PRE-CONGRESS COURSE 8

Practical applications of clinical and basic science genetics to reproductive medicine

ASRM Exchange course
Munich - Germany, 29 June 2014
Practical applications of clinical and basic science genetics to reproductive medicine

Munich, Germany
29 June 2014

Organised by
American Society for Reproductive Medicine (ASRM)
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Objectives

Couples presenting for reproductive care may be at risk for disorders that either are predictable or are yet unknown. Others present with genetic disorders of reproduction that have implications for their care and potentially their children. Finally for others, reproductive technology may provide new risks. This pre-congress course will focus on contemporary genetic issues that are encountered in the practice of reproductive medicine. Topics will include screening for genetic disease, identifying genetic causes of reproductive failure, use of assisted reproductive technologies (ART) for treatment of individuals with reproductive genetic disorders, and potential adverse genetic outcomes of ART.

Learning objectives

At the conclusion of this course, the participant should be able to:

1. Propose appropriate genetic testing for couples presenting with infertility
2. Discuss adverse genetic outcomes of ART
3. Counsel patients with Turner syndrome and Klinefelter syndrome about reproductive options and outcomes
4. Screen for genetic causes of reproductive failure

Target audience

Reproductive Endocrinologists, Andrologists, General Gynecologists, Urologists, and Allied Reproductive Health Professionals
Scientific programme

Chairman: Rebecca Z. Sokol - U.S.A.

09:00 - 09:30  Screening couples for genetic disease presenting for reproductive care. Screening for at-risk and not at-risk individuals

Joe Leigh Simpson - U.S.A.

09:30 - 09:45  Discussion
09:45 - 10:15  Genetics of premature ovarian insufficiency

Marcelle Cedars - U.S.A.

10:15 - 10:30  Discussion

10:30 - 11:00  Coffee break

11:00 - 11:30  Epigenetic modifications due to environmental factors

Linda C. Giudice - U.S.A.

11:30 - 11:45  Discussion
11:45 - 12:15  Genetic and epigenetic factors affecting embryo development and implantation

Christos Coutifaris - U.S.A.

12:15 - 12:30  Discussion

12:30 - 13:30  Lunch break

13:30 - 14:00  Turner Syndrome: Reproductive options and outcomes

Richard Reindollar - U.S.A.

14:00 - 14:15  Discussion
14:15 - 15:00  Klinefelter Syndrome: Reproductive and hormonal options and outcomes

Rebecca Z. Sokol - U.S.A.

15:00 - 15:15  Discussion

15:15 - 15:30  Coffee break

15:30 - 16:00  Sperm aneuploidy and ART

Dolores J. Lamb - U.S.A.

16:00 - 16:15  Discussion
16:15 - 16:45  Imprinting disorders and ARTc

Joe Leigh Simpson - U.S.A.

16:45 - 17:00  Discussion
Screening couples for genetic disease presenting for reproductive care. Screening for not at-risk and for at-risk individuals.

JOE LEIGH SIMPSON, M.D., FACOG, FACMG
Senior Vice President
for Research and Global Programs
March of Dimes Foundation, New York, USA

President, International Federation Fertility Societies (IFFS)

Educational Objectives

- Be able to state single gene disorders for which heterozygote screening must be offered to all couples
- Be able to explain to patients the rationale for undergoing PGD aneuploidy testing (PGS)
- State sensitivity of detecting trisomy 21 and 18 on the basis of cell free fetal DNA in maternal blood

SCREENING DURING PREGNANCY

- Screening asymptomatic individuals to detect couples at risk (two heterozygotes for same mutant allele). Appropriate only if disorders are relatively common, usually in a given ethnic group
- Screening ≠ Testing
PREGNANCY SCREENING TRADITIONAL RECOMMENDATIONS

Blacks: Sickle Cell disease
Jewish: Tay-Sachs disease
Other
Italian and Greek: β-thalassemia
Asian: α-thalassemia
All: Cystic fibrosis, Spinal muscular atrophy (SMA)

ETHNIC DIFFERENCES: HETEROZYGOSITY IN THE ASHKENAZIM (2009)

Tay-Sachs 1/30
Gaucher 1/13
Cystic Fibrosis 1/25
Canavan 1/40
Nieman-Pick 1/90
Familial Dysautonomia 1/30

2009: DNA testing

CYSTIC FIBROSIS

United States:
- Affected 30,000
- Carriers 8,000,000
- Caucasians 1/2,500
- African-Americans 1/18,000
- Asian-Americans 1/90,000
CYSTIC FIBROSIS
TRANSMEMBRANE GENE:
REGULATOR (CFTR)

- Gene spans 250 kb
- 27 exons
- Mature mRNA 6,500 bases
- Encodes a chloride ion channel of 1,480 amino acids (CFTR)
- Three-nucleotide deletion of codon 508 (phenylalanine) in 70% Caucasians: \(\Delta F508\)

RECOMMENDED CORE MUTATION PANEL
FOR GENERAL POPULATION CF CARRIER SCREENING (2001)

Standard Mutation Panel
\(\Delta F508\) G551D W1282X N1303K R553X R117H A455E R560T R1162X G85E R175Q R347P 711+1G>T 1898+1G>A 2184delA 1078delT 3849+10kbC>T 2789+5G>A 3659delC I148T 3120+1G>A

HETEROZYGOTE FREQUENCIES (CF) BY ETHNIC GROUP

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Heterozygote Frequency</th>
<th>% of Heterozygote Detectable</th>
<th>Likelihood of being Heterozygote Despite Negative Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jewish</td>
<td>1/24</td>
<td>94%</td>
<td>1 in 400</td>
</tr>
<tr>
<td>European Caucasian</td>
<td>1/25</td>
<td>88%</td>
<td>1 in 208</td>
</tr>
</tbody>
</table>

**REFERENCES**

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<th>% of Heterozygote Detectable</th>
<th>Likelihood of being Heterozygote Despite Negative Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American</td>
<td>1/65</td>
<td>65%</td>
<td>1 in 186</td>
</tr>
<tr>
<td>Hispanic American</td>
<td>1/46</td>
<td>72%</td>
<td>1 in 164</td>
</tr>
<tr>
<td>Asian American</td>
<td>1/94</td>
<td>49%</td>
<td>1 in 184</td>
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</tbody>
</table>

WHY NOT SCREEN YOUR GENOME?

1. Identify mutations
2. Identify disease-associated alleles
3. Cumulative calculation of risks. Odds ratio based on disease-associated alleles

Genome Wide Screening for Disease Susceptibility

1. Identify mutations
2. Identify disease-associated alleles
3. Cumulative calculation of risks. Odds ratio based on disease-associated alleles
Genome Wide Screening for Disease Susceptibility

**Sample Results**
- Prostate cancer without family history 5 x
- Prostate cancer with family history 9 x
- Colon Cancer 2 x
- Resistance or sensitivity to warfarin none
- Type II Diabetes 2 x
- Huntington Disease not present
- Alzheimers (APO\(\varepsilon\)4; +/- family history 3 x

SCREENING FOR CHROMOSOMAL ABNORMALITIES

DOWN SYNDROME DETECTION THROUGH NONINVASIVE SCREENING

- Second trimester:
  - Three analytes ~ 69%
  - Four analytes ~ 81%
- First trimester (11 weeks) ~ 87%
- First trimester (12 weeks) ~ 85%
- First plus second ~ 92-95%
  (contingent, stepwise, integrated)
5% procedure rate (false positive)

Malone, NEJM, 2005
CELL-FREE DNA IN MATERNAL BLOOD

- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mom and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA

NEXT GENERATION SEQUENCING

- Many DNA fragments sequenced concurrently (massive parallel genomic sequencing) and compared to reference genome
- Cloned bacteria or yeast vectors unnecessary
- Completely automated and rapid / much lower cost
FRAGMENT ASSEMBLY

- Overlap reads and extension to reconstruct the original genomic region
- Given reference genome now known, comparison can be done directly to sample

CELL FREE FETAL DNA FOR ANEUPLOIDY DETECTION

- Strategy: Increased trisomy 21 transcripts (maternal and fetal) in maternal blood of trisomic pregnancies compared to maternal blood of euploid (normal) pregnancies. Massive Parallel Genomic Sequencing (MPGS) [Massive Parallel Shotgun Sequencing – MPSS]
  - Quantitative rather than qualitative difference must be shown for numbered transcripts.

Assessing Fetal 21 Transcripts by Parallel Genomic Sequencing (maternal and fetal transcripts)

Fetal Trisomy Detection With cfDNA

- Each bar represents thousands of cfDNA fragments
- Counting of chromosome cfDNA fragments done by DNA sequencing

Aneuploidy Detection

- Determine total chromosome 21 transcripts (maternal and fetal) in trisomic and non-trisomic pregnancy
- If 5% of cell free DNA in maternal blood is fetal, trisomic pregnancies should have 2.5% greater 21 transcripts than normal pregnancies

Fetal Trisomy Detection With cfDNA

- The overabundance of chromosome 21 cfDNA fragments in trisomy 21, although small, can be measured with DNA sequencing
DETECTION OF TRISOMIES (Verinata)

MPGS: (MELISSA:Verinata) Massively parallel sequencing normalized chromosome values compared with karyotype classifications for chromosomes 21, 18, and 13. Circles display classifications for chromosome 21, squares display classifications for chromosome 18, and triangles display classifications for chromosome 13. Unclassified samples with trisomy karyotypes have been circled. Bianchi. Genome-Wide Fetal Aneuploidy Detection, Obstet Gynecol 212.

DETECTION OF TRISOMIES (Verinata)

Studied in over 6,000 patients, including >2,000 average-risk women

<table>
<thead>
<tr>
<th>DETECTION RATE</th>
<th>FALSE POSITIVE RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T21 99% (214 of 214)</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>T18 97% (101 of 103)</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>T13 80% (55 of 69)</td>
<td>&lt;0.1%</td>
</tr>
</tbody>
</table>

ACOG, SMFM, ISPD and NSGC recommend use in high-risk pregnancy

Preimplantation Genetic Diagnosis (PGD): Testing

- When is PGD applicable?
  - single gene
  - chromosomes

- What pitfalls exist when applying new technologies to PGD?
Table 1: Time To Achieve Pregnancy Without PGD

<table>
<thead>
<tr>
<th>Study</th>
<th>Cumulative Live Birth Rate</th>
<th>Time to Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugura-Ogasawa (2004)</td>
<td>68%</td>
<td>16 year follow up</td>
</tr>
<tr>
<td>Godijn (2004)</td>
<td>70%</td>
<td>6 year (mean)</td>
</tr>
<tr>
<td>Stephenson &amp; Sierra (2006)</td>
<td>71%</td>
<td>4 year (mean)</td>
</tr>
</tbody>
</table>


Table 2: REDUCTION IN MISCARRIAGES IN RECURRENT PREGNANCY LOSS

<table>
<thead>
<tr>
<th>Maternal Age</th>
<th>Cycles</th>
<th>% Loss Expected</th>
<th>% Loss After PGD (FISH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>85</td>
<td>26%</td>
<td>13% p=0.09</td>
</tr>
<tr>
<td>≥35</td>
<td>143</td>
<td>39%</td>
<td>13% p&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>33%</td>
<td>13% p&lt;0.001</td>
</tr>
</tbody>
</table>

Logistic regression that takes into account maternal age, number, prior abortions
Brigham formula Hum Reprod 14: 2868, 1999

PDG Aneuploidy Testing (PGS)

Methods of Analysis: 2013

Stage:
Trophectoderm biopsy (5-10 cells) at 5-6 days or polar body biopsy, but not cleavage stage embryo at 3 days.

Analysis:
Array Comparative Genome Hybridization (Array CGH)
  - All 24 chromosomes
Array CGH and Missing Chromatid 16 (Polar Body)

PGD Aneuploidy Testing Using Array CGH

- Technically less demanding than FISH and less subjective
- Accumulating data show increased pregnancy rates using array CGH

<table>
<thead>
<tr>
<th>Blastocyst Transfers</th>
<th>Array</th>
<th>No array</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schoolcraft, 2012</td>
<td>60.8%</td>
<td>40.9%</td>
</tr>
<tr>
<td>Yang, 2012</td>
<td>69.1%</td>
<td>41.7%</td>
</tr>
</tbody>
</table>

RCT: Advanced Maternal Age

(Rubio et al., 2013)

- Maternal age 41 – 44 years
- Day 3 FISH: 13, 15, 16, 17, 18, 21, 22, X, Y
- Day 5 transfer

<table>
<thead>
<tr>
<th>PGD</th>
<th>No PGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livebirth Rate:</td>
<td>30/93 (32.3%)</td>
</tr>
<tr>
<td>Odds Ratio:</td>
<td>2.585 [CI 1.26-5.29]</td>
</tr>
</tbody>
</table>
### Single Embryo (Array CGH) vs Double Embryo (Morphology)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Preg.</th>
<th>Twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Embryo, Array CGH</td>
<td>89</td>
<td>60.7%</td>
<td>0</td>
</tr>
<tr>
<td>Two Embryos, Morphology</td>
<td>86</td>
<td>65.1%</td>
<td>53.4%</td>
</tr>
</tbody>
</table>


### PGD Testing for Single Gene Disorders

- **At risk for Autosomes Dominant for Autosomal Recessive Disorder**
- Single gene disorders in which nondisclosure desired (adult onset disorders like Huntington disease). Avoids parent learning their own genotype while also avoids transmitting (if present) to their offspring.
- Very high risk – if 2 different disorder in kindred; successive terminations likely statistically.

### BRCA 1 AND ARTHROGRYPOSIS (ARC)

06/2013

ARC, Arthrogryposis, renal, cholestasis (VPS 33B)
<table>
<thead>
<tr>
<th>Mode</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>Dominant</td>
</tr>
<tr>
<td>ARC</td>
<td>Recessive</td>
</tr>
<tr>
<td>Total Abnormal Conceptions</td>
<td>62.5%</td>
</tr>
</tbody>
</table>

**PGD FOR STEM CELL TRANSPLANTATION**

**Genetic Disorders (25% risk)**
- β-thalassemia; Fanconi anemia
  - Non-functioning bone marrow
  - Older affected case treated with stem cells from umbilical cord blood of newborn sibling
  - HLA match ~95% successful
  - PGD Results (Turkey) 40/44 successful (kahraman)
  - Non HLA match ~60% successful
  - Impractical to expect genetically normal embryo that is also HLA compatible

**LIKELIHOOD OF TRANSFERRABLE EMBRYO**

**Autosomal Recessive**
- HLA Compatible = 1/4
- Normal (Autosomal Recessive): 3/4
- Thus, 1/4 * 3/4 = 3/16

Approach: PGD with transfer embryos both HLA compatible and lacking mutation in homozygous form
### REPRODUCTIVE GENETICS INSTITUTE (Chicago)

- **Single gene cases:** >3300
- **Pregnancy rate:** 30-35%
- **Diagnostic errors:** 3 (liveborns or prenatal samples)

### WHOLE GENOME AMPLIFICATION

- **Single cell 6 pg DNA**
- **Must be amplified (polymerase chain reaction, PCR) may allow assay.**
- **Efficiency not 100%.
- **Pitfall:** Allele drop out reflecting less than 100% amplification.

### Clinical Consequences of Allele Drop Out in Heterozygous Cell

- Mutant allele
- Normal allele
- Both alleles
- Allele dropout
- Allele dropout

[Diagram showing the consequences of allele dropout in a heterozygous cell.]
**X-Linked Hydrocephaly**

![Diagram showing X-Linked Hydrocephaly]

**STR markers initially tested and determined by PCR: Phase Unknown**

<table>
<thead>
<tr>
<th>Markers order</th>
<th>B</th>
<th>C</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS8086</td>
<td>239</td>
<td>241, 241</td>
<td>241, 241</td>
</tr>
<tr>
<td>DXS8089</td>
<td>119</td>
<td>117, 117</td>
<td>117, 117</td>
</tr>
<tr>
<td>DXS7423</td>
<td>138</td>
<td>147, 152</td>
<td>147, 147</td>
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<tr>
<td>DXS9929</td>
<td>131</td>
<td>131, 140</td>
<td>131, 131</td>
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<tr>
<td>DXS8103</td>
<td>130</td>
<td>143, 143</td>
<td>143, 143</td>
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<tr>
<td>DXS9061</td>
<td>119</td>
<td>117, 119</td>
<td>119, 111</td>
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<tr>
<td>DXS9067</td>
<td>154</td>
<td>156, 156</td>
<td>158, 158</td>
</tr>
<tr>
<td>Mutated gene</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>DXS8061</td>
<td>119</td>
<td>117, 119</td>
<td>111, 111</td>
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<tr>
<td>DXS8087</td>
<td>154</td>
<td>156, 156</td>
<td>156, 156</td>
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<tr>
<td>F8 Intron 1A</td>
<td>112</td>
<td>121, 112</td>
<td>114, 121</td>
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<tr>
<td>F8 Intron 9A</td>
<td>204</td>
<td>206, 206</td>
<td>206, 206</td>
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<tr>
<td>F8 Intron 13(CA)</td>
<td>97</td>
<td>106, 106</td>
<td>106, 106</td>
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<tr>
<td>DXS1073</td>
<td>176/182</td>
<td>175, 178</td>
<td>178, 178</td>
</tr>
<tr>
<td>SRY</td>
<td>Y</td>
<td>-</td>
<td>-</td>
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</table>

**Determining Phase of Linked Markers**

Red colored alleles are linked to the mutant allele
Black colored alleles are linked to the normal allele
N = normal

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<td>121, 112</td>
<td>114, 121</td>
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<td>206, 206</td>
<td>206, 206</td>
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<td>106, 106</td>
<td>106, 106</td>
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<td>178, 178</td>
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<td>Y</td>
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</table>
Diagnosis by Linked Markers (STRs): Blastomere analysis for X-Linked Hydrocephaly

<table>
<thead>
<tr>
<th>Marker</th>
<th>Blastomere 1</th>
<th>Blastomere 2</th>
<th>Blastomere 3</th>
<th>Blastomere 4</th>
<th>Blastomere 5</th>
<th>Blastomere 6</th>
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<tbody>
<tr>
<td>DXS8069</td>
<td>115/121</td>
<td>115/117</td>
<td>115/121</td>
<td>115/117</td>
<td>117</td>
<td>121</td>
</tr>
<tr>
<td>DXS7423</td>
<td>138/152</td>
<td>138/147</td>
<td>138/152</td>
<td>138/147</td>
<td>147</td>
<td>152</td>
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<td>131/140</td>
<td>131</td>
<td>131/140</td>
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<td>140</td>
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<td>119/117</td>
<td>119</td>
<td>119/117</td>
<td>119</td>
<td>119</td>
<td>117</td>
</tr>
<tr>
<td>DXS8087</td>
<td>154/156</td>
<td>154/158</td>
<td>154/156</td>
<td>154/158</td>
<td>158</td>
<td>156</td>
</tr>
<tr>
<td>DXS1073</td>
<td>202</td>
<td>202/193</td>
<td>202</td>
<td>202/193</td>
<td>193</td>
<td>202</td>
</tr>
<tr>
<td>F8 Intron 1A</td>
<td>112</td>
<td>112/121</td>
<td>112</td>
<td>112/121</td>
<td>121</td>
<td>121</td>
</tr>
<tr>
<td>F8 Intron 13(CA)</td>
<td>97</td>
<td>97/106</td>
<td>97</td>
<td>97/106</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>DXYS154</td>
<td>182/175</td>
<td>176/178</td>
<td>176/175</td>
<td>182/178</td>
<td>178</td>
<td>178</td>
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<tr>
<td>SRY</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N/G452R</td>
<td>N</td>
<td>FA G452R</td>
<td>FA N</td>
<td>G452R/N</td>
</tr>
<tr>
<td>FA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Red colored alleles are linked to the mutant gene
Black colored alleles are linked to the normal gene
N= normal
FA= Failed amplification

Next Generation Sequencing (automated)

Next Generation Sequencing (Pitfalls)

- Expectation that 98% alignment with reference genome. Thus, “allele dropout” still could exist.

Solutions

1. Increased amplification polymerase chain reaction PCR upon amplification can introduce errors, creating and thus masquerading mutations if in a high proportion reads. Thus, increasing number of PCR cycles no panacea.

2. Linked markers still needed until clinical series proves otherwise.
Other Limitations using NGS

- Unable to test for dynamic mutations trinucleotide repeats (fragile X; myotonic dystrophy)
- Unable to take into account pseudogenes (e.g. 21-hydroxylase adrenal hyperplasia)

CONCLUSION (1)

1. All couples should be offered DNA heterozygote screening for single gene disorders, the precise disorders depending on ethnic backgrounds
2. Universal screening to identify pregnancies at risk for aneuploidy is recommended, now using cell free fetal DNA in maternal blood

CONCLUSION (2)

3. PGD Aneuploidy Testing for chromosomal abnormalities has been proved efficacious, using array CGH
4. PGD to detect single gene disorders is accurate and can be applied widely, but must use linked markers given allele drop out
Genetics of Primary Ovarian Insufficiency (POI)

Marcelle I. Cedars, M.D.
Professor and Director
Division of Reproductive Endocrinology and Infertility
UCSF

Primary ovarian insufficiency (POI) Definition
- Age < 40 years
- Amenorrhea > 4 months
  - after normal cycles
- FSH in menopausal range x 2
  - at least 1 month apart
- 1% of women under the age of 40
- Varying and unpredictable
  - 50% may cycle again
  - 5-10% conceive and delivery

POI: Etiology
- Up to 90% - no immediate etiology identified
- Follicular dysfunction
  - e.g. FSH receptor mutation
- Follicular depletion
- Iatrogenic
- Auto-immune
- Genetic
POI: Diagnosis

- On average, women with a diagnosis of POI have seen 3 physicians prior to diagnosis!
- Differential diagnosis for secondary amenorrhea
  - Polycystic ovary syndrome
  - Hypogonadotropic, hypogonadism
  - Hyperprolactinemia
  - Hypergonadotropic, hypogonadism - Primary ovarian insufficiency

POI: can we predict?

Normative AMH data

LaMarca A; European J Obstet Gynecol & Reprod Biol 2012

Impact of AMH on age at menopause

Broer SL, J Clin Endocrinol Metab 2010
POI: Anti-mullerian hormone (AMH)

AMH: conception to menopause

POI: can we predict it?
Identification of causative genes would allow:
- Prospective identification of at-risk women
- Prospective counseling regarding fertility chances with aging to allow reconsideration of family planning
- Counseling regarding fertility preservation – oocyte/ovarian cryopreservation
- Testing of other female relatives

Average age 51.4 years
Heritability 30-85%
15-30% of POI is familial
The life history of a woman’s oocyte endowment

- **Conception**
  - PGCs: <1,000
  - Oocytes with meiosis I & II: 1 million
  - PGC migration & proliferation: 5-7 million

- **Fertilization**
  - 12 wks: 20 wks: Birth

- **Puberty**
  - Age: 12
  - Ovulation begins: 300,000 oocytes
  - Fertility: 25,000 oocytes

- **Menopause**
  - Age: 51
  - Ovulation ends: <1,000 oocytes

---

**Genetic syndromes**

- Genetic Syndromes associated with general aging
  - Fanconi Anemia (FANCA)
  - Werner syndrome (WRN)
  - Bloom syndrome (BLM)
  - Ataxia telangiectasia syndrome (ATM)

- X chromosome imbalances
  - Including Fragile X

---

**X chromosome**

Simpson 2003, Encyclopedia of Life Sciences
POF2 (proximal) & POF1 (distal) often invoked but at arguable validity.

"POF2" extends from Xq 13.3 to 21.1 and harbors DACH2, DIAPH2 and POF1B.

POF1 extends from Xq26-q28 and is home to XPNPEP2 on Xq25 and the FMR1 gene (Xq27.3)

-------------------------------

X-linked genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGRMC1</td>
<td>Xp22</td>
<td>1/67 (1.5%) Swedish and Italian</td>
</tr>
<tr>
<td>AR</td>
<td>Xq12</td>
<td>2/100 (2%) Indian</td>
</tr>
<tr>
<td>FOX04</td>
<td>Xq11.3</td>
<td>0/116 Tunisian</td>
</tr>
<tr>
<td>DACH 2</td>
<td>Xq21.3</td>
<td>2/257 (0.8%) Italian</td>
</tr>
</tbody>
</table>

-------------------------------

Expression of Fragile X gene FMR-1

![Diagram showing mRNA and FMRP levels with typical, premutation, and full mutation stages.]
Premutation (55-200 CGG Repeats) may expand into full mutation (>200 repeat)

- 10-15% of premutation carriers manifest POF.
- Premutation occurs in 1-5% sporadic POF and 10-15% familial POF.
- Prevalence of POF increases as (CGG)_n increases to 80-99, but plateaus thereafter. POF not increased in full mutation.

**FMR1 Xq27 and POF**

- FOXL2 is a master regulatory of ovarian differentiation and primordial follicle activity, suppressing SRY.
- Expressed in human eyelids and oocytes in humans.
- Mutations in Forkhead Transcription Factor (Forkhead bOX) explain BPES.
- FOXL2 mutations uncommon in isolated POF.

**Gonadal differentiation**

**Blepharophimosis-Ptosis-Epicanthus - BPES**
### Forkhead transcription factors and non-syndromic POF

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXL2 (3q23)</td>
<td>0/118, 0/80, 0/120</td>
<td>Chinese, Indian, Italian</td>
</tr>
<tr>
<td></td>
<td>2/70 (2.9%)</td>
<td>New Zealand; Slovak</td>
</tr>
</tbody>
</table>

### Other forkhead transcription factors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX01 (13q14.1)</td>
<td>1/90 (1.1%)</td>
<td>New Zealand; Slovak</td>
</tr>
<tr>
<td>FOX03 (6q21)</td>
<td>4/302 (1.3%), 15/114 (13.2%), 3/150 (2%)</td>
<td>European, Chinese, French</td>
</tr>
<tr>
<td></td>
<td>2/60 (3.3%)</td>
<td>New Zealand; Slovak</td>
</tr>
<tr>
<td>FOX04 (Xq13.1)</td>
<td>0/116</td>
<td>Tunisian</td>
</tr>
</tbody>
</table>

### Genes expressed in early mouse oogenesis

[Diagram showing gene expression during early mouse oogenesis]
Figla knock-out mice
- Normal germ cell migration and proliferation
- No primordial follicle development
- Loose all oocytes shortly after birth
- Regulates zona pellucida glyco-proteins

Haplo-insufficiency has been shown to cause accelerated oocyte loss in humans

Zhao, Chen, Qin, Shi, Wang, Choi, Simpson, Rajkovic, 2008

Represses primordial follicle activation
- SOHLH 1 – induces oocyte specific genes including Zp1 and Zp3
- SOHLH 1 and 2 regulate kit and gdf-9
- SOHLH 1 and 2 regulate HX9 and NOBOX
### Newborn Ovarian Homebox (NOBOX)

- Homeobox transcriptional regulator binds TAATTG, including GDF9 (member of transforming growth factor gene family)
- Murine knockouts lack follicles
- Expressed in human oocytes from primordial follicle through metaphase II

### NOBOX in humans

96 U.S. Caucasian women, 2 missense mutations (Arg 355 His; Arg 360Alu)
Not present in 96 Caucasian controls
Both mutations in conserved region-functional studies
Arg 355 His disrupts binding DNA and, hence, dominant negative effect.

Arg355His (R355H)


### KIT

- LHX8, SOHLH1 and SOHLH2 upregulate Kit expression, as deficiency in these transcriptional regulators cause a severe down-regulation in Kit expression
- Point mutations (in mice) have fewer germ cells and depletion of germ cells shortly thereafter
- Limited human data
### ATK1
- Promotes primordial follicle activation
- Acts through MTOR – a mechanistic target of rapamycin
- Potential for therapeutic intervention
  - Concern: ubiquitous nature of this pathway
- No human correlate with POF identified

### FOX03
- FOXO3 represses primordial follicle activation and its absence leads to widespread activation
- Mice lacking Pten, a negative regulator of AKT1, have elevated FOXO3 phosphorylation, primordial follicle activation
- FOXO3 was found to act upstream of galactose-1-phosphate uridylyltransferase (Galt)

### Oocyte-specific transcription factors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome Location</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOBOX</td>
<td>7q35</td>
<td>0/200</td>
<td>Chinese</td>
</tr>
<tr>
<td>FIGLA</td>
<td>2p13.3</td>
<td>2/100</td>
<td>Chinese</td>
</tr>
<tr>
<td>POU5F1</td>
<td>6p21.31</td>
<td>1/115</td>
<td>Chinese</td>
</tr>
<tr>
<td>LHX8</td>
<td>1p31.1</td>
<td>0/95</td>
<td>Chinese</td>
</tr>
</tbody>
</table>

(Downstream target of NOBOX)
## Transforming growth factors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFBR3</td>
<td>1p33</td>
<td>2/112 (1.8%)</td>
<td>Chinese</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/133 (0.8%)</td>
<td>Indian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/54</td>
<td>New Zealand</td>
</tr>
<tr>
<td>GDF9</td>
<td>5q31.1</td>
<td>1/200 (0.5%)</td>
<td>Chinese</td>
</tr>
<tr>
<td></td>
<td>Dimerizes with BMP15</td>
<td>6/127 (4.7%)</td>
<td>Indian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/284 (0.7%)</td>
<td>Caucasian (mostly)</td>
</tr>
<tr>
<td>NOG</td>
<td>17q22</td>
<td>1/100 (1%)</td>
<td>French</td>
</tr>
</tbody>
</table>

## POI: the role of transcriptional factors

![Diagram](image.png)


## Steroid and related pathway genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGRMC1</td>
<td>Xp22</td>
<td>1/67 (1.5%)</td>
<td>Swedish, Italian (Progesterone Receptor Membrane Component 1)</td>
</tr>
<tr>
<td>GPR3</td>
<td>1p36.1</td>
<td>2/100 (2%)</td>
<td>Chinese</td>
</tr>
<tr>
<td>NR5A1 (SF1)</td>
<td>9q33</td>
<td>0/82</td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/356 (1.4%)</td>
<td>Asian, Caucasian, Mediterranean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/81 (1.2%)</td>
<td>Tunisian</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/180 (1.7%)</td>
<td>French</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/28 (7.1%)</td>
<td>Indian, Senegalese, African</td>
</tr>
</tbody>
</table>
**Conclusions: candidate gene searches**

- Genetic heterogeneity (many different genes). Varies in frequency by ethnic group and usually infrequent if present (1 or 2%)

- Approximately 25% of non-syndromic POF have identified perturbations

**Conclusions: candidate gene searches**

- Molecular heterogeneity: different nucleotide alterations within same gene

- Multiple molecular mechanisms can be causative but involvement of DNA binding and transcription factors pivotal

**POI: BRCA 1 and 2**

- DNA repair genes
- Women with known mutations have increased risk for breast and ovarian cancer
  - BUT – also primary ovarian insufficiency
  - Increased risk for DNA damage
  - Accelerated loss
BRCA 1/2 and menopause

Genome-wide association study

- 800 POF cases with FSH levels > 40 MIL/ml. Case control design
- Two independent replicates
- 8q22.3, most significant (p < 10^{-5} to 10^{-6})

Qin, Zhao, Xu, Sh; Lu... Simpson, Chen. Hum Molec. Genet 21:430, 2012

Genome-wide association study for POF (Negative log10 unadjusted P-values)
8q22.3 and POF

- "Gene desert" region. No genes detected using repository with location of all known genes.
- Significance <10⁻⁹, but still could connote important ovarian regulatory region. Analogous findings in male sex-determining region upstream of SOX9 (17q24).
  - Deletion in XY individuals → female
  - Duplication in XX individuals → male (testes)

Non-coding DNA and enhancers

- Vast majority (98.5%) of the human genome does not code for proteins.
- Non-coding regions could be important for structural integrity.
- Non-coding regions contain stretches of DNA that bind proteins and RNA molecules, bringing them into juxtaposition to cooperatively regulate function and level of expression of other (protein-coding) genes

Non-coding DNA: promoters and enhancers

- >200,000 enhancers (DHSs) identify sites of DNA binding for transcription. Enhancers identified as DNase I hypersensitive sites (DHSs) readily accessible to enzymatic cleavage as result of the displacement of nucleosomes
- Transcription factors sculpt chromatin landscape for generation of organ-specific mRNA → gene products.
- Cell-type-specific enhancers are often located far away from promoters
miRNAs are short (22 nucleotides), non-coding, endogenous RNA molecules that regulate gene function

1/3 of human genes have conserved miRNA targets

Recent study identified a SNP associated with POF

POI: future investigations

Conclusions

- POF genes with mutations detected number ~ 20, explaining ~ 25% of cases.
- Heterogeneous genetically. For any given gene, only 1-2% of POF cases explained, save fragile X premutation (FMR1).
- Additional genetic explanations for POF will become evident as different ethnic groups and different candidate genes are sequenced.

Conclusions (cont)

- Genes responsible for ovarian failure not necessarily predictable based on known endocrine function.
- Whole genome association studies and whole exome/genome sequencing will reveal novel genes not imagined at present.
- Perturbation in regulatory regions (promoters; enhancers) likely to explain other cases and 8q22.3 locus.
<table>
<thead>
<tr>
<th>Current evaluation for genetic etiologies – non-syndromic</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Family history</td>
</tr>
<tr>
<td>▪ Fragile X</td>
</tr>
<tr>
<td>▪ Karyotype</td>
</tr>
<tr>
<td>▪ Microarray analysis to detect submicroscopic chromosome abnormalities</td>
</tr>
<tr>
<td>▪ Whole exome/genome sequencing</td>
</tr>
</tbody>
</table>
Epigenetic Modifications Due to Environmental Factors

ASRM-ESHRE Exchange Pre-Congress Course 8: Practical Applications of Clinical and Basic Science Genetics to Reproductive Medicine

Linda Giudice, MD, PhD
University of California, San Francisco
29th Annual Meeting, ESHRE
Munich
June 29-July 2, 2014

Disclosures

• Quest Diagnostics, Academic Associate
• American Society for Reproductive Medicine, President 2012-1013
• World Endometriosis Society 2014, President
• World Endometriosis Research Foundation Board of Directors, 2011-2014
• March of Dimes Scientific Advisory Board, Member, 2013-2016
• Low Cost IVF Foundation, Board Member, 2011-2015

Learning Objectives

• Review determinants of reproduction
• Understand what epigenetics is
• Learn about the evidence linking environmental factors and epigenetics to health that can affect reproduction and reproductive processes directly
Determinants of Reproduction

The Epigenome

“epi” (Gr. (επί) over, outside of, around)

- Epigenetics is the study of heritable changes in gene expression not caused by changes in the DNA sequence.
- Jean Baptiste Lamarck (1744-1829) formulated the theory that the environment shapes genes and these changes are passed on to offspring.
- Charles Darwin (1809-1882) postulated that genes are not changed by the environment and formulated natural selection.
- Word coined by G.H. Waddington 1942

The Epigenome

- Mechanisms
  - DNA methylation and chromatin remodeling
  - Histone modifications and chromatin remodeling
  - Small interfering RNAs (e.g., miRNAs)
Epigenetics and Biological Processes

- Reprogramming in early development
- X-inactivation
- Cancer
- Obesity
- Biobehavioral
- Reproduction

- Mostly DNA methylation at CpG sites investigated
  CpG site methylation = usually gene repression
  CpG site demethylation = usually gene expression

Developmental origins of health and disease (DOHaD)

Human Disease Trends

- Over the past 50-100 years marked changes in chronic diseases and changes in reproductive potential have been observed, including:
  - Increases in
    - obesity
    - diabetes
    - cancers (e.g., breast, prostate)
  - Declining age at puberty and menarche

Stress, Diet, Environmental Chemicals: Indirect Effects on Reproduction?
Critical Windows of Susceptibility

- Pre/peri-conception
- Prenatal
- Postnatal
- Childhood
- Adolescence
- Adulthood

Considerations for chemical exposures
- dose
- duration
- mixtures

Programming ("Barker Hypothesis"): developmental origins of adult health and disease. Process in which a stimulus or insult at a critical/sensitive period in development or perinatal life has permanent effects on structure, physiology, and metabolism and is transgenerational. Barker 1992

Some Definitions about Generations

F0 generation = Parent
F1 generation = Fetus
F2 generation = Germline of fetus
F3 generation = Trans-generational (no direct exposure)

Maternal Stress Influences Fetal Brain Development via Epigenetic DNA Methylation

Trichostatin rescues the phenotype!
Obesity, Epigenetics, and Gene Regulation

The Agouti Mouse

Genetically identical mice but different color (brown, yellow) and size (normal, obese)

Agouti gene product binds to melanocortin R blocking black pigment involved in feeding behavior involved in weight set point

Normal healthy mice – agouti gene is methylated and is off. Yellow, obese sisters – agouti gene is unmethylated and is on. Higher risk of diabetes and cancer as adults.

Maternal Diet Influences Fetal Development

Changing Epigenetic Marks

Bisphenol A (BPA) to pregnant dams:
- higher ratio of yellow, obese progeny than expected
- global DNA hypomethylation
- agouti gene DNA methylation sites 30% decrease
Obesity, Epigenetics, and Gene Regulation
The Agouti Mouse

Environmental protection
Methyl donors (folic acid, Vit B12) rescue the phenotype
increased DNA methylation
normal ratio of pups

Obesity Develops in F3 Generation with F0
Generation Exposure to Plastics (BPA, Phthalates)

Reproductive outcomes depend on maternal obesity.

Environmental Chemicals: Direct Effects on Reproductive Processes
Germ cells migrate to the fetal ovary and initiate meiosis

Can chemicals affect early meiotic events?

- The only data comes from bisphenol A (BPA) exposure, a weak estrogen
- BPA affects synapsis and recombination
  - increased oocyte loss
  - problems in creating chromosomally normal eggs
  - Susiarjo et al., 2007

Maternal bisphenol A (BPA) exposure disrupts early meiotic events in the fetal ovary

- Susiarjo et al., 2007

- Fetal BPA exposure increases the likelihood of chromosomally abnormal eggs and embryos
  - A grandmaternal (F3) effect
  - DNA methylation?

Meiotic progression and recombination are affected by Bisphenol-A during in vitro human oocyte development

Fetal ovarian follicles were incubated with BPA (1μM, 3-30μM) or E2 (1μM, 30μM)

Main findings BPA:
  - decreased oocyte survival
  - increased MLH1 foci (cross-over marker)

Conclusion:
BPA can affect key events in meiotic prophase and oocyte survival in fetal ovary.

DNA methylation?
As BPA dose increased:
• decrease in % oocytes progressed to MII (P< 0.002)
• increase in % of degenerated oocytes (P< 0.01)
• Increase % oocytes that had undergone spontaneous activation (P< 0.001).

Among MII oocytes, as the BPA dose increased, there were:
• decreased incidence of bipolar spindles (P< 0.0001)
• Decreased incidence of aligned chromosomes (P< 0.02)

DNA methylation?

Bisphenol A Exposure Disrupts Genomic Imprinting

• Maternal BPA exposure during late stages of oocyte development and early stages of embryo development significantly disrupted imprinted gene expression in embryonic day (E) 9.5 and 12.5 embryos and placentas.
• Affect genes: Snrpn, Ube3a, Igf2, Kcnq1ot1, Cdkn1c, and Ascl2 (mutations and aberrant regulation of these genes are associated with imprinting disorders in humans).
• Exposure outside of the epigenetic reprogramming window did not cause significant imprinting perturbations.
• Are these changes associated with changes in DNA methylation?

BPA Exposure Reduces Genome-Wide DNA Methylation in the Placenta, Placentomegaly and Small Labyrinth

• Majority of affected genes were expressed abnormally in the placenta with DMRs including the Snrpn imprinting control region (ICR) and Igf2 DMR1.
• Exposure significantly reduced genome-wide methylation levels in the placenta, but not the embryo.
Transgenerational Effects of Pesticides, EDCs, and Jet Fuel

Gestating F0 generation female rats exposed to plastics (BPA, phthalates), EDCs, jet fuel during embryonic days 8-14 (gonadal sex determination). Incidence of adult onset disease was evaluated in F1 and F3 generation.

Findings:
- Significant increases in the incidence of total disease/abnormalities in F1 and F3 generation male and female animals from plastics lineages.
- Pubertal abnormalities, testis disease, obesity, and ovarian disease (primary ovarian insufficiency and polycystic ovaries) were increased in the F3 generation animals.
- Plastics lineage F3 generation sperm epigenome: 197 differential DMR ("epimutations") in gene promoters correlating with identified pathologies.

Conclusion: A BPA/phthalate mixture can promote epigenetic trans-generational inheritance of adult onset disorders.

F0 Exposure to Plastics Results in F3 Pubertal Abnormalities, Primordial Follicle Loss, Polycystic Ovaries and Tumors

Trans-generational Effects of Pesticides, EDCs, and Jet Fuel on DNA Methylation Regions

Differential DNA methylation regions (DMR) chromosome locations.
BPA and Other Chemicals Show Epigenetic Effects Across Generations

TCDD and JP-8 (jet fuel mixture) exposure of F0 resulted in abnormalities in the F3 generation:
- Primordial follicles decreased 30-40%.
- 2 day earlier puberty (=2 years in humans).
- 50-60% lower T levels in males.
- Global DNA methylation changes
- ? Gene expression changes?

Some things to ponder…

What are the mechanisms underlying effects of parental stress, obesity, and environmental chemicals/endocrine disrupters, air pollution, and heavy metals on human reproduction?

Can we rescue the phenotype(s)?

Does the epigenome change with age?

What do we tell our patients?

What Do Obstetricians Ask About?

100%  < 20%

*Bigger fish to fry*  “Won’t know what to say”  *Pandora’s Box*
Strength of the Evidence

2008

2009

2012

Thank You!
References

Genetic and Epigenetic Factors Affecting Embryo Development and Implantation/Placentation: Lessons from Clinical ART

Christos Coutifaris, MD, PhD
The Nancy and Richard Wolfson Professor of Obstetrics and Gynecology
Chief, Division of Reproductive Endocrinology and Infertility

Perelman School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania, USA

Low Birthweight and Preterm Birth

Can we identify patients at risk for adverse perinatal outcomes following ART?

Can we identify modifiable factors that may prevent these adverse perinatal outcomes following ART?
Educational Objectives

- Review exposures associated with Assisted Reproductive Technologies (ART)
- Describe the time course for the establishment of epigenetic marks during gametogenesis and embryo development
- Describe the rationale for focusing on research on implantation and placentation
- Start the discussion on understanding the molecular basis of adverse perinatal outcomes associated with some infertility treatments

Modified from Woo & Patti, Cell Metabolism, pp. 8-10, July 2008

Egg and Embryo Manipulations

Exposures: Temperature - Light - Oxygen - Embryo manipulation/pipetting

In vitro conditions / Culture media

Page 56 of 138
Epigenetic reprogramming during mammalian development

**Assisted Reproductive Technologies**

- A model to study epigenetic regulation of development in humans and experimental animals?

Loss of imprinted expression occurs primarily in the placenta after pre-implantation culture

*Modified from Smallwood and Kelsey, 2012*

*Loss of imprinted expression in Embryo and Placenta* 

*Moan et al. 2004; Richard Schultz and Maris Bartolomei’s Laboratories*
At least 10% expression from repressed allele to be counted as bi-allelic

In-vitro culture of mouse embryos results in bi-allelic H19 expression

![Graph showing H19 expression in bio-allelic embryos over time.](image)

Whitten's KSOM+aa

R. Rivera in Bartolomei lab

## Mean Methylation of CpG sites

- **Cord blood**
  - 358 CpG sites differed (in vivo vs. in vitro)
  - 277 (77%) more methylated (in vitro)
  - 81 less methylated

- **Placenta**
  - 246 CpG sites differed (in vivo vs. in vitro)
  - 154 (63%) less methylated (in vitro)


## Differential methylation – placenta

Genes which differ between ART and Control placenta in two or more independent experiments

<table>
<thead>
<tr>
<th>AMY2A</th>
<th>CPE</th>
<th>GDF3</th>
<th>MEG3</th>
<th>SERPINF1</th>
<th>VHL</th>
</tr>
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<tbody>
<tr>
<td>BMP7</td>
<td>CYCS</td>
<td>GRB10</td>
<td>MEST</td>
<td>SFRP2</td>
<td>WNT9B</td>
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<tr>
<td>C1QBP</td>
<td>DLX2</td>
<td>GRPEL1</td>
<td>MRPL12</td>
<td>SHC3</td>
<td>WT1</td>
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<td>CART1</td>
<td>DNAJA1</td>
<td>HYMA1</td>
<td>MSX1</td>
<td>SLIT1</td>
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<tr>
<td>CBL</td>
<td>EABBP2</td>
<td>IGF1R</td>
<td>MYC2</td>
<td>SRC</td>
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<tr>
<td>C59</td>
<td>FABP2</td>
<td>IGF2AS</td>
<td>PAX4</td>
<td>STAT3</td>
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<tr>
<td>CDC25A</td>
<td>FAU</td>
<td>IMPDH2</td>
<td>PAX6</td>
<td>TMSL3</td>
<td>H19</td>
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<td>CDH5</td>
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<td>TNNI2</td>
<td>IGF2</td>
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<td>CDKN1C</td>
<td>FMR1</td>
<td>MAPK13</td>
<td>RHOC</td>
<td>UBE2D1</td>
<td></td>
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<tr>
<td>CGB5</td>
<td>GATA4</td>
<td>MAPRE2</td>
<td>SCGB3A2</td>
<td>UBE3A</td>
<td></td>
</tr>
</tbody>
</table>

(Katari, Turan et al. 2009 Hum Mol Genet)
Scatter plots of transcript levels for two genes in which mean transcript levels differ in *in vitro* and *in vivo* populations

Does the expression of any of the genes correlate with birth weight?

DNA methylation profiling identifies epigenetic signatures correlated with birth weight

*Turan et al., (BMC Med Genomics. 5:10, 2012)*

**Differential methylation – placenta**

Genes which differ between ART and Control placenta in two or more independent experiments

(Natari, Turan et al. 2009 Hum Mol Genet)
GRB 10 and Growth

- Adaptor molecule that negatively regulates fetal growth
- Functions downstream of EGFR, IGF1R and IR
- Deletion of the maternal copy of Grb10 leads to 40% INCREASE in fetal weight and a 30% increase in placental weight
- Deletion of the paternal copy has no effect on fetal weight

Charalambous et al. 2010
Liu and Roth 1995

GRB10 and Growth

![Diagram showing normal and maternal Grb10 mutant placenta]

- Normal Placenta
  - Maternal copy Grb10
  - H3K37ME3
  - Normal placenta
  - Maternal expression
  - NORMAL GROWTH
- Maternal Grb10 Mutant
  - No Maternal expression
  - OVERGROWTH
  - H3K37me3

Garfield et al. Nature 2011

GRB 10 in placenta

- Trophoblast cells
- Endothelial cells
- Other
**Experimental Model**

Control female mated with vasectomized male  
Superovulated female mated with vasectomized male

10 naturally conceived blastocysts

Sacrifice at E18.5

---

**Five control (white bars) and eight SO dams (black bars) were sacrificed at E18.5.**

---

**Placenta histology**

Control  
Superovulated recipient

---
Histopathological examination of placentas from control and SO recipients.

Placental expression: Grb10

Serum Estradiol and VEGF in mice following natural mating (white bar) or mating following superovulation with gonadotropins (black bar).
Summary I

- Singletons
- Clinical/translational studies have suggested
  - Placental / Placenta problems (?)
  - Epigenetic regulation problems (?)
- Mouse work
  - Biallelic expression of imprinted genes
  - "Confined" to the placenta
  - Prolonged culture effects on embryo proper
  - GRB 10 and the role(s) of superovulation and VEGF

Summary II

- Human Work
  - Variability in placenta
    - (Normal vs. ART-related?)
    - Consequences?
  - Methylation Microarray data
    - Differences
    - Significance
- GRB10, MEST, GRPEL1, DNAJA1, GRINC1, MSX1, etc.
- The mouse model
- Implications for long term health?
The Most Important Slide

- Nahid Turan, PhD, Sunita Katari, MD
  Raffi Chalian, MD, Mike Foster, BA, Harry Chatzicharalampous, MD, Erica Pocharska, BA
  Christopher Morse, MD, Emelia Bachman, MD

- Karine Chung, MD, MSCE
  Suleena Kalra, MD, MSCE

- Carmen Sapienza, PhD
  Monica Mainigi, MD
  Kurt Barnhart, MD, MSCE
  Marisa Bartolomei, PhD
  Richard Schultz, PhD

- NIH (RO1-HD048730; U54-HD068157; T32-HD40135)
A 36 year old G1 pregnant patient discussed all of the options for aneuploidy screening and elected to have CVS for her singleton pregnancy. She was informed that the karyotype of 50 cells showed 45,X(25 cells)/46,XY(25 cells). You inform her of all phenotypic possibilities for this finding at prenatal diagnosis and state that the vast majority of babies exhibit:

• Turner female with bilateral streak gonads and normal female external genitalia
• Turner female with a unilateral abdominal testes, contra lateral streak gonad, and mild clitoramegaly
• Turner female with a unilateral streak gonad, a contra lateral gonad descended into a labio-scrotum, and marked clitoramegaly
• Normal male phenotype
45,X
- 1 in 2500 females;
- 99% don’t survive > 28 wks;
- 15% of SAB;
- nearly 80% maternal X.

Karyotype of Patients with TS

<table>
<thead>
<tr>
<th>Classical Turner Syndrome (45,X)</th>
<th>24*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-cell Lines:</td>
<td>12</td>
</tr>
<tr>
<td>45,X/46,XY</td>
<td>1</td>
</tr>
<tr>
<td>45,X/47,XY</td>
<td>11</td>
</tr>
<tr>
<td>Structural abnormalities of X:</td>
<td>25*</td>
</tr>
<tr>
<td>Isochromosome</td>
<td>5*</td>
</tr>
<tr>
<td>46,X,i(Xq)</td>
<td>5*</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)</td>
<td>1</td>
</tr>
<tr>
<td>45,X/46,X,del(Xq)</td>
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</tr>
<tr>
<td>45,X/46,X,i(Xq)/46,i(Xq),I(Xq)</td>
<td>1</td>
</tr>
<tr>
<td>45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq)</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>46,X,i(Xq)/q(ter-p22)</td>
<td>1*</td>
</tr>
<tr>
<td>45,X/46,X,del(Xq13)</td>
<td>2</td>
</tr>
<tr>
<td>46,X,q+</td>
<td>1*</td>
</tr>
<tr>
<td>45,X/46,X,q+</td>
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</tr>
<tr>
<td>45,X/46,X,r(X)</td>
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<tr>
<td>45,X/46,X,r</td>
<td>1</td>
</tr>
<tr>
<td>Other X mosaic cell lines:</td>
<td>8</td>
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<tr>
<td>45,X/46,XX</td>
<td>7</td>
</tr>
<tr>
<td>45,X/47,XXX</td>
<td>1</td>
</tr>
</tbody>
</table>

*Single cell lines

Privation of X Chromosomal Material

- Short stature
- Turner stigmata
- Abnormal lymphatics and associated deformations
- Somatic abnormalities
- Endocrine and autoimmune abnormalities
- Ovarian failure
Karyotypic-Phenotypic Correlations (?)

- Often thought dose dependent (i.e., findings more common with higher % 45,X cells)
- Recently when ascertainment considered, better such correlations (those found prenatally fewer findings)
- Gene imprinting suggested: parent of origin of X chromosome partially explains findings (renal abn exclusive with maternal X)
Premature Loss of Germ Cells

<table>
<thead>
<tr>
<th>GESTATION WEEKS</th>
<th>GENETIC CONTROL 46,XX</th>
<th>GONADAL DEVELOPMENT</th>
<th>INTERNAL GENITAL DUCTS</th>
<th>EXTERNAL GENITALIA</th>
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<tbody>
<tr>
<td>1</td>
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<td>INDIFFERENT GONAD</td>
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<td></td>
<td>MULLERIAN AND WOLFIAN DEVELOPMENT</td>
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<td>OVARIAN DEVELOPMENT</td>
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<td>4</td>
<td></td>
<td>CORTICAL PROLIFERATION (OOGONIA)</td>
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<td>5</td>
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<td>MEIOSIS I (OOCYTES)</td>
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<tr>
<td>6</td>
<td></td>
<td>PRIMORDIAL FOLLICLES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>PRIMARY FOLLICLES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>MAXIMUM GERM CELLS (7 MILLION)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>GERM CELLS (2 MILLION)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>OVARIAN DETERMINANTS</td>
<td></td>
<td></td>
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<td>11</td>
<td>1. X-GENES</td>
<td>PRIMORDIAL FOLLICLES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2. AUTOSOMAL GENES</td>
<td>FEMINIZATION</td>
<td></td>
<td></td>
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<tr>
<td>13</td>
<td></td>
<td>MULLERIAN COMPLETION</td>
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<tr>
<td>14</td>
<td></td>
<td>WOLFIAN REGRESSION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>SEENS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TIMETABLE OF NORMAL FEMALE SEXUAL DIFFERENTIATION**

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Loss of Oocytes With Age

- 2 million oocytes at birth
- Total loss over time:
  - Birth
  - Puberty
  - 50 yrs

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Usual Loss of Oocytes With Age

![Graph showing the usual loss of oocytes with age.]

- Birth: 2 million oocytes
- Puberty: Total loss
- 50 yrs: Accelerated loss

Accelerated Loss of Oocytes With Age for Women with Turner Syndrome

![Graph showing the accelerated loss of oocytes with age for women with Turner Syndrome.]

- Birth: 2 million oocytes
- Puberty: Total loss
- 50 yrs: Accelerated loss

A = 85%
B = 15%
C = 5%

Total loss
X Chromosome Loci Important in Maintenance of Germ Cell Complement

- Translocation studies
  - POF1 (Xq26-q28), candidate genes (HS6ST2, TDPF3, GPC3) and known gene (FMR1)
  - POF2 (Xq13.3-q22), candidate genes (DIAPH2)
- TGF-β Superfamily genes: bone morphogenetic protein 15 (BMP15) (Xp11.2)
- Other candidates: DEAD-box 3 (DBX), Ubiquitin-Specific Protease 9 (USP9X) both Xp11.4 (escapes inactivation)
45,X/46,XY Gonadal Dysgenesis  
(5 phenotypes at birth)

1. Bilateral streak gonads (normal female phenotype)
2. Unilateral streak, contra-lateral intra-abdominal testis (clitoromegaly)
3. Unilateral streak, contra-lateral descended testis (ambiguity)
4. Bilateral descended testes (normal male phenotype)
5. Bilateral intra-abdominal testes (normal male/ambiguous)
The majority of 45,X/46,XY found in-utero are normal males.

- Wheeler et al, AJMG, 1988;29:565
  - 6 prenatally dx (all normal); 9 postnatally dx (7 AG, 2 primary amenorrhea)
- Hsu, LYF., Prenatal Diagnosis 1989;9:31
  - 54 prenatally dx (89.4% normal male phenotype; 6.4% asymmetric internal gonads; 2 cases ? Abn)
- Chang et al, AJHG, 1990, 46:156 (Intl survey)
  - 76 cases prenatally diagnosed (95% normal male genitalia, 4/11 testes biopsied abnormal; 5% abn, 3 hypospadias, 1 clitoramegaly. Other Turner abn in 5 cases)

Accelerated Loss of Oocytes With Age for Women with Turner Syndrome

- Birth: 2 million oocytes
- Puberty: 85% loss
- 50 yrs: 15% loss
- Total: 5% loss
What outcomes/options have been reported for TS women with ovarian function?

A. Spontaneous pregnancy
B. IVF with the patients own oocytes and pregnancy
C. Superovulation and oocyte cryopreservation for later use
D. All of the above.

Ovarian Function in TS Patients

- 85% will not have ovarian function for puberty or beyond
- 15% have limited function for pubertal development
- 5% have function for spontaneous menstrual periods of limited time (rarely beyond age 30 years)
- 1% have function for puberty, menstrual periods and pregnancy
54 Spontaneous Pregnancies in Women with Turner Syndrome

- 5 Reports - 45,X patients
- 7 Reports - 45,X/46,XX patients
- 7 Reports - 45,X/46,XX/47,XXX

Dewhurst, 1978

Spontaneous Pregnancy in Turner Syndrome Patients

6 new patients
Review of 160 pregnancies/ 74 patients in literature
- 29% spontaneous abortion
- 7% perinatal deaths
- (approx) 20% TS
- 38% children with 46,XX or 46,XY

Tarani et al, Gynecol Endocrinol 1998;12:83

Loss of Oocytes With Age Turner Syndrome

2 million
Total loss

A = 85%
B = 15%
C = 5%

Birth Puberty 50 yrs
Pregnancy in Turner Syndrome Patient Following Ovarian Stimulation and IVF

- 33 y/o known 45,X/46,XX, and regular cycles
- Turner physical findings
- Laparotomy: resection of right endometrioma, myomectomy, resection communicating left uterine horn and streak ovary
- 4 cycles infertility treatments (OI/IUI)
- IVF: E2 3095 pg/ml, 21 oocytes, 13 fertilized, 5 embryos transferred, pregnant with 46,XY
- Ovarian biopsy: 46,XX (147 cells)

Dilkoff et al, JARG, 1996

Oocyte Freezing in Rare Menstruating Patients

  28 yo mosaic TS, COH, 15 oocytes retrieved, 13 cryopreserved.

- Huang et al, Hum Reprod 2008;23:336
  16 yo 45,X/46,XX, ovarian biopsy, 11 oocytes, 8 matured in-vitro and cryopreserved

Loss of Oocytes With Age Turner Syndrome

<table>
<thead>
<tr>
<th>Year</th>
<th>Loss of Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>A = 85%</td>
</tr>
<tr>
<td>Puberty</td>
<td>B = 15%</td>
</tr>
<tr>
<td>50 yrs</td>
<td>C = 5%</td>
</tr>
</tbody>
</table>

A = 85%
B = 15%
C = 5%

2 million
Total loss
Ruptured Ectopic Pregnancy in Patient with Turner Syndrome Having Achieved Pregnancy Through Donor Oocyte

All of the following scenarios for Turner patients who become pregnant have been reported EXCEPT:

A. Patient known to have hypertension and dilated aorta allowed to become pregnant by donor oocyte and died from aortic rupture.
B. Patient with triplet pregnancy from donor oocyte died at 24 weeks gestation from aortic rupture.
C. Patient found to have dilation of aorta during donor oocyte pregnancy who died three years after delivery.
D. Patient with spontaneous pregnancies at ages 36 and 38 years, who dissected during the third trimester of her second pregnancy and was subsequently diagnosed with mosaic Turner syndrome.
E. Patient with no known risk factors unexpectedly developed dissection and rupture of the aorta and died during singleton donor oocyte pregnancy.
Largest IVF/Donor Oocyte Series for Turner Syndrome Patients

- 17/29 women had 28 pregnancies (U/S) (42%/cycle)
- 10 women: 1 pregnancy; 4 women: 2 pregnancies; 2 women: 3 pregnancies; 1 woman: 4 pregnancies (only 1 with viable pregnancies)
- 2 triplets; remaining singletons
- 50% spontaneous abortion; 1 ectopic
- 13 (19.1%) “take home” baby rate/cycle

Khaustir, 97

Aortic Dilation, Dissection and Rupture in Non-pregnant Turner Syndrome Patients

- Rev Fac Cien Med Cordoba, 1961
- J Thorac Cardiovasc Surg, 1964
- Am J Cardiol, 1969
- J Pediatr, 1971
- J Pediatr, 1975
- JAMA, 1972
- Circulation, 1978
- Am J Dis Child, 1979
- Am Heart J, 1982
- Postgrad Med J, 1982
- J Med Genet, 1983
- Clin Cardiol, 1984
- J Pediatr, 1986

Incidence of aortic dissection in Turner syndrome by age group

This is a summary of Danish epidemiological data. The frequency of aortic dissection is greater in women with Turner syndrome at all ages compared with the general population (500-2000 X), with the highest incidence in young adults.

85 Cases Of Aortic Dissection
Carlson and Silberbach, J Med Genet 2007;44:745

- 1961 - 2006
- Average age 30.7 yrs (usual in females is 68 years, male to female ration 3.2:1)
- Karyotype: 45,X (39/49); mosaics (10/49)
- Dissection: proximal 55%; distal 23%
- CHD 69% (51/74): C (24/51), BAV (15/51), both (9/51)
- Hypertension 54%
- No risk factors 21%
- Cystic medial necrosis (22/22)

85 Cases Of Aortic Dissection
Carlson and Silberbach, J Med Genet 2007;44:745

Symptoms
- Abdominal pain
- “heart burn”
- Back or shoulder pain
- Change in phonation (traction on recurrent laryngeal nerve)

TS NIH Consensus Study Group

- Baseline echocardiogram for CHD
- Frequent BP checks
- In absence of CHD or HTN, MRI measurements of aorta (T-1 images, level of R pulmonary artery, perpendicular to long axis of AA)
- Repeat echocardiogram or MRI q 5 - 10 years

JCEM 2007;92:10 - 25
Elongated transverse arch of the aorta and associated anomalies in a woman with Turner syndrome revealed by Gd-enhanced 3D MRA

(a) flat/elongated arch; (b) aberrant R (*) subclavian; (c) persistent LSCV.

Ascending Aorta Measurements: Historical Thresholds for Repair

- 5.5 cm general population
- 5.0 cm Marfan syndrome
- 4.5 cm Loeys-Dietz syndrome
Aortic Dilation and Dissection in Turner Syndrome

- MRI evaluation of aorta 166 TS women
- When normalized to body surface area, aorta measurements abnormal in 32% of TS women (> 95 percentile)
- Aortic size < 5 cm may be at increased risk for TS patients (in subset > 3.5 cm: 3.69, 4.63, 4.78 ruptured)
- Aortic size index > 2.0 cm/m² close surveillance (> 2.5 highest risk)

Circulation 2007;116:1663

Donor Oocyte for Turner Syndrome: 4 Deaths During Pregnancy

- Two deaths reported in Letter to Editor, Fertil Steril, 1997: one hypertension and slightly dilated aorta.
- One death reported as case report, 1998: hypertension; dx of preeclampsia; died postoperative.
- One death, 2001, reported in pregnancy experience with 50 coarctation patients.
Mosaic Turner Syndrome
Patient with postpartum aortic dissection and survival

- DeBakey type IIIb aortic dissection (not involving proximal aorta)
- Two weeks post cesarean section for eclampsia
- No aortic dilation or other cardiovascular malformation
- Distal extension and uncomplicated dissection: treated medically

Weytjens, C et al., J Cardiovasc Surg 2000;41:295-7

Total (≥ 10) Aortic Dissection Cases Pregnancy Related
Carlson Silverbach Literature Review
(J Med Genet 2007;44:745)
- 6 cases during pregnancy (1 with prior spontaneous pregnancy)
- 1 case, 1 year after ART pregnancy
- 1 case, missed BAV, found 16 weeks, dissection/ death 38 weeks
Bondy C, Rosing D, Reindollar R (Fertil Steril 2009; eLetter to Editor)
- 1 patient, dilation during pregnancy, dissection/ death 3 years later

Unpublished Cases (at least one)

A Survey of 259 Donor Oocyte Programs

- 134 (52%) responses
- 146 Turner patients
- 72 (49%) patients screened with echocardiography
- 6 (8.3%) abnormal echocardiography
A Survey of 259 Donor Oocyte Programs


146 Turner patients
- 101 pregnancies (1 spontaneous abortion)
- 17 multiples, all delivered at least 1 live-born
- Live birth rate: 65% (94/144)
- 7% SAB rate

A Survey of 259 Donor Oocyte Programs: Estimated Death Rate


101 pregnancies / 52% of US programs
- estimated 194 pregnancies (max) / 100% US programs
- Estimated death rate: 4/194 (2%)

Survey by French Study Group for Oocyte Donation


93 TS pregnancies identified > 20 weeks gestation (DO)
- 2 died of aortic dissection and rupture (2.2%)
American Society of Reproductive Medicine (ASRM) Practice Guidelines

Unpublished Concerns

- National Turner Syndrome Society Meeting (8/08)
  - C Bondy
    - Evidence during pregnancy of progressive dilation of aorta
    - Deaths after pregnancy
    - MRI evaluation of aorta is more sensitive than cardiac echo
    - Unpublished deaths during pregnancy
  - Silverbach
  - Unpublished deaths during pregnancy
  - Reindollar
  - Unpublished deaths during pregnancy

ASRM Practice Guidelines: Suggested Revisions (Bondy C, Rosing D, Reindollar R; Fertil Steril, 1990 eLetter to Editor)

1. Women without known CHD require full CV evaluation with MRI at center with expertise (particular attention to AV, aortic diameter, vascular tree)
2. Absolute contraindications to pregnancy
   - known CHD or hypertension
   - aortic size index > 2 cm²/m²
3. TS women may have latent vasculopathy, increasing risk during or following pregnancy in absence of absolute contraindications.
ASRM Practice Committee Report
(2008 Recommendations)

Specific recommendations for surveillance in women with Turner syndrome during pregnancy include:

- Treatment of hypertension.
- Periodic echocardiography and consultation with a cardiologist.
- Women in stable condition having an aortic root diameter less than 4 cm may attempt vaginal delivery under epidural anesthesia.
- Women exhibiting baseline or progressive aortic root dilation should have an elective cesarean delivery prior to the onset of labor under epidural anesthesia.

ASRM Practice Committee Report
(2008 Recommendations)

SUMMARY

- The risk of death during pregnancy may be increased as much as 100-fold for women with Turner syndrome.
- In general, Turner syndrome is a relative contraindication to pregnancy.
- Cardiology consultation and careful screening for cardiac abnormalities is a prerequisite for any planned attempt at pregnancy via oocyte donation.
- An echocardiogram revealing any significant abnormality represents an absolute contraindication to attempting pregnancy in a woman with Turner syndrome.
- Women with Turner syndrome having a normal echocardiogram who intend to attempt pregnancy require careful observation and frequent formal reassessment throughout gestation.

French Government Requested Recommendations

- After death of 2 French TS women during pregnancy

Recommendations

- Check up before pregnancy (multifaceted including PE, endocrine tests, LFTs, Renal FTs, cardiac echo, mandatory MRI)
- Contraindications to Pregnancy:
  - Hx of aortic surgery: aortic dilatation > 25 mm/m2 or 35mm
  - Hx of dissection
  - Coarctation
  - Hx of BAV surgery (BAV in absence of aortic dilatation)
French Government Requested Recommendations (cont.)

- Detailed Counseling
- In absence of contraindications, DO allowed with:
  - yearly US and, if > 10% increase A dilation, MRI
  - SET
  - Cardiac echo end of 1st and 2nd trimester and monthly 3rd trimester
  - MRI if increase in A size >10%
  - If dissection: < 25 weeks, emergency surgery; > 25 weeks cesarean followed by aortic root surgery
  - If increased size 10% or > 25mm/m2: admit, 25 - 34 wks: lung maturation followed by cesarean
  - Cardiac echo 5 - 8 days post delivery

Turner Syndrome:
Unanswered Questions

- What is the real maternal mortality rate? (Some think likely greater than our 2% estimate)
- Is aortic dissection and rupture of the same pathophysiology as in Marfan syndrome or is there a vasculopathy specific to Turner syndrome that increases the risk?
- Are there phenotypes of TS that have different risks?
- Does the physiology of pregnancy damage the aorta (and other vessels) and increase the risk of subsequent rupture?
- Should MRI be performed more frequently?
- Should Turner syndrome be an absolute contraindication to pregnancy?

TS Deaths Following “Successful” Pregnancy

- Gravholt CH, Landin-Wilhelmsen K, Stochholm K et al. (Cardiol Young, 2006)
  1 patient, died 1 year after ART twin pregnancy

- Bondy C, Rosing D, Reindollar R (Fertil Steril 2009; eLetter to Editor)
  1 patient, dilation during pregnancy, dissection/ death 3 years later

106 TS women, 122 deliveries and 131 newborns after DO from Finland, Denmark, Sweden, 1992-2011. SET 70%, twins 7.4%

- Main finding: TS pregnancies carry substantial risk, particularly 35% of pregnancies hypertensive disorders (20% pre-eclampsia)
- Potentially life threatening complications 3.3%: AD (1); tricuspid/ MV regurgitation (1); HELLP in patient with mechanical valve; heart failure (1); post-partum hysterectomy from hemorrhage.
- CV outcomes:
  - 10 pregnant with CVD;
  - One previously normal CV developed mild LVD;
  - One previously normal CV exam; chest pain several days before delivery. CT misread as normal, acute symptoms at 20 days, aortic dissection, successful surgical correction 1.5 years later.
- 22% hypothyroidism
- 2.3% perinatal mortality


Swedish population study, registry, 124 TS women, birth between 1973 and 2010 vs. control group

- No mortality with childbirth (? effect of low risk and need for larger study population, enforced guidelines)
- Morbidity from CV disease vs. control group
  - Before pregnancy increased (HR 3.83; 95% CI 1.02 - 14.43)
  - During pregnancy or within 1 year of delivery increased (HR 5.78; 95% CI 1.94-17.24)
  - ≥ 1 years after pregnancy similar (HR 1.91; 95% CI 0.74-4.96)
- Among TS patients, morbidity similar ≤ 40 years with and without childbirth (HR 1.02; 95% CI 0.53-2.25)
- Of Interest, thyroid disease increased during or within 1 year of pregnancy (HR 5.78; 95% CI 1.94-17.24)

Turner Syndrome: How Do We Reduce the Risk?

- Registry for Turner syndrome patients undergoing donor oocyte badly needed (All French TS DO pregnancies registered)
- In absence of hard data, provide nondirective counseling, and if proceed, do so with extreme caution
  - 2% MM is likely underestimate
  - Pregnancy may or may not place patient at higher risk for later rupture
  - Turner syndrome alone may be absolute contraindication
- MRI needed in everyone
  - Variant vascular anatomy may be important finding regarding risk
  - Serial imaging of ascending aorta necessary
- Cardiac consultation should never be for clearance, rather for assessment
- Surgical option for protection in future.
Thoracic Endovascular Aortic Repair (TEVAR) for the treatment of aortic diseases: a position statement from the European Association for Cardio-Thoracic Surgery (EACTS) and the European Society of Cardiology (ESC), in collaboration with the European Association of Percutaneous Cardiovascular Interventions (EAPCI)

Martin Grabenwoeger1, Fernando Alfonso2, Jean Bachet3, Robert Bonser4, Martin Czerny5*, Holger Eggebrecht6, Arturo Evangelista7, Rossella Fattori8, Heinz Jakob9, Lars Lo¨nn10, Christoph A. Nienaber11, Guido Rocchii12, Herve` Rousseau13, Matt Thompson14, Ernst Weigang15, and Raimund Er

European Heart Journal Advance Access published May 4, 2012

"Indications and contraindications for TEVAR"

TEVAR for TAA

"In asymptomatic TAA patients TEVAR is indicated (by consensus) when the maximum diameter of the aneurysm exceeds 5.5 cm or if rapid expansion (≥5 mm in 6 months) occurs.24,25 In certain morphologic situations which are considered prone to rupture, e.g., saccular aneurysms, TEVAR may be justified at a diameter of less than the above referenced 5.5 cm. Comorbidities and age of the patient have to be considered,26 and it may be appropriate to set a larger aortic diameter threshold in patients with increased operative risk."
Reproductive Options/Outcomes in Women with Turner Syndrome

- Spontaneous pregnancy is a rare occurrence
  - ≥ 40% normal live-born children
  - Approx 20% children with TS
  - ≥ 30% spontaneous abortion rate
- Successful oocyte freezing and IVF for menstruating patients (one report, each)
- "Successful" donor oocyte with real but unknown risk of aortic dissection, rupture, and death.
- Donor oocyte with gestational carrier
- Adoption

References on Slides of Presentation
Learning Objectives

At the conclusion of this presentation, the participant should be able to:

1. Discuss the pathophysiology, genetics, and associated medical conditions of Klinefelter Syndrome
2. List the signs, symptoms, and laboratory results associated with Klinefelter Syndrome
3. Discuss the treatment of patients with Klinefelter Syndrome
Overview

- Overview of Klinefelter Syndrome
- Evaluation of patients with KS
- Controversies in the treatment of KS

Testicular Compartments

- Germ Cells
- Sertoli Cells
- Leydig Cells
**Klinefelter Syndrome**

- Most common cause of primary testicular failure
- Incidence 1.2-1.5/1000 male births
- Under-diagnosed
- May be increasing

**Genetics of Klinefelter Syndrome**

- Non-disjunction during meiosis
  - 53% paternal, 42% maternal
  - 5% post zygotic mitosis
  - XXY or XXY mosaic
  - XXXY maternal origin
  - XYXY paternal origin
  - Short arm of X chromosome likely region of KS genes

**KS and the Androgen Receptor Gene**

- X-linked
- Differences in AR sequence found in CAGn in exon 1
- Normal length 9-37
**KS and the Androgen Receptor Gene**

- CAGn polymorphism modulates androgen action
- Preferential inactivation of the more functional short CAGn allele in KS
- Long allele associated with lower androgenic activity
  - Taller, gynecomastia, osteoporosis, learning disabilities

**Symptoms**

- Learning disabilities
- Behavioral problems
- Delayed puberty
- Poor coordination
- Head aches/hot flushes
- Weak muscle strength
- Increased BMI/fat
- Fractures
- Decreased libido
- Erectile dysfunction
- Infertility

**Signs**

- Broad range of presentation from normal body type to eunichoid
- Female body habitus
- Gynecomastia
- Decreased body hair
- Small penis
- Skeletal disorders
- Poor muscle strength
- Anemia
- Low sperm count
- Small testes
Vast array of phenotypes

- Midfacial hypoplasia
- Narrow shoulders
- Scoliosis and kyphosis
- Pectus carinatum or excevatum
- Pes planus and narrowing of forearm
- Clinodactyly

Associated Medical Issues

- Motor, cognitive, behavioral dysfunction
- Cancer
- Vascular and Cardiac
- Endocrine/Metabolic
- Autoimmune
- Mortality RR 1.63 (CI 1.40-1.91)

Laboratory testing

- Pre puberty
  - Karyotype
  - Educational testing
- Puberty and Adulthood
  - Karyotype
  - T, LH, FSH, E2
  - Androgen receptor studies
  - SA
  - Educational testing
**Klinefelter Syndrome**

- Low testosterone
- Elevated LH and FSH
- Oligospermia or Azoospermia

---

**Treatment with Testosterone**

**Preparations**
- Oral
- IM
- Patch
- Gel
- Skin Implants

**Controversies**
- Timing
  - At 3 mo
  - Pre-pubertally
  - Pubertal age
  - Adulthood
- Dose
  - High vs low
  - Topical vs IM

---

**Hormone and germ cell changes with age**
**Testosterone declines with age**

- **Prenatal**
  - Unclear if T increases at 12 wks

- **Neonatal**
  - "Mini-puberty" at 3mo may be absent

- **Pre-puberty**
  - T does not increase in majority of pts
  - T increases in some

- **Puberty**
  - T decreases by 15 yrs in majority

- **Adulthood**
  - T low in 80%
  - E2 elevated in most

**FSH & LH Increase and Inhibin Decreases With Age**

- **Longitudinal study**
  - 36 untreated KS boys

**Testicular Histology Changes With Age**

- Germ cell migration to genital ridge is normal
- Infant testicular histology near normal
- Mitotic proliferation is reduced as testis develops neonatally
- Spermatogonia start to decrease prepubertally
- Apoptosis occurs at puberty
- Sertoli cells decline around puberty
- Adults have extensive fibrosis & hyalinization of seminiferous tubules and hyperplasia of interstitium
**Testicular Histology Changes With Age**

- A. Fetal testis
  - Germ cells
- B. 4 yr old boy
  - Still with germ cells
- C-E. 10-12 yr old boys
  - Decreasing germ cells
- F. 14 yr old boy
  - No germ cells
  - Degeneration of tubules

**Etiology of Testicular Degeneration**

- Increased expression of genes on the extra X chromosome
- Intratesticular hormone imbalance
- Abnormal apoptotic activity of Sertoli and Leydig Cells
- Defects in spermatogonial stem cells

**Fertility of KS Patients**
What is the Source of the Fertile sperm?

- The sperm originate from clones of spermatogonia that have randomly lost one of the X chromosomes
- Suggests that the sperm that are "lost" after puberty are the 47XXY sperm that never had the potential to become haploid
- Thus harvesting after puberty is futile

TESE/Micro-TESE and ICSI for Adults

- Sperm retrieval 29-57%
- Pregnancy rate 50%
- No predictive factors of sperm retrieval
- 2-3X increase in autosomal and sex chromosome abnormalities in KS sperm than in controls
- Offspring usually with normal karyotype
- Genetic Counseling recommended
Should aggressive fertility management be offered to peripubertal boys? PRO

- Some studies report progressive decline in spermatogonia
- Puberty is associated with accelerated germ cell depletion
- Some TESE studies reported a correlation between sperm aspirated and younger age of patient
- Science is evolving

TESE for Adolescent KS patients

Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor


Should aggressive fertility management be offered to all peripubertal boys? CON

- There are known negative effects of TESE/micro-dissection
- There is no evidence that the sperm cells harvested will increase fertility potential
- Data suggest that it is only sperm that have haploid potential that are the "fertile" sperm harvested and may be preprogrammed in utero.
- There are no clinical parameters available to detect patients who might benefit
- Successful recovery of sperm by TESE in adults is 50%
It is Not all About the Testes

Etiology of KS Cognitive Phenotype

KS Cognitive Phenotype

- Executive dysfunction
- Poor judgment & decision making
- Poor self-control
- Poor problem solving & reasoning
- Don’t learn from their mistakes
**KS Cognitive Phenotype**

- Learning disabilities
  - Language and language-based learning
    - Delayed early speech
  - Auditory processing and memory
  - Less difficulty with non-verbal and spatial testing

**Left Hemisphere Dysfunction**

- Cerebral blood flow
  - KS: symmetric in temporal & parietal (language)
  - Controls: asymmetric
- MRI
  - Reduction of left temporal gray matter
  - Increase in lateral ventricle volume
  - Correlated with poor language skills

**KS Cognitive Phenotype**

- Not clear of role of testosterone exposure
- KS animal model
  - Rate of learning slower in XXY mice
  - Decreased X inactivation + Decreased T
- CAGn (trinucleotide repeat in exon 1) Polymorphism
  - Higher functioning with shorter length
Other Medical Conditions

Tumors

- Mediastinal germ cell tumors
  - Incidence in general population is 1.13-1.53
  - Incidence in KS is estimated at 60X
- Breast Cancer
  - Controversial
  - Incidence in KS is 3.7%-7.5%
  - 50X that of the general male population

Vascular and Cardiac Disease

- Phlebitis and thrombosis
  - HR 5.29 (95% CI 3.29-8.5)
- Pulmonary embolism
  - HR 3.60 (95% CI 1.92-6.74)
- Ischemic heart disease
  - HR 1.71 (95% CI 1.28-2.29)
Autoimmune Diseases

- SLE
- Sjogren syndrome
- Rheumatoid Arthritis

Endocrine and Metabolic Diseases

- Osteoporosis
- DM2
- Obesity
- Metabolic Syndrome
- Hypothyroidism

Etiology of Associated Disease

- Not known
- Misbalance of ratio of Testosterone: estradiol proposed
- Part of genetic syndrome
Summary

- Incidence of KS is underestimated and under diagnosed
- KS is a syndrome with many associated medical issues
- Early diagnosis improves quality of life
- Etiology for decline in germ cells with age is unclear
- Testosterone therapy is indicated at puberty
- Fertility can be achieved with TESE/ICSI
- TESE/BX for preservation of sperm at puberty is experimental
SOKOL REFS for KS Talk


Sperm Aneuploidy and ART

Dolores J. Lamb, Ph.D., HCLD

ASRM President (2011-2012)
Director, Center for Reproductive Medicine
Professor and Vice-Chairman for Research
Scott Department of Urology
Professor of Molecular and Cellular Biology
Baylor College of Medicine

Disclosures

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Learning Objectives

- At the conclusion of this presentation, the attendee should be able to:
  - Discuss the relationship of sperm aneuploidy, recurrent pregnancy loss and ART
  - Understand the known causes of sperm aneuploidy
  - Know the tests used to diagnose sperm aneuploidy and the test interpretation
The Problem of Recurrent Miscarriage

- Three or more consecutive pregnancy losses
- Known

A Majority of First Trimester Abortuses Are Chromosomally Abnormal

- Idiopathic

When Do Chromosome Abnormalities Arise?

- Gametogenesis
  - Meiotic Non-Disjunction During
  
The Male Contribution to Recurrent Pregnancy Loss At the Sperm Chromosomal Level Is Not Always Considered During the Evaluation of the Couple With Current Pregnancy Loss Especially If the Male Is Normozoospermic

Chromosome Deletion or Gain (Aneuploid) in Offspring May Result From Defective Spermatogenesis

- During spermatogenesis, meiosis results in a haploid germ cell (one set of chromosomes)
- The loss of or presence of an extra chromosome results in:
  - Down’s Syndrome (Trisomy 21)
  - Klinefelter (XXX)
  - Turner Syndrome (Monosomy X)
  - Edwards Syndrome (Trisomy 18)
  - Patau Syndrome (Trisomy 13)
- Fetal loss
Spermatogenic Defects Leading to Genetic Abnormalities in Sperm

- How Do We Measure Sperm Aneuploidy?

Fluorescent In Situ Hybridization (FISH)

- Uses multicolor fluorescently labeled DNA probes specific for each chromosome
- Allows detection of number of specific chromosomes in a cell
  - Haploid = single copy of each chromosome (germ cells)
  - Diploid = double (disomic) copy of each chromosome (somatic cells)
  - Aneuploid = any deviation from haploid or diploid state

Examples of Sperm Aneuploidy
How Does Aneuploidy Occur?

- Non-dysjunction (main mechanism in sperm)
  - Reduced or absent meiotic recombination
- Anaphase lag (nullisomic sperm)
- Ineffective checkpoint control
  - Synaptic and recombination errors cause abnormal chromosome segregation and meiotic arrest
  - Failure to arrest meiosis with aneuploidy


One Example of Aneuploidy

What is Normal? Average Percentages of Sperm Disomy in Healthy Individuals

<table>
<thead>
<tr>
<th>Chr.#</th>
<th>%</th>
<th>Chr.#</th>
<th>%</th>
<th>Chr.#</th>
<th>%</th>
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<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>13</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>15</td>
<td>0.10</td>
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<tr>
<td>3</td>
<td>0.20</td>
<td>16</td>
<td>0.07</td>
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<td></td>
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<tr>
<td>4</td>
<td>0.08</td>
<td>18</td>
<td>0.06</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>0.16</td>
<td>X,Y</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numerical Chromosomal Abnormalities in Sperm

- Infertile males exhibit a 10-fold increase in the incidence of sperm-specific chromosomal abnormalities, even when a normal somatic karyotype is present.

Selected Infertile Men Exhibit High Levels of Sperm Aneuploidy

Nearly 25% of Patient #2’s sperm have an extra chromosome 21. With ICSI, predict 25% of embryos with Down’s Syndrome.

High Sperm Aneuploidy in a Subset of Recurrent Pregnancy Loss Male Partners
Which Categories of Spermatogenic Deficiency Exhibit Elevated Sperm Aneuploidy?

- Oligoasthenoteratozoospermia (OAT)
- Oligoteratozoospermia
- Oligozoospermia
- Non-obstructive azoospermia
- Normozoospermic men with Recurrent Pregnancy Loss

Other Types of Patients With Increased Sperm Aneuploidy

- Unilateral or bilateral cryptorchidism after orchidopexy
- Varicoceles
- Chemotherapy (Beiomycin, doxorubicin, vincristine, dacarbazine, novantrone, oncovin, velban, prednisone)
  - Choy and Brannigan, Fert Steril 100: 1187-1190, 2013
- Germline TP53 Mutations (Li-Fraumeni syndrome (p53))
  - Paulasova, et al., Cancer Genetics 204: 278-281, 2011

Lifestyle Issues May Affect Sperm Aneuploidy Levels

- Cigarette smoke
- Alcohol consumption
- Diazepam (>0.3 mg/kg/day > 6 mo)
  - Baumgartner, et al., 2001
- Finasteride
- Benzene
- Pesticides/Fenvalerate/Carbaryl/Polychlorinated biphenyl, \( p,p'\)-DDE/Organophosphate pesticides/Pyrithroids
  - Perry Hum Reprod Update 14:233-242, 2008
  - McAuliffe, et al., Env Health Perspec 120: 535-540, 2012

Reviewed in Hwang, et al., Therapeutic Advances in Urology, 2010
What Level of Sperm Aneuploidy Can Result in Aneuploidy in the Offspring?

- There is an association between fathering aneuploid offspring or recurrent abortions and moderately increase levels of sperm aneuploidy
  - Moderate increases in the rates of aneuploidy are clinically significant (2-3x)
    - Down syndrome, Turner
      - Nagvenkar, et al., Fert Steril 84:925-931, 2005
    - Controversial

Who Should Be Tested for Sperm Aneuploidy?

- Oligospermic men
- Oligoasthenozoospermic men
- Recurrent pregnancy loss

Take Home Message

- Sperm aneuploidy is important to evaluate in male partners of women with recurrent pregnancy loss
- Sperm fluorescent in situ hybridization is the method commonly used to detect sperm aneuploidy
  - Chr. 13, 18, 21, X, Y
  - Consistent with a viable but affected offspring
- Normal fertile men display low levels of sperm aneuploidy
- Men with increased levels of sperm aneuploidy should undergo genetic counseling
- Options
  - PGD with ICSI, natural conception, sperm donor, adoption, remain childless
OBJECTIVES

1. State the relative increase (odds ratio) in birth defects associated with ART
2. Be able to state at least two potential explanations for the increased frequency of birth defects in ART
3. Be able to state how you would inform couples embarking upon ART concerning risk for birth defects in their offspring

META-ANALYSES: ART and Birth Defects

<table>
<thead>
<tr>
<th>Year</th>
<th>Studies</th>
<th>Accepted</th>
<th>OR</th>
<th>95% C.I.</th>
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<td>Rimm (2004)</td>
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<td>1.29</td>
<td>(1.01-1.67)</td>
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<tr>
<td>Hansen (2005)</td>
<td>25 *</td>
<td>1.29</td>
<td>(1.21-1.37)</td>
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<tr>
<td>Wen (2012)</td>
<td>46</td>
<td>1.37</td>
<td>(1.26-1.48)</td>
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<tr>
<td>Hansen (2013)</td>
<td>45 *</td>
<td>1.32</td>
<td>(1.24-1.42)</td>
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</tr>
</tbody>
</table>

* 14 reports accepted in 2005 report excluded in 2013
## ART AND BIRTH DEFECTS (Reports 2005-2013)

### Significant Differences

<table>
<thead>
<tr>
<th>ART Years</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982-2001</td>
<td>1.44</td>
<td>(1.32-1.57)</td>
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<tr>
<td>1994-1998</td>
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<td>Australia</td>
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<tr>
<td>2001-2007</td>
<td>1.25</td>
<td>(1.15-1.37)</td>
<td>Sweden</td>
</tr>
<tr>
<td>1996-1999</td>
<td>1.31</td>
<td>(1.10-1.57)</td>
<td>Finland</td>
</tr>
<tr>
<td>2006</td>
<td>1.17</td>
<td>(0.81-1.69)</td>
<td>Japan</td>
</tr>
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</table>

### ART AND BIRTH DEFECTS (Reports 2005-2013)

### Significant Differences

<table>
<thead>
<tr>
<th>ART Years</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
<th>Country</th>
</tr>
</thead>
<tbody>
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<td>1986-2002</td>
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<tr>
<td>1991-2004</td>
<td>1.36</td>
<td>(1.19-1.55)</td>
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<tr>
<td>1995-2000</td>
<td>1.25</td>
<td>(1.12-1.39)</td>
<td>California, USA</td>
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<tr>
<td>1984-2002</td>
<td>1.53</td>
<td>(1.30-1.79)</td>
<td>Perth, Australia</td>
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<tr>
<td>2007-2011</td>
<td>1.01</td>
<td>(0.67-1.52)</td>
<td>Colorado, USA</td>
</tr>
</tbody>
</table>

### PITFALLS AND ANALYSIS

- Current ART methods and embryo culture methods earlier era and lack generalizability
- Multiple ovulation stimulation and embryo culture techniques
- In population-based studies lack of consistent anomaly surveillance, leading to differing anomaly rates reflecting inclusion minor anomalies
### ASSISTED CONCEPTIONS AND BIRTH DEFECTS STUDY DESIGN
(DAVIES, 2012)

- South Australia Registry (1986-2002)
- Birth defects sought before 5th birthday
- Included terminations for anomalies <20 weeks, within 28 days birth, or reported from multiple other sources
- 6163 Assisted conceptions / 308,974 Births
- Multiple (p<0.001) differences between assisted and spontaneous conceptions – age, socioeconomic status, race, nulliparity, paternal occupation, smoking, multiple gestation, diabetes, anemia.  
  *Davies et al., 2012*

### BIRTH DEFECTS ADJUSTED AND UNADJUSTED

<table>
<thead>
<tr>
<th>Conceptions</th>
<th>Percentage</th>
<th>Odds Ratio</th>
<th>Unadjusted</th>
<th>Adjusted</th>
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<tr>
<td>All Assisted</td>
<td>8.3%</td>
<td>1.47</td>
<td>1.28</td>
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<tr>
<td>All Spontaneous</td>
<td>5.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF alone</td>
<td>7.2%</td>
<td>1.26</td>
<td>1.07</td>
<td>(0.90-1.26)</td>
</tr>
<tr>
<td>ICSI alone</td>
<td>9.9%</td>
<td>1.77</td>
<td>1.57</td>
<td>(1.30-1.90)</td>
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</tbody>
</table>

Includes terminations <20 weeks, live births 1-28 days and any reported anomaly <5 years  
*Davies et al., 2012 NEJM*
BIRTH DEFECTS: ICSI / IVF versus Traditional IVF

• No consistent difference observed in overall frequency of structural birth defects
• Hypospadias increased in offspring of ICSI pregnancies. Could reflect transmission of pleiotropic paternal genes that contributed to male infertility
• Sex chromosomal abnormalities seem increased in ICSI offspring
  – Relationship to balanced autosomal translocation in ICSI father (2%) and mothers (1.2% - 2.0%)

HYPOSPADIAS IN ICSI OFFSPRING

- Wennerholm et al. (2000): RR 3.0 (1.09-6.50) compared to Swedish Medical Birth Registry and Registry of Congenital Malformation
- Ericson and Kallen (2001): RR 1.5 (1.0-2.1)
- Klemetti et al (2005)- 76/10,000 v 29/10,000

Explanations for Increased Risk Birth Defects

• Are increased risks related to ART per se?
  Or,
• Do increased risks relate to underlying reason why ART needed?
TECHNICAL VARIABLES IN ART

- Ovulation stimulation regimes
- Obtaining and handling gametes
- Embryo culture
- Cryopreservation
- Many potential confounders previously employed in IVF/ICSI no longer utilized (2013)

EMBRYO CULTURE

- Media composition not also disclosed (Proprietary)
- Supplementation has varied:
  - ± human or maternal serum albumin
  - ± synthetic serum substitute
- Considerable changes decade by decade

BLASTOCYST (DAY 5-6)
INCREASED LENGTH OF TIME IN CULTURE FOR BLASTOCYSTS

One third of cleavage stage embryos do not survive in vitro to day 5.

- Selection against aneuploidy?
- Traditional culture medias developed for first three days, but now cultures must extend to five days. Appropriate culture media for day 6?

DIFFERENTIAL GENE EXPRESSIONS WITH DIFFERENT EMBRYO CULTURE MEDIA

- Mouse embryos in Whitten’s media misexpressed 114 genes *(Affymetrix microarray chip)* compared to in vivo embryos
- Incubation in KSOM / AA medium misexpressed 29 genes

*Rinaudo and Schultz, Reproduction 128:301, 2004*

CONCLUSION: TECHNICAL VARIABLES

- Potential disturbances at each step
- Plausible that certain genes differentially expressed compared to in vivo conception, either through ovulation stimulation or embryo culture
- Few contemporaneous population-based reports and these show lower OR *(Fuji 2006 OR 1.17; Moses 2007-11 OR 1.01)*
IS IMPRINTING THE EXPLANATION FOR INCREASED BIRTH DEFECTS?

- Plausible give potential effects of culture media and incubation. However, also hypothesis of last resort
- Deleterious effect would not necessarily be embryonically lethal ("all or none" phenomenon) and could be manifested as birth defects
- But, absolute rate low given rarity of known imprinting syndrome (Beckwith-Wiedemann, Russell Silver, Prader-Willi, Angelman)

Bi-Allelic Expression

- From both chromosomes, maternally and paternally inherited alleles. Some genes designed to be expressed only by allele from a parent of given sex

Genomic Imprinting

The unequal expression of maternal and paternal alleles
### Maintaining Parent of Original Imprinting

- Parental mark set in germ cells
- Reversible on passage through the opposite parental germline (switch)
- Differential expression of the new parental imprint in the offspring
- Stable transmission through mitosis in somatic cells

### IMPRINTING AND CHROMATIN MODIFICATIONS

- DNA methylation = Inactive

### ART AND BECKWITH - WIEDEMANN SYNDROME (BWS)

- Overgrowth syndrome. IGF2 normally expressed only from paternal allele. Maternal H19 restrains growth by suppressing maternal IGF2. Usual mechanism in BWS: hypomethylation of maternal KCNOT1 (60%) leading to IGF2 of maternal origin.
- De Braun (2003): 7 of 65 BWS cases associated with ART, 5 requiring ICSI
  - Imprinting perturbation (H19) involved maternal allele, uncommon molecular basis for BWS
BECKWITH-WIEDEMANN SYNDROME

6 kb band: methylated
4.2 kb band: unmethylated

IMPRINTING DISORDERS IN ART REGISTRIES

• Population-based (vital statistics) studies in Scandinavia show no increased risk overall
• When odds ratio for selected disorders is increased, the absolute effect is small because imprinting disorders are rare
• No technical feature in common with cycles resulting in birth defects.

Are increased risks related to ART per se?

Or, • Do increased risks relate to underlying reason why ART needed?
  – Different populations: Fertile v Infertile
  – Populations could alter differ? re: status of imprint
CHARACTERISTICS OF POPULATION REQUIRING INFERTILITY TREATMENT

• 10% of population (equal male and female)
• Differs from general population
  – Older age
  – May have genetic disorders with implications for offspring (e.g., Kartagener syndrome, cystic fibrosis), in both male and female partners
  – Increased balanced translocations in both male and female partners

SPERM ANEUPLOIDY AND ICSI

• 3 fold increase in sex chromosome aneuploidies after ICSI (prenatal diagnosis data)

ICSI FATHERS AND HYPOSPADIAS IN OFFSPRING

• Hypospadias is polygenic/multifactorial with 2.5% recurrence risk for first-degree relatives
• Gonadal abnormalities that necessitate ICSI for fertilization could result in decreased hormone production in father and fetus and thus hypospadias
EPIGENETIC PERTURBATION IN SPERM OF INFERTILE MALES

- MTHR, PAX8, NTF3, SFN, HRAS
- IGF2, H19
- RASGRF1, GTL2, PLAG1, MEST, KCND1, LIT1, SNRPN
- H3K4me, H3K27me

<table>
<thead>
<tr>
<th>Hypermethylation</th>
<th>Decreased methylation</th>
<th>Locus – specific hypermethylation</th>
<th>Histone retention (nucleosomes)</th>
</tr>
</thead>
</table>

INCREASED TRANSLOCATIONS IN COUPLES UNDERGOING ICSI (PER 1,000)

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>Newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rcp</td>
<td>6.9</td>
<td>12.3</td>
<td>1.52</td>
</tr>
<tr>
<td>Rob</td>
<td>6.9</td>
<td>8.2</td>
<td>0.90</td>
</tr>
<tr>
<td>Inv</td>
<td>6.9</td>
<td>1.4</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>20.7</td>
<td>21.9</td>
<td>2.84</td>
</tr>
<tr>
<td>(2.07%)</td>
<td>(2.19%)</td>
<td>(0.28%)</td>
<td></td>
</tr>
</tbody>
</table>

Gekas et al., Hum. Reprod., 2001

EPGENETIC (METHYLATION) IN SILVER – RUSSELL SYNDROME

- PEG\textsubscript{i} / MEST locus ordinarily demethylated (active gene)
- Infertile father requiring ICSI showed unscheduled methylation at four cytosine residues. During father’s meiosis four additional residues became methylated = Silver-Russell syndrome transmitted to twin offspring

Eggermann et al., Amer J Med Genet C 2010
SUBFERTILITY AND BIRTH DEFECTS

• Danish national birth cohort; interviews determined infertility history
• 50,897 singleton and 1366 twins of fertile couples; Time to pregnancy (TTP) < 12 months
• 5764 singleton and 100 twins; TTP > 12 months, but natural conception
• 4588 singleton and 1690 twins undergoing infertility treatment (singleton 398 ICSI; 1483 IVF; others “hormonal”, surgery, IUI)

OR 1.2 Birth Defects

Zhu et al., BMJ 2006

BIRTH DEFECTS: SUBFERTILITY (>12 months) versus “normal” (<12 months)

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu, 2006</td>
<td>1.20</td>
<td>1.07-1.35</td>
</tr>
<tr>
<td>Jacques, 2010</td>
<td>1.30</td>
<td>0.98-1.72</td>
</tr>
<tr>
<td>Davies, 2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Subfertile no prior pregnancy</td>
<td>1.29</td>
<td>0.99-1.68</td>
</tr>
<tr>
<td>– Spontaneous conception after prior ART</td>
<td>1.25</td>
<td>1.01-1.56</td>
</tr>
</tbody>
</table>

SUBFERTILITY AND BIRTH DEFECTS

• Naturally conceived pregnancies occur in couple “infertile” by traditional definitions (12 months unprotected coitus)
• Frequency of birth defects: Increased compared to controls
• Thus, increased rate in ART offspring likely reflects underlying biologic reason necessitating ART. May or may not account for all (Rimm) increase
CONCLUSIONS (1):  
BIRTH DEFECTS AND ART

1. Overall malformation rate increased (relative risk 1.3). Only specific abnormalities: hypospadias and sex chromosomal abnormalities in intracytoplasmic sperm injection (ISCI)
2. Increase in birth defects probably similar in IVF alone versus IVF/ISI

CONCLUSIONS (2):  
BIRTH DEFECTS AND ART

3. Whether increase is due to ART or to characteristics of underlying population is unclear.
   - ART couples not representative of the general population; thus, true control group not possible.
   - Birth defects increased in sub-fertile couples not requiring ART.

CONCLUSIONS (3):  
BIRTH DEFECTS AND ART

4. Epigenetic phenomena plausible explanation but unproven. Unclear whether alterations observed are a component of subfertility or arose in ART, but former certain in selected cases
UPCOMING ESHRE EVENTS
// ESHRE CAMPUS EVENTS

ESHRE's 30th Annual Meeting
- www.eshre2014.eu
  - Munich, Germany
  - 29 June - 2 July 2014

Epigenetics in reproduction
- www.eshre.eu/lisbon
  - Lisbon, Portugal
  - 26-27 September 2014

Endoscopy in reproductive medicine
- www.eshre.eu/endoscopyoct
  - Leuven, Belgium
  - 15-17 October 2014

Making OHSS a complication of the past:
State-of-the-art use of GnRH agonist triggering
- www.eshre.eu/thessaloniki
  - Thessaloniki, Greece
  - 31 October-1 November 2014

From gametes to blastocysts –
a continuous dialogue
- www.eshre.eu/dundee
  - Dundee, United Kingdom
  - 7-8 November 2014

Controversies in endometriosis and adenomyosis
- www.eshre.eu/liege
  - Liège, Belgium
  - 4-6 December 2014

Bringing evidence based early pregnancy care to your clinic
- www.eshre.eu/copenhagen
  - Copenhagen, Denmark
  - 11-12 December 2014

An update on preimplantation genetic screening (PGS)
- www.eshre.eu/rome
  - Rome, Italy
  - 12-13 March 2014

For information and registration: www.eshre.eu/calendar
or contact us at info@eshre.eu