PGT-A for all: Con

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PGT-A: Why the numbers (still) don’t add up

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Disclosures

• None
Learning Objectives

1) To understand why PGT-A has intuitive appeal
2) To describe limitations of PGT-A
3) To counsel patients about the appropriate application of PGT-A

Purpose of PGT-A

- Increase in pregnancy rates
- Method:
  - Selection of best embryos
  - Elimination of abnormal embryos
  - Elimination of embryos with lower implantation potential
  - No improvement in quality of remaining embryos

Limitations of PGT-A

- Inaccurate testing
  - Discarding normal embryos
- Harm from the procedure
  - Decreased implantation rates of normal (biopsied) embryos
- Substantial numbers of potential implantations lost
How do we estimate losses due to PGT-A?
How do we estimate losses due to PGT-A?

- Implantation rate of untested embryos
  - “unpurified sample”
- Implantation rate of tested embryos
  - “purified sample”
- Aneuploidy rate
  - “amount discarded”

Generalized Efficiency Equation

Embryo implantation (EI) must increase if we are removing lower quality embryos from the population

\[
\text{EI (expected)} = \frac{\text{EI (untested)}}{\text{percent normal}}
\]

Efficiency = \frac{\text{EI (observed after testing)}}{\text{EI (expected)}}
Sample calculation

• Idealized good prognosis patient
  – 50% implantation rate before testing
  – 67% implantation rate after testing
  – PGT-A testing
    • 40% aneuploidy

100 embryos

100 embryos, 50% implantation rate

50 implant
50 no implant
100 embryos, 50% implantation rate
40% aneuploidy

40 aneuploid
50 implant
50 no implant

After PGT-A, 60 embryos left

If there are NO LOSSES
New implantation rate:
50/60 = 83%
If there a 5% loss,
New implantation rate:
48/60 = 80%

If there a 10% loss,
New implantation rate:
45/60 = 75%

Actual implantation rate is:
67% ≈ 40/60
Improvement over 50%
Actual advertised numbers

- Women < 35
  - 49.4% implantation rate before testing
  - 65.0% implantation rate after testing
  - PGT-A testing = 45.5% aneuploidy

Incidence of euploidy
(based on age and # of embryos)

<table>
<thead>
<tr>
<th>No. of day 5 embryos</th>
<th>Patient Age</th>
<th>Euploid Patients</th>
<th>Euploid Patients</th>
<th>Euploid Patients</th>
<th>Euploid Patients</th>
<th>Euploid Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;35</td>
<td>54%</td>
<td>39%</td>
<td>35%</td>
<td>31%</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>35-37</td>
<td>57%</td>
<td>47%</td>
<td>43%</td>
<td>36%</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>38-40</td>
<td>58%</td>
<td>49%</td>
<td>44%</td>
<td>38%</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>40+</td>
<td>58%</td>
<td>50%</td>
<td>44%</td>
<td>38%</td>
<td>28%</td>
</tr>
</tbody>
</table>

Average Euploid rate: 54.5% (= 45.5% aneuploid)
Generalized Efficiency Equation

Embryo implantation (EI) must increase if we are removing lower quality embryos from the population

\[
\text{EI (expected)} = \frac{\text{EI (untested)}}{\text{(percent normal)}}
\]

\[
\text{Efficiency} = \frac{\text{EI (observed after testing)}}{\text{EI (expected)}}
\]

Generalized Efficiency Equation

Embryo implantation (EI) must increase if we are removing lower quality embryos from the population

\[
\text{EI (expected)} = \frac{49.4\% \text{ implantation}}{54.5\% \text{ normal}} = 90.6\%
\]

\[
\text{Efficiency} = \frac{65.0\%}{90.6\%} = 71.7\%
\]

How do we estimate losses due to PGT-A?

- Number of potential implantations lost
  \[= 1 - \text{Efficiency}\]
- Women < 35
  - Efficiency = 71.7%
  - % implantations lost = 28.3%
Actual advertised numbers

• Women 35 - 37
  – 42.3% implantation rate before testing
  – 64.5% implantation rate after testing
  – PGT-A testing = 52% aneuploidy
  – Efficiency = 73.0%

27% of potential implantations lost

Actual advertised numbers

• Women 38 - 40
  – 32.9% implantation rate before testing
  – 61.1% implantation rate after testing
  – PGT-A testing = 63.25% aneuploidy
  – Efficiency = 68.0%

32% of potential implantations lost
Actual advertised numbers

- Women 40+
  - 20.7% implantation rate before testing
  - 60.2% implantation rate after testing
  - PGT-A testing = 73.5% aneuploidy
  - Efficiency = 77.1%

23.9% of potential implantations lost

When is it OK to lose 20-30%?

- Specific reason for genetic diagnosis
- Excellent prognosis patient
  - More embryos than she needs

When is it NOT OK to lose 20-30%?

- Limited number of eggs
  - Fertility preservation patients
  - Patients over 40
What are actual “real life” implantation rates?

- STAR Trial
  - Approximately 50% live birth rates
  - Both PGS and control groups

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The STAR Trial

A randomized controlled trial (RCT) comparing pregnancy rates following VeriSeq™ PGS versus standard morphology for elective single embryo transfer (eSET)

What is VeriSeq PGS?
VeriSeq PGS is the Illumina next-generation sequencing (NGS) solution for PGS. VeriSeq PGS provides comprehensive testing for copy number on all 24 chromosomes from the embryo biopsy.

https://www.illumina.com/content/dam/Illumina-marketing/documents/clinical/hr/gly/star-one-pager-web.pdf

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Figure 1: 20-week ongoing pregnancy rate

<table>
<thead>
<tr>
<th>35-week CPR</th>
<th>NS</th>
<th>53%</th>
<th>49%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>169</td>
<td>25-34 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGS</td>
<td>152</td>
<td>25-40 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>145</td>
<td>All ages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGS</td>
<td>122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>213</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGS</td>
<td>274</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant
“Real World” numbers (STAR trial)

- Women <35
  - 50% implantation rate before testing
  - 50% implantation rate after testing
  - PGT-A testing = 45.5% aneuploidy
  - Efficiency = 54.5%

45.5% of potential implantations lost

Conclusions

- Trophectoderm resembles a soccer ball
  - Inherent inaccuracy of sampling
- Current practice of PGT-A results in the loss of 20% - 45% of potential implantations
  - Small part is due to inaccuracy
  - Large part is due to damage to embryos
Counseling patients about PGT-A

- PGT-A will provide information about the embryo
- PGT-A will likely increase implantation in 1st ET
- PGT-A will add cost
- You will lose 20% - 45% of embryos that might have implanted
- Cumulative pregnancy rate will be decreased

Conclusions – PGT-A

- Useful:
  - Specific diagnosis, e.g. translocation, sex selection
  - Recurrent aneuploidy (RPL) (likely)
  - Age 36-40, with many blastocysts
- Unnecessary:
  - Young good prognosis patients (< 35 yo)
- Not worth it:
  - Limited number of eggs
    - Fertility preservation, women over 40

The End

- Thank you!
- Merci!
- Danke!
- Gracias!
- Dekuji Vam!
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Actual advertised numbers

Igenomix.com
https://www.igenomix.us/hubfs/USA/PGS/PDF/PGS%20patient%20handout%20US.pdf?r=1511491539910
Actual advertised numbers

- Women < 35
  - 49.4% implantation rate before testing
  - 65.0% implantation rate after testing
  - PGT-A testing = 51.8% aneuploidy
  - Efficiency = 63.4%

- 36.6% of potential implantations lost
<table>
<thead>
<tr>
<th>Women Age Range</th>
<th>Implantation Rate Before Testing</th>
<th>Implantation Rate After Testing</th>
<th>PGT-A Testing</th>
<th>Efficiency</th>
<th>Potential Implantations Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 - 37</td>
<td>42.3%</td>
<td>64.5%</td>
<td>54.4% aneuploidy</td>
<td>69.5%</td>
<td>30.5%</td>
</tr>
<tr>
<td>38 - 40</td>
<td>32.9%</td>
<td>61.1%</td>
<td>67.9% aneuploidy</td>
<td>59.9%</td>
<td>40.1%</td>
</tr>
<tr>
<td>41 - 42</td>
<td>20.7%</td>
<td>60.2%</td>
<td>77.9% aneuploidy</td>
<td>64.2%</td>
<td>35.8%</td>
</tr>
</tbody>
</table>
Actual advertised numbers

- Women > 42
  - 7.8% implantation rate before testing
  - 53.7% implantation rate after testing
  - PGT-A testing = 79.8% aneuploidy
  - Efficiency = 139.1%
- 39.1% additional implantations
  (Impossible)

Unusual aspects of Rubio study

- Day 3 biopsy
  - No damage or loss

### Unusual aspects of Rubio study

- **538 d3 embryos**
  - 112 euploid (20.8%)

- **335 blastocysts (62.3% blastocyst rate)**
  - 104 euploid blastocysts (31.0%)
    - 93% blastocyst progression for euploid embryos
    - Blastocyst culture selected for euploid embryos

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PGO-A</th>
<th>Non-POG-A</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles performed</td>
<td>160</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cycles with transfer (%)</td>
<td>110</td>
<td>125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of embryos transferred (%)</td>
<td>13.0 (13.1)</td>
<td>14.0 (14.1)</td>
<td>&lt; .0001</td>
<td>0.32 (0.19-0.55)</td>
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<tr>
<td>Implantation rate (%, n)</td>
<td>49/145 (33.6)</td>
<td>48/134 (35.8)</td>
<td>&lt; .0001</td>
<td>2.34 (1.47-3.68)</td>
</tr>
<tr>
<td>Clinical pregnancy (%, n)</td>
<td>35/145 (24.0)</td>
<td>40/134 (30.0)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>25/145 (17.1)</td>
<td>30/134 (22.4)</td>
<td>&lt; .0001</td>
<td>0.86 (0.59-1.25)</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>150</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cycles with transfer (%)</td>
<td>108</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
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*The table includes some missing data.

Unusual aspects of Rubio study

• PGT-A group
  – 335 blastocysts, 104 euploid (31%)
  – Aneuploidy rate = 69%

Unusual aspects of Rubio study

• Women 38 – 41
  – 14.9% ongoing implantation rate without PGT-A
  – 49.4% ongoing implantation rate with PGT-A
  – 69% aneuploid
  – Efficiency = 102.8%

Appropriate utilization of PGT-A

• Individualization
  – Balance of benefits and "costs" in each case
Limitations of PGT-A

- Mosaic nature of trophectoderm
  - Analogous to a soccer ball
  - Inherent inaccuracy of sampling (about 10%)
- Significant “cost” of obtaining PGT information
  - 20% - 45% of potential implantations lost
    - Partly due to inaccuracy and sampling error
    - Partly due to trauma of embryo biopsy

The End

- Thank you!
- Merci!
- Danke!
- Gracias!
- Děkuji Vám!
How do we estimate False Positives?

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study

Richard T. Scott Jr., MD,1,2,3,4,10 Kathleen Perry, BS,5 Ling Yu, M.S.,1,2 Vin Tap, M.S.,5 Katherine Scott, M.S.,3 and Nathan J. Tefft, M.D.1

NCT 01219517
NCT 01219504

Scott et al, Fertil Steril 2012;97:870

Predictive Value of CCS

- 255 embryos biopsied
  - Average age = 34
- 232 evaluable microarray results
  - 133 euploid
    - 55 (41.4%) of these resulted in normal children
  - 99 (42.7%) aneuploid
    - 4 (4%) normal children (96% negative predictive value)

Scott et al, Fertil Steril 2012;97:870
41% of the “Euploid” group implanted
4% of the “Aneuploid” group implanted
Error rate: 10/99 (10%) “aneuploid” were actually euploid
4/59 (6.8%) implantations would have been discarded

Scott et al, Fertil Steril 2012;97:870

Trauma from Embryo Biopsy?

Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial

Richard T. Scott Jr., M.D.,* Kathleen M. Upham, B.S.,† Eric J. Forsten, M.D.,‡ Tian Zhao, M.S.,§ and Nathan R. Tefft, M.D.**

“Seminal Contribution” NCT01219504

Scott et al, Fertil Steril 2013;100:624
What does a day 5 embryo look like?

“Buckyball”
- Naturally occurring C_{60}
  - 32 faces
  - 20 hexagons
  - 12 pentagons
- Trophectoderm with 64 cells
  - 2 cells/face
- Imagine removing 5 cells
  - Is this really NOT traumatic?

PGT-A 1.0
- Cleavage stage biopsy
- FISH analysis
- Widely utilized

PGT-A 1.0 meta-analysis

Mastenbroek et al, Human Reprod Update 2011;4:454
PGT-A 2.0

- Blastocyst stage biopsy
- Comprehensive Chromosome Screening (CCS)
  - Comparative Genomic Hybridization (CGH)
  - Next-Gen Sequencing (NGS)

PGT-A 3.0

- Cell-free DNA in culture dish
- WGA
  - Next-Gen Sequencing (NGS)
- Unproven

Ho et al, Fertil Steril 2018;110:467-75