PGD and PGS: Indications, techniques and future perspectives

Antonio Capalbo, PhD
Senior Embryologist, GENERA reproductive medicine Laboratory Director, GENETYX, molecular genetics
DEFINITION OF PGD AND PGS

PGD

- Developed to avoid the transfer of embryos affected by a specific genetic disease
- Usually, it is applied for fertile patients
- An preliminary “ad hoc” work-up is required for each couple

Embryological aspects

PGS

- Developed to avoid the transfer of chromosomally abnormal embryos due to “de novo” aneuploidies
- Usually, it is recommended for infertile patients
- No preliminary work up. The genetic test is the same for every couple.
• No misdiagnosis was reported for monogenic PGD cycles in ESHRE PGD consortium data collection X, XI and XII.
Techniques: biopsy stage for PGD

- **PBs biopsy**
  - High diagnostic failure rate
  - Exclude paternal genome
  - Impact on embryo development
  - Most expensive and time-consuming approach
  - Time for fresh ET

- **Blastomere biopsy**
  - High worldwide experience
  - Time for fresh ET
  - High diagnostic failure rate (10%)
  - Reduction in embryo viability

- **TE biopsy**
  - More robust analysis
  - Reduced number of embryos/cycles
  - Less time consuming
  - No impact of biopsy
  - Limited time for analysis
PGD: FUTURE DIRECTIONS

• Use new genetic technologies that can reduce the time-consuming work-up required to set-up a monogenic PGD cases.

**Karyomapping**: a high density genome wide map of the parental origin of each chromosome in the embryo.

Combines genome wide linkage based detection of single gene defects with chromosomal aneuploidy.

(Handyside et al., 2010)
<table>
<thead>
<tr>
<th>Population</th>
<th>Methodology*</th>
<th>Timeframe of studies</th>
<th>Incidence of aneuploidy‡</th>
<th>Most common aneuploidies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns</td>
<td>Karyotyping</td>
<td>1960s–1970s</td>
<td>0.3%</td>
<td>+13; +18; +21; XXX; XXY; XYY</td>
</tr>
<tr>
<td>Stillbirths</td>
<td>Karyotyping</td>
<td>1970s–1980s</td>
<td>4%</td>
<td>45,X; +13; +18; +21; XXX; XXY</td>
</tr>
<tr>
<td>Spontaneous abortions</td>
<td>Karyotyping</td>
<td>1970s–1980s</td>
<td>&gt;35%</td>
<td>45,X; +15; +16; +21; +22</td>
</tr>
<tr>
<td>Preimplantation embryos</td>
<td>Karyotyping</td>
<td>1990s</td>
<td>20–40%</td>
<td>+16; +17; +18</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>1990s–present</td>
<td>25–70%</td>
<td>Various</td>
</tr>
<tr>
<td></td>
<td>CGH, SNP array,</td>
<td>2000–present</td>
<td>30–60%</td>
<td>+15; +16; +21; +22</td>
</tr>
<tr>
<td></td>
<td>CGH array</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs or polar bodies</td>
<td>Karyotyping</td>
<td>1990s</td>
<td>10–35%</td>
<td>+16; +17; +18; +21; +22</td>
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<tr>
<td>Sperm</td>
<td>Karyotyping</td>
<td>1980s–1990s</td>
<td>1–4%</td>
<td>XY disomy; +21; +22</td>
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BLASTOCYST MORPHOLOGY CAN NOT BE RELIED ON TO ENSURE THE TRANSFER OF CHROMOSOMALLY NORMAL EMBRYOS

956 blastocysts with CCS results (mean female age 37.8)

- Excellent: 56.4%
- Good: 39.1%
- Average: 42.8%
- Poor: 25.5%

Capalbo et al. Hum Reprod 2014
Aims of Preimplantation Genetic Screening

Expected advantages in IVF

- Increase implantation rate
- Decrease in abortion rate
- Less abnormal pregnancies
- Single ET
- Decrease time to pregnancy
- Increase the cost-effectiveness
WE NEED TO PERFORM A BIOPSY AND CCS, WHEN?

No affect to embryo development

Reliable and informative results

Clinical evidences of effectiveness

Easy to implement in IVF:
- to reduce the cost
- reduce lab workload

Christopikou 2013; Capalbo 2013; Levin 2011; Geraedts 2011; Scott 2012

Scott 2013; Mertzanidou 2013; Robio 2012; van Echten-Arends 2011; Treff 2010; Hardason 2008; Vanneste 2009; Mastenbroek 2007; Staessen 2004
Confirmation studies based on FISH reanalysis of aneuploid blastocysts found 98-100% of correct aneuploid prediction of meiotic errors (Fragouli et al., 2010; Capalbo et al., 2013; Novik et al., 2014).

Method: qPCR blinded reanalysis of 120 second biopsies of aneuploid blastocysts previously screened by TE aCGH

A consistent chromosome copy number diagnosis was observed in 99.4% (2561/2576; 95%CI 99.0-99.7) of the chromosomes analysed.

Technical variation between CCS methods

0.6% Mosaicism
BLASTOCYST BIOPSY PROVIDES A HIGH PREDICTIVE VALUE ON CLINICAL OUTCOMES OF IVF CYCLES

Prospective Randomized non selection study

Scott et al., 2013
BLASTOCYST STAGE CCS IS A VALIDATED TECHNOLOGY TO IMPROVE SUSTAINED IMPLANTATION RATE IN IVF

<table>
<thead>
<tr>
<th>Study design</th>
<th>Methods</th>
<th>Conclusions</th>
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<td>RCT</td>
<td>Good prognosis patients; aCGH based CCS and fresh ET</td>
<td>Increased sustained implantation rate of euploid blastocysts</td>
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<td>RCT</td>
<td>qPCR based CCS and fresh or frozen ET; Mean female age 37</td>
<td>Similar pregnancy rate of eSET of euploid blastocysts and lower multiple pregnancies compared with DET of untested blastocysts.</td>
<td>Forman et al., 2013 and 2014</td>
</tr>
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**RCT evaluating live birth rate per cycle**
Maximizing live birth rate in blastocyst stage PGS cycles: biopsy all!!!

Ongoing implantation rate of euploid blastocysts according to morphology

Capalbo et al., Hum. Rep, 2014
No impact of biopsy

Reliable and informative results

Evidence of clinical effectiveness

Easy to implement
Data from GENERA based on 370 PGS cycles, mean female age 39.4

Overall abortion rate 6.1%

Aneuploidy rate

No transferable blastocysts cycles rate

Ongoing implantation rate

- ≤35yr: 35.5% Aneuploidy, 11.4% No transferable blastocyst, 47.1% Ongoing implantation
- 36-38yr: 51.7% Aneuploidy, 25.2% No transferable blastocyst, 52.7% Ongoing implantation
- 39-41yr: 64.0% Aneuploidy, 50.0% No transferable blastocyst, 52.7% Ongoing implantation
- 42-45yr: 81.2% Aneuploidy, 63.2% No transferable blastocyst, 47.1% Ongoing implantation
Do we need indication for PGS when 30% of IVF derived embryos from “young” patients are aneuploid considering also that invasive prenatal diagnosis is recommended when the risk of a chromosomally affected pregnancy is less than 1%?
TAKE HOME MESSAGE

• **PGD is a well established PGD and effective technology** that applies when one or both parents carry a gene mutation or a chromosomal rearrangement. Future works will be focused on the application of blastocyst biopsy and in the development of new genetic technologies to reduce the lab-workload and costs.

• **Blastocysts stage PGS** is also an already validated technology to improve clinical outcomes per transfer in IVF cycles in terms of higher implantation rate and reduced miscarriage rate. RCTs are still needed to evaluate live birth rate per cycle.

  • Emerging technologies will briefly reduce the cost of PGS and increase the patient population that can benefit from aneuploidy screening in IVF cycles.

  • The increase in usage of blastocyst culture and “freeze all” approach will further stimulate the application of this technology in IVF in the future.

  • Considering the high risk of aneuploidies of preimplantation embryos, all patient should be informed and advised about the possibility of performing CCS on their embryos.
GENERA, Reproductive Medicine Centres, Italy;
Clinical Director: Filippo Maria Ubaldi
Laboratory Director: Laura Francesca Rienzi

GENETYX, Molecular genetic laboratory, Italy;
Laboratory Director: Antonio Capalbo
Biologist team: Danilo Cimadomo, Cristina Patassini, Ludovica Dusi, Cristina Dusi, Emiliano Scepi,