Epigenetic Gene Regulation During Development and Under Adverse Environmental Conditions

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LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

- Describe epigenetic gene regulation in the mammalian embryo.
- Describe the role and epigenetic regulation of imprinted genes in development.
- Identify potential epigenetic risks associated with ART.

DISCLOSURE

- No Disclosures
Mammals Have Thousands of Cell Types, Which Originate from Single Genome in the Fertilized Egg

1 Genome
DNA
Chromatin
Genetic information obtained from gametes
Cell type specific organized information
> 1000 Epigenomes

Epigenetics

“Epi” = above, over, outside or beside

Events that are above and beside genetics; beyond DNA sequence

Molecular definitions: defined chemical changes to the DNA and the proteins that package DNA

Epigenetics Vs Genetics
There are Many Levels of Epigenetic Information

Two Main Components to the Epigenetic Code

DNA methylation

Histone Modifications

Structure of the Nucleosome

Histone tails protrude from the nucleosome and can serve as templates for epigenetic modifications

Luger et al., Nature 1997; 389:251-260
Post-translational modifications of amino acids occur primarily on the histone tails.

Chromatin and Gene Expression
Euchromatin vs Heterochromatin

Two Main Components to the Epigenetic Code
DNA methylation
Histone Modifications

DNA Methyltransferase (DNMT)

Cytosine
Guanine

Nucleosome
Histone Tails
DNA
Histone
DNA Methylation

- Mostly occurs at cytosines in CpGs, which are sparsely distributed (70-80% are methylated)
- Under-represented in the genome largely because 5mC is mutagenic – deamination of 5mC generates T
- Generally a repressive mark through preventing binding of transcription factors or assembling repressive chromatin structure
- Dynamically regulated during mammalian development

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Most of the Mammalian Genome Reprograms DNA Methylation During Development

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Remodelling Sperm Genome After Fertilization

Sperm genome:
- Packaged with protamines
- Only 5-15% of histones retained in mature spermatozoa (human)

Post fertilization:
- Removal of protamines
- Deposition of new histones; Histone chaperones (Hira)
Barton et al., 2001

Zygotic reprogramming : DNA demethylation

5mC
DAPI

Genomic Imprinting is Mammalian-Specific and Results in Monoallelic, Parent-of-Origin-Specific Expression

Non-imprinted genes
Imprinted genes

Functions of Imprinted Genes

From Kalish et al., 2014
Imprinted Genes Reside in Clusters and are Regulated by ICRs which are DNA Methylated on a Single Parental Allele

ICRs are Differentially Methylated in the Germline and Escape DNA Methylation Reprogramming after Fertilization

In Vitro Fertilization
In Vitro Fertilization

IVF is associated with a higher than expected incidence of the imprinting disorders Beckwith-Wiedemann syndrome and Angelman Syndrome

WHY???
The Problem

How can we address whether procedures used in ART disrupt epigenetic gene regulation?

Ultimately, our goal is to improve the technology.

Strategy—Use a Mouse Model

Investigate morphology and epigenetic gene regulation in the mouse embryo and placenta

ART Paradigm—Can We Associate Specific Procedures with Pathology?
Imprinting Control Regions Regulate Imprinting and Exhibit Parental-Allele Specific DNA Methylation

ICR=Imprinting Control Region

Maternal Allele

Paternal Allele

Unmethylated CpG

Methylated CpG

Imprinted Genes

CH3

C G

Placental Phenotype at Term-ICR Methylation

H19/Igf2 ICR

Peg3 ICR

Snat4 ICR

weight (ET, SET, IVF)

junctional zone (SET, IVF)

loss of ICR methylation (IVF)

Placental Phenotype at Term-Global DNA Methylation

LUMA Assay

weight (ET, SET, IVF)

junctional zone (SET, IVF)

Loss of ICR methylation (IVF)

Loss of Global DNA methylation (IVF)
CONCLUSIONS I

In comparison to naturally conceived groups

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<tr>
<th>Procedure</th>
<th>Placental weight</th>
<th>Junctional Zone Overgrowth</th>
<th>ICR DNA Methylation</th>
<th>Global DNA Methylation</th>
<th>Aberrant Imprinted Gene Exp</th>
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Lisa Vrooman and Eric de Waal: HMG, 2015

ART Paradigm-Can We Associate Specific Procedures with Pathology?

Natural
- Mating
- Gestation to term

ET
- Mating
- Gestation to blastocyst
- Transfer

S + ET
- Superovulation
- Mating
- Gestation to blastocyst
- Transfer

S + EC + ET
- Superovulation
- Culture to blastocyst
- Transfer

S + IVF + EC + ET
- Superovulation
- IVF
- Culture to blastocyst
- Transfer

CONCLUSIONS II

- Embryonic time course reveals that embryo is initially smaller
- Placenta subsequently overgrows
- Embryo culture alone is no worse than IVF procedure
- Epigenetic abnormalities are associated embryo culture

Lisa Vrooman & Eric Rhon-Calderon
Assisted Reproductive Technologies (ART) Expose the Early Embryo to Suboptimal Conditions

Modified from Smallwood and Kelsey, 2012

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