The Clinical Assessment of Sperm Aneuploidy in Male Infertility Patients

University of Utah Andrology & IVF Labs

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Patient #1

- 26 year old healthy
- Normal sperm count and motility. 0% normal morphology
- TEM: Type 2 Round Head Syndrome
- Brother: Type 1 Round Head Syndrome
- Brother: 48% total aneuploidy with 5 probes
- ???
Patient #2

- 29 year old, 5 years of unexplained infertility.
- 8 AI cycles w/o pregnancy.
- IVF cycle 1: 7/10 oocytes fertilized. One level 2 embryo, six level 3 (fragmented) embryos.
- IVF cycle 2: 8 embryos, all level three.
Patient #3

- 28 year old, 2 years primary infertility
- OAT (3.5 M/mL)
- 13/14 Robertsonian Translocation
- IVF/ICSI/PGD for Translocation
- 9/9 embryos unbalanced
Objectives of Lecture

- Is it technologically feasible to implement aneuploidy analysis in a clinical setting?
- Is sperm aneuploidy clinically relevant?
- What is the incidence of elevated aneuploidy?
- What causes sperm aneuploidy?
- Can therapy lower the sperm aneuploidy rate?
- What are reasonable guidelines for testing?
**History of Aneuploidy Testing in Sperm**

- **Interphase FISH in decondensed sperm nuclei.** (Martin, 1993; Wyrobek, 1994)

Rudak et al, 1978
Constraints:
1) Development and Standardization of Good Hybridization Techniques and Counting Criteria
2) Costs – Probes/Tech time
3) Counting Time – 10,000 sperm, 5+ probes
4) Low sperm counts in some samples (i.e. biopsies)

Result: Lack of Proper Validation and Large-Scale Studies
Automation of Chromosome Enumeration
Manual vs Automated Enumeration

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean % Aneuploidy (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5%</td>
</tr>
<tr>
<td>2</td>
<td>5.3%</td>
</tr>
<tr>
<td>3</td>
<td>4.7%</td>
</tr>
<tr>
<td>4</td>
<td>6.1%</td>
</tr>
</tbody>
</table>

Carrell & Emery, 2008

% Deviation (COV x 100%)
Is Sperm Aneuploidy Relevant?

- 15% of recognized pregnancies result in SAB
  - 50% of these are chromosomally abnormal

  Hassold & Jacobs, 1984

- Aneuploidies in gametes are predominately from errors of meiosis during oogenesis.

- Is sperm aneuploidy clinically relevant?
Fertilization Does Not Select for Euploid Sperm

- Marchetti et al. (Wyrobek lab), 1999
  - Double heterozygous mice for 2 Robertsonian translocations Rb(6.16)24Lub and Rb(16.17)7Bnr.
  - Chromosome painting of chromosomes 8, 16, 17, Y of sperm and blastomeres.
  - Aneuploidy rates equal in sperm and first cleavage blastomeres.
  - No interchromosomal effects noted.
Clinical Relevance of Aneuploidy

- Sperm aneuploidy does not influence fertilization capacity. (Marchetti, 1999)
- Correlation between sperm and PGS aneuploidy rates. (Tempest et al, 2009)
- The prevalence of sperm aneuploidy is higher in cases of repeated IVF failure with male factor infertility. (Magli, 2009)
- Screening for the prevalence of a known translocation and/or associated aneuploidies in the male germline prior to ICSI. (Munne, 2005; Carrell, 2008)
Anecdotal Evidence of Anomalies

- Elevated chrss 15 aneuploidy transmitted to fetus. Carrell et al., 2001
- 4 consecutive trisomic pregnancies with elevated frequency of associated sperm. Thomascik-Cheeseman et al., 2006
- Other reports: 21, 18, X
Sperm Aneuploidy and the Resulting Embryo

- Study design:
  - 32 Couples enrolled in the study (mean mat age 32)
  - Tested chromosomes: 13, 16, 18, 21, 22, X & Y
  - t-test

<table>
<thead>
<tr>
<th>Sperm aneuploidy</th>
<th># embryos</th>
<th># blastomeres</th>
<th>% chr abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.2%</td>
<td>35</td>
<td>628</td>
<td>58</td>
</tr>
<tr>
<td>2.2%-4.4%</td>
<td>25</td>
<td>318</td>
<td>63.5</td>
</tr>
<tr>
<td>&gt;4.4%</td>
<td>23</td>
<td>354</td>
<td>73.45</td>
</tr>
</tbody>
</table>

Significant correlation between increased sperm aneuploidy and chromosome abnormalities in blastomeres

Tempest et al, 2009
Sperm Aneuploidy and the Resulting Embryo
(Univ of Utah Ongoing Study)

- Study design:
  - 85 couples, Mat Age = 34.4
  - Tested chromosomes: 13, 18, 21, X & Y

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<th>% chr abnormality</th>
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</thead>
<tbody>
<tr>
<td>&lt; 3.0</td>
<td>58</td>
<td>812</td>
<td>53.2</td>
</tr>
<tr>
<td>3.0 – 5.0</td>
<td>35</td>
<td>396</td>
<td>66.8</td>
</tr>
<tr>
<td>&gt;5.0</td>
<td>37</td>
<td>362</td>
<td>81.4</td>
</tr>
</tbody>
</table>
Chromosomal Abnormalities Reported in IVF Embryos are Correlated to Sperm Parameters

<table>
<thead>
<tr>
<th></th>
<th>Normozoospermia</th>
<th>Oligoasthenoteratozoospermia</th>
<th>Obstructive azoospermia</th>
<th>Non-obstructive azoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos diagnosed</td>
<td>594</td>
<td>695</td>
<td>127</td>
<td>133</td>
</tr>
<tr>
<td>FISH abnormal (%)</td>
<td>328 (55)</td>
<td>431 (62)</td>
<td>80 (63)</td>
<td>92 (69)</td>
</tr>
<tr>
<td>Monosomies and trisomies (%)</td>
<td>147 (45)</td>
<td>160 (37)</td>
<td>36 (43)</td>
<td>23 (25)</td>
</tr>
<tr>
<td>Haploidy and polyploidy (%)</td>
<td>30 (9)</td>
<td>60 (14)</td>
<td>5 (6)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Complex abnormalities (%)</td>
<td>151 (46)</td>
<td>211 (49)</td>
<td>39 (49)</td>
<td>63 (68)</td>
</tr>
<tr>
<td>No. day-3 embryos with 7–8 regular cells, no fragmentation (%)</td>
<td>237 (40)</td>
<td>243 (35)</td>
<td>37 (29)</td>
<td>21 (16)</td>
</tr>
</tbody>
</table>

Values with same superscript letter are significantly different: \( a \leq P < 0.025; \( b \leq P < 0.005; \( c \leq P < 0.05; \( d \leq P < 0.01; \( e \leq P < 0.001.

FISH = fluorescence in-situ hybridization.

Magli, 2009
Gonosomal Aneuploidies in 1549 Embryos

Magli, 2009
## Recurrent Miscarriage

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>XY</th>
<th>13</th>
<th>18</th>
<th>21</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPL patients</td>
<td>24</td>
<td>0.77 ± 0.11*</td>
<td>1.02 ± 0.10†</td>
<td>0.51 ± 0.05*</td>
<td>0.47 ± 0.06*</td>
<td>2.77 ± 0.22†</td>
</tr>
<tr>
<td>General population</td>
<td>16</td>
<td>0.40 ± 0.05</td>
<td>0.44 ± 0.06</td>
<td>0.33 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>1.48 ± 0.12</td>
</tr>
<tr>
<td>Fertile donors</td>
<td>10</td>
<td>0.31 ± 0.06</td>
<td>0.39 ± 0.03</td>
<td>0.25 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>1.19 ± 0.11</td>
</tr>
</tbody>
</table>

RPL = recurrent pregnancy loss.

Data are expressed as mean ± standard error. Total aneuploidy indicates the percentage of sperm with one or more aneuploid chromosomes. Greater than 5000 sperm were analyzed in all samples.

* \( P < .05 \) compared with general population and fertile donors.
† \( P < .005 \) compared with general population and fertile donors.

Carrell et al, 2003
How Common is Elevated Sperm Aneuploidy?
What is its Clinical Relevance?

1. What is Normal?
2. Few Systematic Analyses
3. Standardization
4. Improper QC
# Reported Incidences of Aneuploidy

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Aneuploidy (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klinefelter syndrome (mosaic)</td>
<td>1.5Š7</td>
<td>Kruse et al, 1998; Lim et al, 1999</td>
</tr>
<tr>
<td>Klinefelter syndrome (nonmosaic)</td>
<td>2Š25</td>
<td>Rives et al, 2000; Estop et al, 1998</td>
</tr>
<tr>
<td>Robertsonian translocation</td>
<td>10Š23 unbalanced</td>
<td>Ogur et al, 2006</td>
</tr>
<tr>
<td></td>
<td>1Š19 aneuploid</td>
<td>Ogur et al, 2006</td>
</tr>
<tr>
<td></td>
<td>7Š36 unbalanced*</td>
<td>Fryndman et al, 2001</td>
</tr>
<tr>
<td>Reciprocal translocation</td>
<td>19Š77 unbalanced</td>
<td>Martin and Spriggs, 1995</td>
</tr>
<tr>
<td>Severe morphology defects</td>
<td>15Š100</td>
<td>Benzacken et al, 2001</td>
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<tr>
<td>Multiflagellar, macrocephalic</td>
<td></td>
<td>Devillard et al, 2002</td>
</tr>
<tr>
<td>Tail agenesis</td>
<td></td>
<td>Carrell et al, 2004; InOl Veld et al, 1997</td>
</tr>
<tr>
<td>Round headŠonly syndrome</td>
<td>15Š60</td>
<td>Carrell et al, 1999, 2001</td>
</tr>
<tr>
<td>Nonobstructive azoospermia</td>
<td>1Š51</td>
<td>Burrello et al, 2005</td>
</tr>
<tr>
<td>Unexplained recurrent pregnancy loss</td>
<td>1Š34</td>
<td>Bernardini et al, 2004; Carrell et al, 2003</td>
</tr>
<tr>
<td>Repeated IVF failure</td>
<td>2Š7</td>
<td>Petit et al, 2005</td>
</tr>
</tbody>
</table>
Mean Aneuploidy Rate

- 5 Chromosomes (13, 18, 21, X, Y)
- A – Fertile Normozoospermic: 1.2% (n=59)
- B – General Population: 1.8% (n=238)
- C – General Infertile: 2.7% (n=364)
- D – Teratozoospermic: 3.6% (n=176)
- E – Poor Embryogenesis: 4.3% (n=51)
- F – Recurrent Miscarriage: 5.1% (n=86)
- G – Severe Ultrastructure Defects: 14.8% (3-78) (n=65)
Incidence of Elevated Aneuploidy
(% of Samples >3% Total Aneuploidy for 5 Probes)

- 5 Chromosomes (13, 18, 21, X, Y)
- A – Fertile Normozoospermic: 0% (n= 59)
- B – General Population: 0.4% (n= 238)
- C – General Infertile: 3.7% (n= 364)
- D – Teratozoospermic: 3.9% (n= 176)
- E – Poor Embryogenesis: 5.8% (n= 51)
- F – Recurrent Miscarriage: 5.7% (n = 86)
- G – Severe Ultrastructure Defects: 58% (n = 65)
Intra-Individual Variation

- Tempest et al., 2009
  - Sporadic variation in all 10 subjects over time.
  - Single sample variable over trial.
- Rubes et al., 2005
  - Interchromosomal differences.
  - Generally consistent at 2 years, some variability at 5 years.
Segregation Analysis for Translocation Patients

- Vozdova et al., 2008: Different segregation patterns and unbalanced sperm rates even with similar translocations.
- Different rates with similar Robertsonian translocations. Chen et al., 2007
- Perrin et al., 2009: Different segregation patterns for similar sex/autosome translocations affecting chance of IVF success.
- Yakut et al., 2006: Sperm FISH unbalanced rate related to blastomeres.
- Wiland et al., 2008: Suggest high unbalanced rate (>60%) is still conducive to IVF pregnancy. *Note: High miscarriage rate.
- Breakpoint variability related to the variability in segregation patterns and subsequent unbalanced rates.
What Causes Aneuploidy?
Prophase 1: Synaptonemal Complex

Axial Elements: SCP 2&3, Rec 8
  Hold sister chromatids together
Transverse Filaments: SCP1
  Holds pairs of sister chromatids together
Axial Elements become Lateral Elements of SC.

http://219.221.200.61/ywwy/zbsw(E)/edetail11.htm
Progression of Meiosis During Spermatogenesis

Chromosomal Non-Disjunction May Result in Aneuploidy

• Recombination is essential for normal segregation.
What Causes Nondisjunction?
Recombination Mechanics – Biopsy Samples

- Number of crossovers (MLH foci)
- Positioning in subtelomeric regions
- Fidelity of the synaptonemal complex
The Frequency of Crossovers and MLH Foci

- “Normal” Spermatogenesis
  - Mean $49.8 \pm 4.8$ (S.D.) recombination sites
  - Range $46.2 \pm 3.3$ to $55.3 \pm 3.7$ (Hassold et al 2004)

Number of MLH Foci


NOA Infertile Men

- Mean $40.4 \pm 6.1$ recombination sites
- Range of $32.3 \pm 15.1$ to $48.9 \pm 7.4$ (Sun et al., 2006)
The formation of synaptonemal complexes without a crossover event is increased in infertile men.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean No. of autosomal SCs where No. of foci is</th>
<th>MLH1 foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Controls</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Obstructive azoospermia</td>
<td>0.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Nonobstructive azoospermia</td>
<td>1.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*a P < 0.0001, nested ANOVA.

Sun et al. 2005
Crossovers Occur in Subtelomeric Regions

Sun et al. 2006
Meiotic Errors are Associated with Male Infertility

Mean Distance from the Centromere to the Proximal Crossover Sight on the p arm

- Patients
  - Mean Distance 2.84 ± 0.096 μm
- Controls
  - Mean Distance 3.20 ± 0.105 μm

P= 0.01

Mean Distance from the Centromere to the Proximal Crossover Sight on the q arm

- Patients
  - Mean Distance 4.23 ± 0.11 μm
- Controls
  - Mean Distance 4.58 ± 0.129 μm

P= 0.04
Meiotic Errors are Associated with Male Infertility

Gaps and splits of the synaptonemal complex are associated with infertile

- Gaps
  - 35% controls
  - 45% NOA patients

- Splits
  - 7.5% controls
  - 35% patients

- Sun et al. 2005
Conclusion: Analysis of Recombination

- The mechanics of meiosis affect non-disjunction.
  - Number of crossovers (MLH foci)
  - Positioning in subtelomeric regions
  - Fidelity of the synaptonemal complex
- Recombination is not just important for genetic variation, but also to assure proper segregation. (Hassold, 2007)
Sequencing of Genes Involved in Meiosis

Azoospermic and Oligozoospermic Patients
– SPO-11 – Low frequency of Novel SNPs, Carrell et al., 2006
– Rec 8 – No Difference from Controls, Carrell et al., 2008
– MMRs – Early Data– Low level SNPs Carrell and Sanderson, 2009
– Genomewide Analysis – No significant SNPs, Aston and Carrell, 2009 J Androl

Increased Aneuploidiy Patients
– Ongoing Studies Only
Position of Chromosomes in Nucleus


- Centromeres shifted from center in patients with disomy, compared to fertile controls. Olszewska et al., 2009

Finch et al., 2008
Positioning of Chromosomes in Sperm

Zalensky and Zalenskaya, 2007
Therapy and “Sperm Selection”
Sperm Selection

Sperm Preparation:
• Swim-up lowers aneuploidy rate. Jakab et al., 2003
• Selection of motile sperm (DG, SU, GW) does not lower aneuploidy rate. Samura et al., 2001
• Mosaic Translocation – Unbalanced chromosome increased following Density Gradient. Iwarsson et al., 2009

Sperm Selection:
• Lower aneuploidy in sperm selected by high power magnification and w/o vacuoles. Garolla et al.
• HA–mediated selection resulted in 4–6 fold reduction in disomy. Huszar et al., 2007, 2008; Paasche, 2009
• Normal strict criteria morphology does not predict euploidy. Ryu et al, 2001; Sun et al., 2006.
• Severe abnormal morphology is associated with an elevated aneuploidy rate. Tang et al., 2009; Carrell et al, 2004; Prissant, 2007; Collodel et al., 2006; Perrin et al., 2008
Medical Therapy

• 90 days of FSH Therapy lowered total aneuploidy rate. Piombi et al., 2009
• Traditional Chinese Medical Therapy – Tempest et al., 2005 (not RCT)
Varicocele Repair

• Elevated rate of 17, 18 aneuploidy improved by repair. Acar et al., 2009

• Animal Model: No elevation in aneuploidy (Carrell, Unpub)
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- TEM: Type 2 Round Head Syndrome
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- **Aneploidy Analysis:**
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- **Aneuploidy Analysis:**
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- OAT (3.5 M/mL)
- 13/14 Robertsonian Translocation
- IVF/ICSI/PGD
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• Aneuploidy Analysis:
  - 74% Unbalanced 13/14
  -- 8.9% Aneuploidy for 5 Chromosomes
Conclusions and Clinical Recommendations

• New advances facilitate of sperm aneuploidy testing analysis in a clinical setting; however, more data are needed in establishing reference ranges, etc.
• Further studies are needed on sperm separation techniques to select euploid sperm.
• Further data are needed on specific sperm aneuploidy rates and embryo aneuploidy to assess relative risks.
• Recombination is essential for normal segregation (Quantity and Quality)
• Clinical screening may be useful in certain pathologies, which include:
When To Use Sperm Chromosome Testing

Clinical syndromes for which sperm chromosome aneuploidy testing may be advisable

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Carrell, 2008
Technical and Logistical Considerations

• Which chromosomes should be evaluated? (All?)
  – Most commonly tested Chromosomes:
    • X, Y, 13, 16, 18, 21, 22
  – Most Predictive of Recurrent Miscarriage:
    • 1, 15, 17, 21, 22

• Number of sperm to be counted for relevant data?
• Standardization and automation of hybridization and enumeration protocols?
• Relative risk?
Caveat: Non-chromosomal Aneuploidy

- Functional Aneuploidy via Epigenetic Markings (Silencing) Nature, 2009
Acknowledgements

Ben Emery
Matt Sanderson
Jeanine Griffin

Terry Hassold, Washington State