Debate: PGT-A for all (Pro)
P
PGT-A increases pregnancy rates per transfer and shortens time to pregnancy

Carmen Rubio, PhD

DISCLOSURE

I am a fully employee of Igenomix and Igenomix Foundation. Both institutions dedicated to Reproductive Genetics, providing genetic test and as a research foundation focused in the same field.

LEARNING OBJECTIVES

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

1) Describe the potential advantages and disadvantages of PGT-A
2) Counsel patients about the use of PGT-A
3) Understand the relative magnitude of implantation loss due to false positive testing and by the trauma of the embryo biopsy
4) Discuss the state of the art of non-invasive PGT-A to avoid the trauma of the embryo biopsy and/or manipulation
Benefits and indications of PGT-A

- Improved implantation
- Decreased miscarriage rates
- Decreased risk of multiples and abnormal offspring
- Decreased time to pregnancy and emotional burden
- Potential cost-effectiveness

Indications
- Advanced maternal age (≥38 years)
- Prior pregnancy/child chromosomally abnormal
- Multiple implantation failures (≥2 failed IVF)
- Recurrent miscarriage (≥2 miscarriages)
- Severe male factor (low sperm count)
- Ovum donation cycles & good prognosis patients

Why perform aneuploidy testing in embryos?

Most aneuploid embryos in IVF do not implant or end in a miscarriage

Morphology selection does not avoid the transfer of aneuploid embryos

Single Aneuploidy: high incidence in young women and donors (~30%). No maternal age effect.
Morphokinetics can predict only specific aneuploidy types

Why perform aneuploidy testing in embryos?

- Complex Aneuploidies (2-5 chr.)
- Shorter kinetic parameters in chaotic embryos
- Faster early development, but arrest before

Similar kinetics in euploid and trisomic embryos

Advantages of PGT-A versus conventional IVF

PUBLISHED STUDIES

1) Meta-analysis of RCT & cohort studies
2) Observational & RCT studies
3) Time to delivery and cost models

Improved sustained implantation with PGT-A in RCTs and cohort studies

(Dahdouh et al., F&S, 2015)
1) Meta-analysis of RCT & cohort studies

Decreased miscarriage with PGT-A in cohort studies

RCTs

<table>
<thead>
<tr>
<th>Study</th>
<th>Total miscarriage</th>
<th>Total</th>
<th>Total</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGT-A</td>
<td>1,256</td>
<td>600</td>
<td>656</td>
<td>0.945</td>
</tr>
<tr>
<td>No PGT-A</td>
<td>572</td>
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<td>1,144</td>
<td>1.000</td>
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Cohorts

<table>
<thead>
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2) Observational & RCT studies

Observational study in ovum donation

Increased live birth transfer with PGT-A for DET and a trend for SET

<table>
<thead>
<tr>
<th>Group</th>
<th>Live birth rate (DET)</th>
<th>Live birth rate (SET)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGT-A</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>No PGT-A</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

Gestational Carrier

Own recipient

Parkin et al. JARG, 2017
2) Observational & RCT studies

### RCT in AMA (≥38 years; ≥5MII oocytes)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PGT-A</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Patients with fresh transfer</td>
<td>90.5</td>
<td>68.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>39.0</td>
<td>2.7*</td>
<td>0.0007</td>
</tr>
<tr>
<td>Delivery rate/transfer</td>
<td>24.2</td>
<td>52.9*</td>
<td>0.0002</td>
</tr>
<tr>
<td>Delivery rate/patient</td>
<td>21.9</td>
<td>36.0*</td>
<td>0.0009</td>
</tr>
<tr>
<td>Cumulative delivery rate/ patient</td>
<td>33.3</td>
<td>37.0</td>
<td>NS</td>
</tr>
<tr>
<td>No. of livebirths/patient</td>
<td>39 (37.1)</td>
<td>45 (45.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

(Rubio et al. F&S, 2017) (ClinicalTrials.gov NCT01571076 Igenomix-IVI)

2) Observational & RCT studies

### RCT in AMA (≥38 years)

- **Time to pregnancy (weeks) vs. No. of transfers**
  - PGT-A: 12 weeks
  - Control: 16 weeks

- **Time to clinical pregnancy**: shorter mean time to reach a clinical pregnancy leading to a live-birth (104.8 days vs 140.6 days, *P* < 0.05).

(Rubio et al. F&S, 2017) (ClinicalTrials.gov NCT01571076 Igenomix-IVI)

3) Time to delivery and cost models

### Models for time to delivery and cost-effectiveness

- Decrease in time to pregnancy and cost in patients ≥38 years with at least 1 embryo (Neal et al. F&S, 2018)
- Cost-effectiveness increases with age and number of available blastocysts; superior for women >35 yrs and for 35 yrs with at least three blastocysts (Somigliana et al. F&S, 2019)
- Half cost ET
**How to perform aneuploidy testing in embryos**

**THE KEY: the methodology**

1) **Embryo Biopsy: impact on embryo viability**

2) **Genetic Analysis: NGS and mosaicism**

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**Embryo Biopsy: impact on embryo viability**

- **Sustained implantation rate**
  - Biopsied blastocysts: 60%
  - Non-biopsied blastocysts: 40%

- **Equivalence**
  - Class I data from paired randomized study
  - TE biopsy DOES NOT affect embryo reproductive potential

- **Good results in experienced labs**
- Impact of number of removed cells
- Impact of cell integrity in the results

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**Present**

Conventional PGT-A

- Embryo biopsy: Day 5-6
- 5-8 TE cells
- NGS

**What is coming**

Non-invasive PGT-A

- Embryo cultured from day 5-6
- cfDNA in spent media
- NGS

**NGS in blastocyst biopsy**

- 45, -16, XX

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**NGS in Spent Culture Media**

- 45, -16, XX
Customized algorithms for mosaicism determination

- Cell lines mixes: 30% - 70%.
- Thresholds established with the means of the medians.

Low Mosaic  
High Mosaic

Bioinfoserver & Machine learning and transfer of data

Incidence of Mosaic in human blastocysts
(>30,000 biopsies Oct-Dec 2018)

- Mosaicism rate: 5.8% (3.8% low mosaic degree and 2% high mosaic degree).
- High consistency: 9 diagnostic laboratories with similar low and high mosaicism rates (low mosaic: 2.4-4.4 and high mosaic: 1-2.6).

Euploid embryos
(<30% aneuploidy)

Low Mosaic Degree
(30-50% aneuploidy)

High Mosaic Degree
(50-70% aneuploidy)

Aneuploid embryos
(>70% aneuploidy)

Capalbo et al., Hum Reprod 2016
Incidence of aneuploidy with two different NGS approaches

**Graph**

- **ASRM 2018**
- **Lab A** = 659 biopsies
- **Lab B** = 740 biopsies

<table>
<thead>
<tr>
<th>No call rate</th>
<th>Euploidy &lt;37</th>
<th>Euploidy &gt;37</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lab A</strong></td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Lab B</strong></td>
<td><img src="image3" alt="Graph" /></td>
<td><img src="image4" alt="Graph" /></td>
</tr>
</tbody>
</table>

Summary of up-to-date data from preclinical and clinical studies on PGT-A

**Expected/Observed advantages**
- Increase implantation rate per ET
- Decrease miscarriage rate
- Decrease abnormal pregnancies
- Decrease time to pregnancy
- Potential for being cost-effective

**Potential disadvantages**
- Potential for minimal damage of embryos (overcome with nPGT-A)
- Needs expertise

- We should offer information about PGT-A to ALL patients describing the expectations according to their age and number of embryos
- Informed choice of the treatment

**REBUTTAL**
Non invasive PGT-A (embryonic cfDNA)

Spent culture medium in comparison to TE biopsies

<table>
<thead>
<tr>
<th>Study</th>
<th>Informativity</th>
<th>Concordance (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shamoni et al., Fert&amp;Star 2016</td>
<td>3.5%</td>
<td>concordance (N=55)</td>
</tr>
<tr>
<td>Feichtinger et al., RBMonline 2017</td>
<td>27%</td>
<td>concordance (N=22)</td>
</tr>
<tr>
<td>Vera et al., HR 2018</td>
<td>30.4%</td>
<td>concordance (N=56)</td>
</tr>
<tr>
<td>Ho et al., FertiSteri 2018</td>
<td>65.9%</td>
<td>concordance (N=61)</td>
</tr>
<tr>
<td>Xu et al., PNAS 2016</td>
<td>85.7%</td>
<td>concordance (N=42)</td>
</tr>
<tr>
<td>Kuznyetsov et al., PloSOne 2018</td>
<td>87.5%</td>
<td>concordance (N=47)</td>
</tr>
<tr>
<td>Li et al., Scientific Reports 2018</td>
<td>76.0%</td>
<td>concordance (N=40)</td>
</tr>
</tbody>
</table>

High variability between results

Non invasive PGT-A (embryonic cfDNA)

niPGT-A: comparison of TE biopsies and SBM (n=108)

<table>
<thead>
<tr>
<th>RESULTS Day-5 (N=27)</th>
<th>RESULTS Day 6-7 (N=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informativity</td>
<td>Concordance</td>
</tr>
<tr>
<td>81.8%</td>
<td>63%</td>
</tr>
<tr>
<td>100%</td>
<td>84%</td>
</tr>
</tbody>
</table>

Rubio et al., ESHRE 2018; submitted F&S 2019
Non invasive PGT-A (embryonic cfDNA)

**DAY 6/7 comparison of TE biopsies and SBM (n=81)**

| CONCORDANT RESULTS | With similar gender | 84% | CONCORDANT RESULTS | With different gender | 5% |
| FALSE NEGATIVES: | Aneuploid biopsy | Euploid media | 2.5% | FALSE POSITIVES: | Euploid biopsy | Aneuploid media | 8.5% |

Rubio et al., ESHRE 2018; submitted F&S 2019

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Non invasive PGT-A (embryonic cfDNA)

**niPGT-A: preliminary clinical results**

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Euploid Y/ Euploid SBM</th>
<th>Euploid Y/ Aneuploid SBM</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of transfers</td>
<td>17</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>Mean maternal age (SD)</td>
<td>37.5 (2.5)</td>
<td>37.4 (2.3)</td>
<td>37.5 (2.4)</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>11 (64.7)</td>
<td>4 (33.3)</td>
<td>15 (51.7)</td>
</tr>
<tr>
<td>Biochemical pregnancy loss</td>
<td>2 (18.2)</td>
<td>0</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>9 (52.9)</td>
<td>4 (33.3)</td>
<td>13 (44.8)</td>
</tr>
<tr>
<td>Clinical miscarriage</td>
<td>0</td>
<td>2 (50.0)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>9 (52.9)</td>
<td>2 (16.7)</td>
<td>11 (57.9)</td>
</tr>
</tbody>
</table>

Rubio et al., ESHRE 2018; submitted F&S 2019

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Non invasive PGT-A (embryonic cfDNA)

**Impact of number of aneuploidies in concordance rates**

![Graph showing impact of number of aneuploidies in concordance rates](image)

Rubio et al., ESHRE 2018; submitted F&S 2019
Non invasive PGT-A (embryonic cfDNA)

Transfer prioritization, could it be an alternative?

- **Euploid SBM**: 98% euploid TE
- **Non-Informative SBM**: 50% euploid TE
- **Chaotic (> 6 aneuploid chr)**: 47% euploid TE
- **Complex Aneuploid (3-5 chr)**: 25% euploid TE
- **Aneuploid (1-2 chr)**: 5% euploid TE

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FINAL CONCLUSIONS

- **PGT-A**
- **niPGT-A**

- Conventional PGT-A is beneficial for MOST couples attending IVF clinic (>35 years of age).
- Non-invasive PGT-A could be applied to ALL patients. More studies needed including not only delivery rates, but also cost-effectiveness (no biopsy), and time to pregnancy.