New perspectives on embryo biopsy, not how, but when and why

PGS

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“ESHRE 10 Years”, Harper et al., HRU 2012; 18:234

CRM-PGD, 1992 to 2015

Key:
- Aneuploidy screening
- Single-gene disorders
- Chromosomal abnormalities
- Sex selection for X-linked disorders
- Social seeing

59.4%
22.8%
10.4%
5.8%
2.3%
A Statement on the use of Preimplantation Genetic Screening (PGS) of chromosomes for IVF patients

CONSENSUS STATEMENT ON PGS
For all practitioners of IVF there is the clinical imperative

➢ to achieve the highest chance of a live birth per single attempt, reducing the time to delivery for each patient;
➢ to reduce the incidence of miscarriage; reduce the number of multiple pregnancies;
➢ decrease the number of non-viable embryo transfers (unnecessary IVF transfer cycles);
➢ eliminate the freezing of embryos that are chromosomally abnormal;
➢ to diagnose patients with no chance to deliver with IVF; and,
➢ given the high incidence of embryo aneuploidy in all IVF cycles, to minimize the chance of transferring an aneuploid embryo.

➢ The Undersigned have issued the Statement below and welcome debate and comment in this forum.

Contradictions in Recent Literature


The three RCTs demonstrated benefit in young and good prognosis patients in terms of clinical pregnancy rates and the use of single embryo transfer. However, studies relating to patients of advanced maternal age, recurrent miscarriage and implantation failure were restricted to matched cohort studies, limiting the ability to draw meaningful conclusions.

Aneuploidy screening was the most common indication for PGD. Use of PGD was not observed to be associated with an increased odds of clinical pregnancy or live birth for women <35 years. PGD for aneuploidy was associated with a decreased odds of miscarriage for women >35 years, but an increased odds of a live-birth and a multiple live-birth delivery among women >37 years.

Technical advancement & limitations

- Biopsy from D5/6 embryos,
- Specimens undergone WGA (noise and background) and WGA products subject to array or NGS to obtain chromosome copy number analysis
- WGA products subject to array or NGS to obtain chromosome copy number analysis
- Software makes “Call” or “Not to Call”, “A SPECILIST” will make the final “CALL” and prepare the report.
- Variations of unknown significance
Use of SNP Array for few cells (2009)

The SNP call rate from 1C, 1+1C and 2C groups showed no significant difference (p>0.05), but when the cell number increased to 5-10 cells, the call rate presented significant difference (p<0.05).
Detection of Mosaicism

Chromosome complements of the blastomeres analyzed by aCGH

Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos

Mertzanidou et al., 2013
D5/6 Mosaicism

1) Can mosaicism be detected in the biopsied specimens with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?

Mix of 46,XX and 47,XX,+21, aCGH

(a) G1, 0% trisomic  (d) G4, 60% trisomic
(b) G2, 20% trisomic  (e) G5, 80% trisomic
(c) G3, 40% trisomic  (f) G6, 100% trisomic

20%
NGS Cell Mix Validation Test

46,XY, del(4p) : 47,XY,+13 (0 : 10)

Wolf Hirschhorn Syndrome, WHS (~26MB)

46,XY, del(4p) : 47,XY,+13 (1 : 9)
46,XY, del(4p) : 47,XY,+13 (2 : 8)

Copy Number

20%

80%

46,XY, del(4p) : 47,XY,+13 (3 : 7)

Copy Number

30%

70%
46,XY, del(4p) : 47,XY,+13 (4 : 6)

46,XY, del(4p) : 47,XY,+13 (5 : 5)
46,XY, del(4p) : 47,XY,+13 (6 : 4)

Copy Number vs Chromosomal Position

40%

46,XY, del(4p) : 47,XY,+13 (7 : 3)

Copy Number vs Chromosomal Position

30%

70%
46,XY, del(4p) : 47,XY,+13 (8 : 2)

46,XY, del(4p) : 47,XY,+13 (9 : 1)
46,XY, del(4p) : 47,XY,+13 (10 : 0)

D5/6 Mosaicism

1) Can mosaicism be detected with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?
**Incidence of mosaicism: 4%, 16%, 21%, 33% or 69%**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Description</th>
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<tbody>
<tr>
<td>3.9%</td>
<td>Johnson et al., (Mol. Hum. Reprod., 2010) observed 49/51 (96.1%) ICM samples were concordant with TE biopsies derived from the same embryos.</td>
</tr>
<tr>
<td>~16%</td>
<td>Northrop et al. (Mol. Hum. Reprod., 2010) found 16% of embryos are mosaic.</td>
</tr>
<tr>
<td>21.2%</td>
<td>Capalbo et al., (Hum. Reprod. 2013), by FISH reanalysis of previously aCGH-screened blastocysts, a total of 66 aneuploidies were scored, 52 (78.8%) observed in all cells and 14 (21.2%) mosaic.</td>
</tr>
<tr>
<td>~33%</td>
<td>Fragouli et al., (Hum. Reprod., 2011) demonstrated that about one-third of all blastocysts are mosaic.</td>
</tr>
<tr>
<td>69%</td>
<td>Liu et al. (Biol. Reprod., 2012) reported 69% of abnormal blastocysts from women of advanced age are mosaic.</td>
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</table>

**D5/6 Mosaicism**

1) Can mosaicism be detected with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?
Mosaic patterns and risk of misdiagnosis

D3, cleavage stage

D5/6, blastocysts

What is the incidence of mosaicism that may cause false positives?

TE biopsy

Correct diagnosis

Correct diagnosis ()?

False negative

False positive?

Low rate of mosaicism

Likely, will not implant

20% Diploid/aneuploid

Diploid ICM

Diploid (euploid) TE

Aneuploid cells

Should we transfer mosaic embryos?

Transfer of Mosaic (monosomic) Embryos

Our study shows that mosaic embryos can develop into healthy euploid newborns. These findings have implications for women who undergo IVF resulting in mosaic embryos but no euploid embryos.

Euploidy with age (PGS 24 chromosomes)

Total #: 3507

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<thead>
<tr>
<th>Age (weeks)</th>
<th>CRM</th>
<th>Referral</th>
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<tbody>
<tr>
<td>≤30</td>
<td>63.90%</td>
<td>61.80%</td>
</tr>
<tr>
<td>31-34</td>
<td>53.00%</td>
<td>44.20%</td>
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<tr>
<td>35-36</td>
<td>44.20%</td>
<td>31.10%</td>
</tr>
<tr>
<td>37-38</td>
<td>31.10%</td>
<td>21.70%</td>
</tr>
<tr>
<td>39-40</td>
<td>21.70%</td>
<td>7.50%</td>
</tr>
<tr>
<td>41-42</td>
<td>7.50%</td>
<td>0.00%</td>
</tr>
<tr>
<td>≥43</td>
<td></td>
<td>10.00%</td>
</tr>
</tbody>
</table>

Aneuploidy detected by CRM and Referral Labs (D5/6)

Data are remarkably comparable

CRM PGS vs Referral PGS

Data are remarkably comparable

CRM = 4390
Referral = 1071
Among patients ≤37, IVF-PGS does not improve CIG, LB, and miscarriage rates.

IVF-PGS in women >37 improved CIG and LB rates.

However, per cycle, the PGS advantage in this age group does not persist.
Discussions

Aneuploid embryos can be identified accurately when gain or loss in one or more chromosomes are involved. Although mosaicism can be accurately detected in a model system, it is difficult to know exact number of cells biopsied, therefore, the extent of mosaicism in the specimen can only be estimated. Knowledge of mosaicism on D5/6 embryos is limited.

Present views on PGS are controversial. With PGS, improvement of overall IVF outcome, particularly for woman of advanced age, is not yet clear. Specific indications need to be identified, discussed with patients, number of “BIOPSIABLE” should be evaluated for each individual patient/PGS-Cycle.

PGS may not be applied for “ALL” patients. Rebiopsy maybe considered when there is a doubt on the results and the embryo quality appears to be “good”. Research on D5/6 mosaicism is urgently needed. Artificial gametogenesis will address the issues.

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