
  - ↓ Mitochondrial stress

- **Female**
  - ↑ Oocyte ROS
  - ↑ Mitochondrial stress

- **Male**
  - ↑ Sperm ROS
  - ↑ Motility

- **Female**
  - ↑ Oocyte meiosis
  - ↓ Mitochondrial stress

- **Male**
  - ↑ Sperm ROS
  - ↓ Motility
  - ↓ Testosterone

F0 sperm have increased ROS and DNA damage with abnormal microRNA content & germ cell methylation

Fullston 2012, 2013
The Barker Hypothesis: How Can We Optimize the Health of Our IVF Offspring?

- Discuss metabolic and environmental health with the couple before beginning fertility therapy.
- Optimize IVF cell culture conditions during preimplantation embryogenesis.
- Consider liberal use of frozen embryo transfer to reduce the risk of elevated estradiol on placentation.
- Monitor IVF-conceived offspring into adulthood to determine their risk of developing metabolic disease.
Question 1: Clinical

Which of the following factors most commonly impairs the health of adult human offspring?

A. IVF cell culture  
B. Fresh embryo transfer  
C. Maternal obesity  
D. Paternal obesity

Question 2: Basic Science

Which of the following statements regarding epigenetics is true?

A. Nearly global erasure of cytosine methylation on the paternal genome occurs after fertilization.  
B. The paternal genome is primarily packaged with protamines and susceptible to demethylation.  
C. Parts of the gamete epigenome are protected from erasure at fertilization by a factor that associates with the genome.  
D. All of the above
References


