Genetic methods for assessing embryo viability and improving IVF treatment

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Points for discussion

Diagnosis of inherited disorders in preimplantation embryos

Sampling DNA from embryos (embryo biopsy)

Frequency of chromosome abnormalities in human embryos

The use of aneuploidy screening to improve IVF treatment

Clinical data from preimplantation genetic screening

What next for genetic methods of embryo viability assessment
Genetic testing of embryos produced using IVF

Originally an alternative to prenatal testing for high-risk patients

Patients undergo in vitro fertilisation treatment

Ovarian stimulation

Fertilisation with sperm

Embryo biopsy and genetic analysis

Transfer of unaffected embryos

Multiple oocytes collected

Several embryos produced

Unaffected embryos revealed

Healthy pregnancy and birth

PGD is a rapid process

Oocyte retrieval Day-0

Fertilisation Day-1

Biopsy Day-3 or -5

Transfer ~24 hours later

Increasingly embryos are frozen (vitrified) after biopsy
Genetic testing of embryos produced using IVF

Originally an alternative to prenatal testing for high-risk patients

Genetic analysis

Transfer of unaffected embryo

Prevent affected pregnancy and avoid pregnancy termination
PGD of single gene disorders

To date diagnosis performed diagnosis of >300 disorders

Diagnosis possible for any disorder provided mutation is known

Tests are typically 99% accurate

Results within 24 hours
The use of genetics to improve IVF outcomes

Preimplantation genetic screening (PGS)
In vitro fertilisation (IVF)

A highly successful medical intervention

Infertility treatment revolutionised

Estimated that >5 million babies born following IVF

1-5% of all births in industrialised countries

But....

....the process is very inefficient
In vitro fertilisation (IVF)

Worldwide only 30% of IVF cycles produce a pregnancy

Choose most viable embryo - based (primarily) on morphology

Methods are subjective and provide only rough guide

85% of embryos transferred do not implant

Solution to poor embryo selection – transfer more embryos!

20-25% of IVF pregnancies are multiple gestations

Significant risks of complications for mother and child
In vitro fertilization (IVF)

Improved methods needed for embryo selection (eSET)

Could genetic tests provide a more definitive, less subjective assessment?
Genetic abnormalities explain most implantation failures and miscarriages

Chromosome abnormality is extremely common in oocytes

Problem increases with advancing maternal age

Data from >50,000 embryos analyzed by Reprogenetics
Genetic abnormalities explain most implantation failures and miscarriages

Aneuploidy is almost always lethal (failed implantation/miscarriage)
While aneuploidy increases with age, implantation rate decreases

Data from >50,000 embryos analyzed by Reprogenetics
Ideally, one embryo is transferred to the uterus after chromosome screening. Standard embryo evaluations do not reveal embryos with the wrong number of chromosomes. IVF treatment usually results in the production of several embryos. Munne et al., 1993
Microarray comparative genomic hybridization

Monosomy 1 and monosomy 14

Chromosome number
Does PGS work?
Evidence that PGS has clinical value

New comprehensive methods shown to be highly accurate: ~98%

Highly predictive:
<2% of aneuploid embryos transferred produced a viable pregnancy
(Scott et al., Fertil Steril 2012)

RCTs have now been carried out using the modern PGS methods
All show that PGS provides a significant advantage
None have presented any negative findings
## 1st Randomized trial:
aCGH + single embryo transfer, <35 years old

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<tr>
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<th>Control</th>
<th>PGS</th>
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<tbody>
<tr>
<td>patients</td>
<td>48</td>
<td>55</td>
</tr>
<tr>
<td>age</td>
<td>&lt;35</td>
<td>&lt;35</td>
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<tr>
<td>replacement</td>
<td>Day 6</td>
<td>Day 6</td>
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<tr>
<td>replaced</td>
<td>48 (1)</td>
<td>55 (1)</td>
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<td>pregnancy rate</td>
<td>45.8%</td>
<td>70.9%</td>
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<td>ongoing preg rate</td>
<td>41.7%</td>
<td>69.1%</td>
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<tr>
<td>multiples</td>
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*Yang et al. (2012)*
2\textsuperscript{nd} randomized trial: qPCR, <42 years old

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<th>PGS</th>
<th>Control</th>
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<tr>
<td>age</td>
<td>32.2</td>
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<tr>
<td>N</td>
<td>72</td>
<td>83</td>
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<td>embryos replaced</td>
<td>1.9</td>
<td>2.0</td>
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<tr>
<td>implantation</td>
<td>79.8%</td>
<td>63.2%</td>
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<tr>
<td>sustained implant</td>
<td>66.4%</td>
<td>47.9%</td>
<td>$0.03$</td>
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<tr>
<td>delivery rate</td>
<td>84.7%</td>
<td>67.5%</td>
<td>$0.01$</td>
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Scott et al., 2013 Fertil Steril.
3rd randomized trial: Transfer of 1 euploid embryo vs. 2 untested

<table>
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<tr>
<th></th>
<th>1 euploid blastocyst (PGS)</th>
<th>2 untested blastocysts</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Fresh transfer</td>
<td>65%</td>
<td>70%</td>
<td>NS</td>
</tr>
<tr>
<td>Twins/triplets</td>
<td>0%</td>
<td>53%</td>
<td>P&lt;0.001</td>
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</table>

Forman et al. (2013) Fertil Steril
Mean maternal age 35 (patients <43)
PGS eliminates the effect of maternal age on miscarriage

* SART,  ** Harton et al. (2013) Fertil Steril, and unpublished data
PGS eliminates the negative effect of maternal age on implantation

*SART, ** Harton et al. (2013) Fertil Steril, and unpublished data
What can PGS potentially offer?

- Achieve very high efficiency eSET
- Faster time to pregnancy
- Avoid unnecessary embryo transfers
- Avoid cryopreservation of non-viable embryos
- Reduce miscarriage rate
- Reduce risk of Down syndrome

Chromosome screening conclusions
Future of perspectives on PGS
In USA - growing acceptance that PGS should be widely applied

European medical community waiting for further evidence?

Cost of PGS - in Europe usually adds >30% to the cost of IVF cycle
- in USA adds <20% to the cost
Lower cost PGS

Next generation sequencing now allows cheaper PGS

NGS is revolutionizing genetic research and diagnostics (basis of noninvasive prenatal testing)

Vast quantities of DNA sequence information at low cost

Cost of PGS reduced by 25% this year using NGS

Wells et al., 2014 Journal of Medical Genetics
Additional genetic information relevant to viability

Example: patient 35 year old, blastocyst transfer

- **Euploid implanting** (40%)
- **Euploid not implanting** - Unknown reason (25%)
- **Aneuploid** (35%)

**PGS selection:**
- 62% implantation

**no PGS selection:**
- 40% implantation
Approximately 35% of euploid embryos fail to implant. Why?

Chromosomally normal blastocysts with elevated mtDNA levels do not implant.

Fragouli et al., 2015 PLoS Genetics

Explains ~1/3 of implantation failures involving euploid embryos.
28% of euploid blastocysts have elevated mtDNA

Data obtained using the MitoGrade test

n=100 chromosomally normal blastocysts

Fragouli et al., 2015 PLoS Genetics
Added information from NGS - mtDNA

Example: patient 35 year old, blastocyst transfer

- **Euploid implanting** (40%)
- **Euploid not implanting** - Unknown reason
- **Euploid not implanting** - Elevated mtDNA (8%)
- **Aneuploid** (35%)

PGS + MitoGrade selection: 70% implantation (estimated)

PGS selection: 62% implantation
Key point

There is increasing evidence that genetic screening of embryos is of value to the majority of patients undergoing IVF.

Has the use of genetics to select embryos reached its zenith?

No! The best is still to come...

Methods will become cheaper

Viable embryos will be revealed with more certainty.
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