The Many Advantages of Trophectoderm Biopsy Compared to Day 3 Biopsy for Pre-Implantation Genetic Screening (PGS)

Mandy Katz-Jaffe, PhD

Chromosomal Aneuploidy

Aneuploidy is the most common chromosome abnormality in human conceptions, and is the leading cause of miscarriage and congenital birth defects.
Maternal age is the highest risk factor for the incidence of fetal trisomies.

Maternal age is also the major contributor to human infertility.

Primarily due to:
- Progressive oocyte depletion
- Increase in maternal meiotic errors resulting in chromosome aneuploidy

**AIM: To select euploid embryos for transfer**

Aneuploidy screening of IVF embryos allows infertile couples to transfer chromosomally normal embryos, thereby increasing the likelihood of implantation and a chromosomally normal live birth, as well as reducing the chance of pregnancy loss.

*In 1993 the first PGD baby was born following aneuploidy screening using fluorescent in situ hybridization (FISH)*

Interphase FISH can rapidly analyze a handful of chromosomes on individual single cells.
Day 3
Blastomere Biopsy

Based on the premise that all blastomeres are totipotent

Animal studies indicated minimal impact on developmental competence

PGD for Chromosome Aneuploidy

Testing for chromosomes X, Y, 13, 16, 18, 21 and 22 enabled the detection of 72% of the chromosomal abnormalities found in spontaneous abortions (Simpson, 1987)

Initial reports of D3 FISH were promising with increases in pregnancy rates and decreases in miscarriage rates

However, despite the need to avoid the transfer of aneuploid embryos, RCT studies indicated a lack of beneficial impact from D3 FISH aneuploidy screening
Multicenter RCT comparing 3 cycles of IVF with or without PGS ages 35-41

<table>
<thead>
<tr>
<th>Screening</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing PR</td>
<td>52 (25%)</td>
<td>74 (37%)</td>
</tr>
<tr>
<td>Live Birth</td>
<td>49 (24%)</td>
<td>71 (35%)</td>
</tr>
<tr>
<td>SAB</td>
<td>18%</td>
<td>18%</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>11.7%</td>
<td>14.7%</td>
</tr>
</tbody>
</table>

Total of 11 RCTs
- 5 with good prognosis IVF patients
- 6 with poor prognosis IVF patients
- NONE showed that D3 FISH PGD aneuploidy screening improved delivery rate
- Some even showed that D3 FISH PGD aneuploidy screening was detrimental to outcome
- FISH with 5-10 chromosomes on a biopsied blastomere
Limitations of Day 3 Biopsy

- Studies of embryo polarity suggest not all blastomeres are equal
- Cell polarization (TE and ICM) is not definitive but serves to bias patterning
- Potential developmental impact
- Chromosomal mosaicism
- Does the biospied cell represent the chromosome constitution of the remaining blastomeres?

Limitations of Day 3 Biopsy

- High rate of discrepant results, 10-70% of cleavage stage aneuploid embryos found to be euploid on D5
- Northrop et al, 2010 showed no evidence of embryo self correction
- Apoptosis not observed until morula stage (day 4) – possibly eliminate cells with chromosomal aneuploidy
- FISH technique itself
- Half the chromosomes not tested
### Experience with Blastocyst Biopsy and FISH

<table>
<thead>
<tr>
<th></th>
<th>D5 PGD FISH</th>
<th>AH only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>55</td>
<td>46</td>
</tr>
<tr>
<td>Live Birth</td>
<td>20 (36%)</td>
<td>27 (59%)</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>22/55 (39%)</td>
<td>27/46 (59%)</td>
</tr>
</tbody>
</table>


### Two Randomized Trials – RIF and AMA

<table>
<thead>
<tr>
<th></th>
<th>D5 Transfer</th>
<th>D3 FISH &amp; D5 Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF Patients (≥3; &lt;40 years)</td>
<td>43</td>
<td>48</td>
</tr>
<tr>
<td>Live Birth</td>
<td>12/43 (27.9%)</td>
<td>23/48 (47.9%)</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>21.4%</td>
<td>36.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>D5 Transfer</th>
<th>D3 FISH &amp; D5 Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA Patients (41-44 years)</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>Live Birth</td>
<td>14/90 (15.5%)</td>
<td>30/93 (32.3%)*</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>13.1%</td>
<td>35.1%*</td>
</tr>
</tbody>
</table>

*P<0.05; Rubio et al, (2013) Fertil Steril
Despite most evidence that FISH based PGD for aneuploidy screening does not improve IVF outcomes

The concept of aneuploidy screening to select euploid embryos in ART remains valid

Comprehensive Chromosome Screening (CCS)

All 23 pairs of human chromosomes
Metaphase Spread CGH

Monosomy 15 Embryo

Clinical application of comprehensive chromosomal screening at the blastocyst stage metaphase CGH

<table>
<thead>
<tr>
<th></th>
<th>Contemporary comparison group (n = 113)</th>
<th>Comprehensive chromosome screening group (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average maternal age (y)</td>
<td>37.1</td>
<td>37.7</td>
</tr>
<tr>
<td>Average no. of previous failed IVF treatments</td>
<td>1.24</td>
<td>2.42</td>
</tr>
<tr>
<td>Day 3 FSH (IU)</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Average no. of oocytes retrieved per cycle</td>
<td>19.4</td>
<td>18.6</td>
</tr>
<tr>
<td>Average no. of blastocysts transferred per cycle</td>
<td>2.7 (299 transfused in 113 cycles)</td>
<td>2.0 (90 transferred in 45 cycles)</td>
</tr>
<tr>
<td>Biochemical pregnancy (positive pregnancy test) per cycle</td>
<td>84.0% (95/113)</td>
<td>82.2% (37/45)</td>
</tr>
<tr>
<td>Implantation rate (proportion of transferred embryos producing a fetal sac)</td>
<td>46.5% (139/299)</td>
<td>72.2% (65/90)</td>
</tr>
<tr>
<td>Implantation rate (proportion of transferred embryos producing a fetus with heartbeat)</td>
<td>44.8% (134/299)</td>
<td>68.9% (62/90)</td>
</tr>
</tbody>
</table>

DNA microarrays can detect aneuploidies in single cells and are now being clinically applied:

SNP arrays, aCGH, Oligonucleotide arrays & Chromosome-specific libraries

Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and SNP microarray CCS in infertile patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>37.8 (range 30–42)</td>
</tr>
<tr>
<td>Day 3 FSH</td>
<td>7.39 ± 2.2</td>
</tr>
<tr>
<td>Antimullerian hormone</td>
<td>2.98 ± 2.6</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>17.3 ± 8.1</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>19.1 ± 8.3</td>
</tr>
<tr>
<td>No. of oocytes fertilized by ICSI</td>
<td>12.8 ± 5.5</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>52.3%</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>86.9 million/mL</td>
</tr>
<tr>
<td>Good blastocyst development (grade ≥3BB)</td>
<td>38%</td>
</tr>
<tr>
<td>No. of blastocysts biopsied and vitrified</td>
<td>5.9 ± 3.5</td>
</tr>
</tbody>
</table>
Live birth outcome with trophoderm biopsy, blastocyst vitrification, and SNP microarray CCS in infertile patients

<table>
<thead>
<tr>
<th>Outcome results (n = 100 FETs)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No result</td>
<td>4.5%</td>
</tr>
<tr>
<td>All aneuploid cycle</td>
<td>20%</td>
</tr>
<tr>
<td>Euploid blastocysts</td>
<td>47.4% (356/751)</td>
</tr>
</tbody>
</table>

| Bacteriolytic survival after warming | 96.8% (179/185) |
| Mean no. of euploid blastocysts transferred | 1.78 |
| Biochemical pregnancy           | 87% (87/100) |
| Clinical pregnancy (fetal heart tone) | 73% (73/100) |
| Missed abortion                 | 2.7% (2/73) |
| Implantation rate (fetal heart tone) | 64.6% (115/178) |
| Euploid babies born             | 113 = 71% live birth rate per transfer = 55.9% live birth rate per oocyte retrieval |

Array Comparative Genomic Hybridization

- Rapid-results within 12 hours of sample receipt
- Allows the copy number of every chromosome to be determined

24Genes array-CGH (Illumina)
- Hundreds of thousands of clinical biopsies performed to date
- Publications in peer-reviewed journals and abstracts at conferences
- More than 100 spots tested per chromosome
- Greater than 3000 unique areas tested across the entire genome
- Requires whole genome amplification (WGA)
Quantitative Real-Time PCR

- Accurate, rapid & cost effective qualitative and quantitative detection of nucleic acids
- Multi-well plates, Microtiter plates, Integrated Fluidic Circuits, Digital PCR
- Hundreds of simultaneous real-time PCR measurements in a single experiment with only nanoliters of reagents and samples
- Ability to run both CCS and single gene PGD without the need for WGA or an additional embryo biopsy (Zimmerman et al, 2016 *Fertil Steril*).

qPCR = 98.6% consistency with SNP arrays
Next Generation Sequencing (NGS)

Fiorentino et al, 2014 *Hum Reprod*

NGS based aneuploidy screening of biopsied blastocysts (n=192) showed concordant results (99.5%) with array-CGH. NGS demonstrated as a reliable methodology, especially in terms of reliability, high-throughput, automation, allowing identification and transfer of euploid embryos resulting in ongoing pregnancies.

Yang et al, 2015 *BMC Med Genomics*

Randomized clinical study on the efficiency of NGS (n=86) for preimplantation genetic screening in comparison to aCGH (n=86). Implantation rates were comparable between the NGS and aCGH groups (70.5% vs. 66.2%, respectively, ns).

Embryo Biopsy

Biopsy should be performed by a clinical embryologist who has the relevant certification, training and experience, including placing cells into tubes.
Impact of Embryo Biopsy

Scott et al., 2013, Fertil Steril

Polar Body Biopsy

Advantages include:
- Avoids chromosomal mosaicism
- Results available for a day 3 transfer
- Reduced impact of biopsy technique?

Disadvantages include:
- Only accesses maternal contribution
- Difficulties with polar bodies due to degeneration = more “no result”
ESHRE PGD Task Force

n=42 cycles (mean mat age = 40yrs)
n=226 oocytes from 42 cycles (14% no results)
72% aneuploid zygotes (all aneuploid cycle = 45%)
30% ongoing pregnancy rate
94% concordance between oocyte and PB

Gerada et al., 2010 Human Reprod

All 23 oocyte chromosomes were involved in meiotic chromosome errors (gains & losses)
All chromosome aneuploidies were compatible with embryo cleavage
Blastocyst TE Biopsy

Advantages include:
- Competent in vitro embryo

A meta-analysis reviewed 23 RCTs and concluded that blastocyst transfer resulted in a significant increase in live birth rates (Glujovsky et al, 2012).

Blastocyst TE Biopsy

Advantages include:
- Competent in vitro embryo
- Reduced chromosomal mosaicism

Mitotic errors are observed during human preimplantation development resulting in chromosomal mosaicism (defined as the presence of more than one chromosome complement). Several studies have observed a lower rate of mosaicism in blastocysts compared to cleavage stage embryos (Reviewed by Mantikou et al, 2012).
Blastocyst TE Biopsy

Advantages include:

• Competent \textit{in vitro} embryo
• Reduced chromosomal mosaicism
• Several cells for testing
• Minimal impact of TE biopsy

Potential disadvantages:

• Only testing TE cells

Isolation and re-analysis of ICM and TE cells from aneuploid blastocysts have revealed no preferential allocation of abnormal cells between the two cell lineages (Capalbo et al, 2013)
Blastocyst TE Biopsy

Potential disadvantages:
• Only testing TE cells
• Cryopreservation

<4hrs for CCS analysis between a TE biopsy and fresh D5 transfer.

Roy et al, 2014 reported a 94.4% survival rate of vitrified-warmed blastocysts and excellent neonatal outcomes following SET (n=645).

FET Results in Healthier Babies and Better Overall Outcomes

Roque et al, 2013
• Meta-analysis revealed significantly higher clinical pregnancy rates following FET versus fresh transfer

Wennerholm et al, 2013
• Population based cohort study revealed FET singletons have a better perinatal outcome compared with singletons born after fresh IVF and ICSI

Ishihara et al, 2014
• Improved general perinatal outcome of pregnancy but increased risk of maternal complications including placenta accreta and pregnancy-induced hypertension
**Randomized Controlled Trials (RCT)**

- **Definition:**
  - A randomized controlled trial (RCT) (or randomized comparative trial) is a specific type of scientific experiment, and the gold standard for a clinical trial (Level 1 or Class 1 Data)

- RCTs are often used to test the efficacy and/or effectiveness of various types of medical intervention within a patient population

- RCTs may also provide an opportunity to gather useful information about adverse effects, such as drug reactions

---

**Negative and Positive Prediction for Reproductive Potential**

Scott et al., 2012
**Single Blastocyst Fresh D6 Transfer – Randomized Pilot Study**

*Yang et al., 2013*

**Study Eligibility:**
- <35 years maternal age
- Regular ovulation
- No previous IVF
- Infertility etiology was tubal factor or male factor or both
- D3 FSH <10IU/l
- D3 Estradiol <60pg/ml
- Normal intrauterine contour

<table>
<thead>
<tr>
<th>Condition</th>
<th>aCGH (n=55)</th>
<th>Morphology alone (n=48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 5/6</td>
<td>31</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>21</td>
<td>19</td>
<td>0.677</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clinical Pregnancy</td>
<td>70.9%</td>
<td>45.8%</td>
<td>0.017</td>
</tr>
<tr>
<td>Ongoing Pregnancy</td>
<td>69.1%</td>
<td>41.7%</td>
<td>0.009</td>
</tr>
<tr>
<td>MA8</td>
<td>2.6%</td>
<td>9.1%</td>
<td>0.597</td>
</tr>
</tbody>
</table>

**RCT – CCS versus Nonintervention**

- n=155 patients; 21-42 years and 0-1 previous failed IVF cycle
- Study Group = Euploid blastocyst transfer on D6 after D5 biopsy
- Control Group = Day 5 blastocyst transfer based on morphology

<table>
<thead>
<tr>
<th></th>
<th>Study (CCS)</th>
<th>Control (Morphology)</th>
</tr>
</thead>
<tbody>
<tr>
<td># Patients</td>
<td>72</td>
<td>83</td>
</tr>
<tr>
<td>Age</td>
<td>32.2</td>
<td>32.4</td>
</tr>
<tr>
<td>Clinical Implantation</td>
<td>79.8 %</td>
<td>63.2 %*</td>
</tr>
<tr>
<td>Sustained Implantation</td>
<td>66.4 %</td>
<td>47.9 %*</td>
</tr>
<tr>
<td>Delivery per Cycle</td>
<td>84.7%</td>
<td>67.5 %*</td>
</tr>
</tbody>
</table>

*P<0.05; Scott et al., 2013*
RCT – CCS versus Morphology Selection

- <42 maternal years and 0-1 previous failed IVF cycle
- Study Group = single euploid blastocyst transfer
- Control Group = double blastocyst transfer based on morphology

<table>
<thead>
<tr>
<th></th>
<th>Study (SET)</th>
<th>Control (DET)</th>
</tr>
</thead>
<tbody>
<tr>
<td># Patients</td>
<td>89</td>
<td>86</td>
</tr>
<tr>
<td>Age</td>
<td>34.5</td>
<td>35.1</td>
</tr>
<tr>
<td>Clinical PR</td>
<td>69 %</td>
<td>81 %</td>
</tr>
<tr>
<td>Ongoing PR</td>
<td>61 %</td>
<td>65 %</td>
</tr>
<tr>
<td>Multiples</td>
<td>0</td>
<td>48 %*</td>
</tr>
</tbody>
</table>

*P<0.05; Forman et al., 2013

Meta-Analysis of Three RCTs

1. Yang et al, 2012
2. Forman et al, 2013

- All good prognosis IVF patients
- Increases in clinical implantation rates
- Increases in ongoing pregnancy rates
- Improves embryo selection
- Trophectoderm biopsy has a negligible effect on embryo development

Dahdouh et al, 2015
Infertile patients of maternal age >35 years were computer randomized at oocyte retrieval into either:

**Control Group**
- All embryos are grown to the blastocyst stage

**Test Group**
- Blastocyst biopsy for CCS on either D5 or D6 (mean = 5.8)
- CCS using SNP microarray technology (RMA-NJ; no result = 2.6%)

**Day 5 Fresh Transfer**
- Embryo selection based on morphology

**Frozen Embryo Transfer**
- Euploid embryos only

**Surplus blastocysts biopsied for CCS prior to vitrification**

**Meta-Analysis of 4 RCTs and 7 Cohort Studies**

**CCS versus Morphological Criteria:**
- Euploid embryos more likely to successfully implant
- Increased clinical pregnancy rate with CCS
- Increased live birth rate with CCS
- Decreased miscarriage rate with CCS
- Decreased multiple pregnancy rate with CCS

**Conclusion:**
- CCS is comparable to traditional morphological embryo selection methods, with better IVF outcomes
- Transfer of euploid embryos improves implantation rates

Chen et al, 2015
Conclusion:

• Trophoderm biopsy with CCS increases the likelihood that an individual blastocyst will result in a chromosomally normal live birth, including for infertile AMA women.

• All current published RCTs have shown a significant improvement in implantation rates and a decrease in miscarriage rates following blastocyst CCS.

• Ongoing RCT studies are evaluating PB and blastocyst biopsy with CCS for clinical efficacy and live birth rates.