Indications for chromosome screening

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Chromosome imbalance
(aneuploidy)
Uncontroversial data
Aneuploidy is extremely common in human oocytes and increases with advancing age.

This trend is also reflected in the dramatic increase in Down syndrome pregnancies with maternal age.
The incidence of aneuploidy

The high incidence of oocyte aneuploidy has been demonstrated using multiple techniques in laboratories worldwide.

Aneuploid oocytes produce embryos abnormal in every cell.

For women over 40, over 50% of cleavage stage embryos are chromosomally abnormal in every cell.

What is the impact of aneuploidy?
Aneuploidy and IVF failure

As aneuploidy increases age, so implantation rate decreases

~65% of 1st trimester miscarriages are aneuploid
Preimplantation genetic screening (PGS)

Standard embryo evaluations do not reveal embryos with the wrong number of chromosomes.

Regular or after chromosome screening
Preimplantation genetic screening

Chromosomal indications

- Biopsy
- 1st round of FISH
- 2nd round of FISH
- NRR

• Theoretical benefits for patients undergoing routine IVF
  - Increase embryo implantation/pregnancy rate
  - Reduce aneuploid syndromes
  - Reduce miscarriage
Advanced maternal age
The positive
Reduction in aneuploid pregnancies
Reduction in aneuploidies 13, 18, 21, XY achieved using PGS

From 2,300 cases with follow-up data available, mean age 37

Munne et al 2006 and Reprogenetics data to 10/2007
Are patients interested in PGS for this purpose?

Recent study of subfertile women (Twisk et al., 2007)

If PGS was assumed to have no effect on pregnancy rate
83% of patients would request PGS (75% if 80% detection)

If PGS was assumed to reduce pregnancy rate from 20% to 14%
36% of patients would still request PGS (31% if 80% detection)
Reduction in miscarriage rate
IVF pregnancy loss and maternal age

<table>
<thead>
<tr>
<th>Age Range</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>13.3%</td>
</tr>
<tr>
<td>35-37</td>
<td>17.7%</td>
</tr>
<tr>
<td>38-40</td>
<td>26.2%</td>
</tr>
<tr>
<td>41-42</td>
<td>39.4%</td>
</tr>
<tr>
<td>43-44</td>
<td>53.3%</td>
</tr>
</tbody>
</table>

SART-ASRM (2005)
## Reduction in spontaneous abortion

### Pregnancy loss rates in the general IVF population and after PGD

<table>
<thead>
<tr>
<th>Age</th>
<th>35-40</th>
<th>&gt;40</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF population*</td>
<td>19%</td>
<td>41%</td>
</tr>
<tr>
<td>PGD**</td>
<td>14%</td>
<td>22%</td>
</tr>
</tbody>
</table>

- $p<0.05$
- $p<0.001$

considering pregnancies as the presence of a gestational sac, and pregnancy loss as the loss of the whole pregnancy.

Munne et al., 2006
Increase in pregnancy rates
# PGS – live birth rate

**Patients 38-42**

Chromosomes analyzed: XY, 13, 15, 16, 17, 18, 21, 22

SART data of 5 centers with >10% PGS cases, 2003-2005

<table>
<thead>
<tr>
<th>clinic</th>
<th>Non-PGS cycles</th>
<th>loss rate</th>
<th>live birth</th>
<th>PGS cycles</th>
<th>loss rate</th>
<th>live birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>505</td>
<td>27%</td>
<td>35%</td>
<td>70</td>
<td>22%</td>
<td>40%</td>
</tr>
<tr>
<td>2</td>
<td>210</td>
<td>36%</td>
<td><strong>14%</strong></td>
<td>72</td>
<td>27%</td>
<td><strong>15%</strong></td>
</tr>
<tr>
<td>3</td>
<td>1204</td>
<td>34%</td>
<td><strong>12%</strong></td>
<td>120</td>
<td>15%</td>
<td><strong>23%</strong></td>
</tr>
<tr>
<td>4</td>
<td>509</td>
<td>29%</td>
<td>15%</td>
<td>236</td>
<td>26%</td>
<td>22%</td>
</tr>
<tr>
<td>5</td>
<td>191</td>
<td>25%</td>
<td>17%</td>
<td>208</td>
<td>16%</td>
<td>25%</td>
</tr>
<tr>
<td>total</td>
<td>2619</td>
<td><strong>30%</strong>(^a)</td>
<td><strong>18%</strong>(^b)</td>
<td>706</td>
<td><strong>21%</strong>(^a)</td>
<td><strong>24%</strong>(^b)</td>
</tr>
</tbody>
</table>

\(^a\): p<0.01  
\(^b\): p<0.001  

Losses reduced by ~1/3  
Live births increased by ~1/3

Munne et al 2007; Colls et al 2007
Problems with positive PGS studies

BUT....

- Not randomized
- In some cases control groups questionable
The negative
Increase in implantation/pregnancy- controversy

Implantation rate

- Mastenbroek et al (2007), NEJM
- Maternal age $\geq 35$
- 8 chromosomes assessed, randomised
- No significant improvement in implantation
Problems with negative PGS studies

BUT....
- Many patients with <5 embryos included in study (mean 4.8)
  Little selection possible
Problems with negative PGS studies

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  Developmental potential drastically reduced
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  Literature 6-20 times less failure, little selection possible
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- Did not test chromosomes 15 & 22 (only 28% of aneuploidies detected)
Poor selection of chromosome probes

Percentage of total aneuploidies

Chromosome

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 XY
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**BUT....**

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  - Little selection possible
- Many 4-cell embryos biopsied
  - Developmental potential reduced
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  - Literature 6-20 times less failure, little selection possible
- Did not test chromosomes 15 & 22 (only 28% of aneuploidies detected)
  - Many abnormal embryos undetected, little selection possible
- Implantation rate for biopsied, non-diagnosed embryos = 6%
  - Developmental potential reduced. Lack of biopsy experience?
Problems with negative PGS studies

- Critically damaged by biopsy
- Only 28% of aneuploidies detected
- No result

Pool of embryos reduced while little selective advantage has been gained
<table>
<thead>
<tr>
<th>Legitimate criticisms of traditional PGS methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Current methodologies are not robust, limiting application</td>
</tr>
<tr>
<td>• Biopsy can have a serious impact if poorly performed</td>
</tr>
<tr>
<td>• Mosaicism will lead to the exclusion of a small number of potentially viable embryos</td>
</tr>
<tr>
<td>• No randomized study has proven that PGS is beneficial</td>
</tr>
</tbody>
</table>
Chromosome screening for repeated implantation failure (RIF)
So far there is no evidence that PGS improves outcome for RIF patients (studies 1-5)

1: Gianaroli et al. 1999
2: Kahraman et al. 2000
3: Munné et al., RBO 2003
4: Pehlivan et al. 2002
5: Werlin et al. 2003

Aneuploid rate in one cycle is usually highly predictive of aneuploidy rate in the next

PGS may help patients with 100% abnormal results to consider alternative options such as gamete donation
Chromosome screening for patients with previous trisomic conception
## Patients (<35 years) with previous trisomic conception

### CHROMOSOME ABNORMALITIES:

<table>
<thead>
<tr>
<th></th>
<th>Aneuploidy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with previous trisomy</td>
<td>41%</td>
</tr>
<tr>
<td>Control</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

### IMPLANTATION RATE:

<table>
<thead>
<tr>
<th></th>
<th>% pregnancy</th>
<th>implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with PGS</td>
<td>57%</td>
<td>50%</td>
</tr>
<tr>
<td>Controls</td>
<td>43%</td>
<td>22%</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.025</td>
<td></td>
</tr>
</tbody>
</table>

*Munne et al. 2004b*
Chromosome screening for recurrent pregnancy loss (RPL)
Patients with recurrent pregnancy loss

Controlled studies on idiopathic RPL:


- Munné et al. (2005) Preimplantation genetic diagnosis reduces pregnancy loss in women 35 and older with a history of recurrent miscarriages. Fertil Steril 84:331

- Munné et al. (2006) PGD for recurrent pregnancy loss can be effective in all age groups. Abstract PGDIS

- Garrisi et al. (2008) Preimplantation genetic diagnosis (PGD) effectively reduces idiopathic recurrent pregnancy loss (RPL) among patients with up to 5 previous consecutive miscarriages after natural conceptions. Fertil. Steril in press

- Rubio et al. (in press) Prognosis factors for Preimplantation Genetic Screening in repeated pregnancy loss. Reprod Biomed Online, in press

All show a decrease in miscarriage rate
Patients with recurrent pregnancy loss

N=122
With ≥3 previous losses

- 94% before PGD (P<0.05)
- 85% expected after PGD (P<0.001)
- 89% observed after PGD (P<0.001)

* Munné et al. 2005 and unpublished data, **Brigham et al. 1999

*Patients with recurrent pregnancy loss

8% 16% 12% 33% 44% 39%
Limitations of conventional embryo screening techniques

- **Cells are in interphase - use FISH**
- **Limited range of fluorochromes**
- **Less than half the chromosomes tested**
- **Spreading requires skill and can be inconsistent**
- **Mosaicism** poses a significant problem for diagnosis. However, most mosaic cleavage stage embryos are aneuploid in every cell.
- **Cleavage stage biopsy may represent a cost to the embryo**
Comparative genomic hybridization - CGH

Chromosome 15

Loss
Normal
Gain

Normal DNA
Trisomy
Monosomy
Gain
Loss
Normal DNA Test DNA Test DNA
Embryo screening using CGH

Benefits

• All chromosomes tested
• No spreading of cells on slides

But what about mosaicism and the impact of biopsy?
### Comprehensive chromosome screening of blastocysts

#### Analysis of blastocyst stage

- **Biopsy of several cells is possible**
  - Diagnosis more robust and accurate
  - Less risk of misdiagnosis due to mosaicism
  - Reduced impact of embryo biopsy

- **Blastocyst cryopreservation (vitrification) necessary**

- **Can overcoming the principal challenges to accurate screening allow PGS to fulfill the potential predicted by theory?**
### Blastocyst CGH - clinical results

- **170 patients**, mean age 38 years, 1-6 previous failed IVF cycles (mean 2)

- **Near 100% survival** after biopsy, freeze and thaw

- **Pregnancy rate per cycle with transfer**: 87% 72%

- **Birth rate per cycle with transfer**: 79% 60%

- **Implantation rate per embryo**: 67% 28% *

Control group matched for: maternal age, day-3 FSH, day of transfer, # oocytes retrieved, # of failed cycles

*p<0.0003 - Extremely promising for single embryo transfer*
Blastocyst CGH- rates of pregnancy loss

- **Embryo loss rates are low**
- **91% of embryos that produced a fetal sac resulted in an ongoing third trimester pregnancy or live birth**
- **97% of embryos that produced a fetal heart beat resulted in an ongoing third trimester pregnancy or live birth**

- **Expected pregnancy loss rate for IVF patients in this age range is ~25%**
Blastocyst CGH - clinical results

- Implantation rate
- Aneuploidy rate
- Cycles with all embryos aneuploid
Questions

- Can the results obtained in the current study be replicated in a randomized controlled trial?
- How much of the observed benefit is due to transfer in a subsequent cycle?
- Aneuploidy explains most of the decline in IVF success with advancing maternal age. What explains the remainder?
- What patient groups will benefit the most from this type of screening?
United Kingdom (Oxford)
Elpida Fragouli
Samer Alfarawati

United States (Livingston, NJ)
Pere Colls
Tomas Escudero
N-neka Esprit-Ngachou
Jill Fischer
Cristina Gutierrez-Mateo
Santiago Munne
Renata Prates
Jorge Sanchez
Sophia Tormasi
John Zheng

Colorado Center for Reproductive Medicine
Mandy Katz-Jaffe
John Stevens
Bill Schoolcraft

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