Pre-ESHRE advanced course in embryology
IVF today: is it possible to further improve the clinical outcomes?
2 July 2016 - Helsinki, Finland
Comprehensive chromosome screening and embryo biopsy: advantages and difficulties

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Disclosure

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GENER A, Reproductive medicine centers
GENETYX, molecular genetics laboratory.
Italy

Received grant from Merck (GFI 2013 and 2015) for the study of a non-invasive approach for embryo selection based on miRNAs analysis in blastocyst culture media.
Objectives

1. Acquire knowledge about the potential benefit of using aneuploidy testing in IVF
2. Learn how to optimize embryo biopsy and CCS strategy in order to select the best embryo without compromising live birth rates per cycle;
3. Review the limitation of embryo testing for aneuploidies;
4. Recapitulate the state of the art concerning preimplantation genetic techniques with a look to the implementation and future
The current selection of embryos to transfer based on non-invasive evaluation of morphological parameters is relatively not effective.

In the Advanced Maternal Age population (36-43 years):

~ 30% cumulative clinical pregnancy rate;

~ 10% of transferred embryos delivered.

~ 20% multiple pregnancy rate;

~ 30% miscarriage rate;

Ubaldi et al., Human Reproduction, 2015

72% of IVF patients over 35 years (40,000 cycles per year in Italy)
Blastocyst transfer enhance embryo selection
...but still not enough

Cochrane review of 12 RCTs

No difference in **multiple pregnancy rate** and **miscarriage rate** (13 RCTs, OR 1.18, 95% CI 0.86 to 1.60) per couple between the two treatment groups (16 RCTs, OR 0.92, 95% CI 0.71 to 1.19)

Impact of Aneuploidies in human Reproduction

The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophoderm biopsies evaluated with comprehensive chromosomal screening


Gynecology and Reproductive Science, Robert Wood Johnson University, New Brunswick, and * Reproductive Medicine Associates of New Jersey, Morristown, New Jersey.

Implantation failures
Spontaneous miscarriages
Affected childs

PGS = Preimplantation Genetic Screening; PND = PreNatal Diagnosis
Morphology cannot be rely on to select chromosomally normal embryos

**Figure 3** Comprehensive chromosome screening data for 956 blastocysts according to morphology (A) and developmental rate (B).
Blastocyst biopsy does not compromise implantation potential

Scott et al., 2013
Selecting the most effective TE biopsy approach

Day 3 hatching and biopsy on TE cells
Schoolcraft et al., Fertil Steril 2010

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image 1" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
<td><img src="image3.png" alt="Image 3" /></td>
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</tbody>
</table>

Cons:
- Extra-source of stress at the cleavage stage;
- Herniation of ICM cells;
- Low synchronization;

TE biopsy without hatching at cleavage stage
Capalbo et al., Hum. Reprod. 2014

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td><img src="image4.png" alt="Image 4" /></td>
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<td><img src="image8.png" alt="Image 8" /></td>
<td><img src="image9.png" alt="Image 9" /></td>
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</table>

Pro:
- Undisturbed embryo culture;
- Selection of TE cells to biopsy;
- High synchronization;
- Well suited with the use of single media and TLM
Preclinical evidences of blastocyst biopsy effectiveness

1. No impact of TE biopsy on blastocyst implantation  
   (Scott et al., F&S 2013-paired randomized study)

1. No major diagnostic impact of chromosome mosaicism at the blastocyst stage  
   (Capalbo et al., HR 2013);

2. High positive and negative clinical predictive value of TE biopsy and CCS (Scott et al., 
   F&S 2013- prospective non-selection study);

3. High laboratory and clinical consistency of TE biopsy and qPCR based aneuploidy 
   testing (Capalbo et al., HR 2016)

4. 3 RCTs showing improved sustained implantation rate and reduced miscarriages after 
   euploid embryo transfer compared to standard care (Chen et al., Plos one 2016)
Difficulties: the impact mosaicism on chromosome testing

Estimates of preimplantation stage mosaicism frequency range from 3% to as high as 90% (Taylor et al., 2014)
Mosaicism is not common in blastocysts

<table>
<thead>
<tr>
<th></th>
<th>Northrop</th>
<th>Fragouli</th>
<th>Capalbo</th>
<th>Johnson</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northrop</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.2%</td>
</tr>
<tr>
<td>Fragouli</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.1%</td>
</tr>
<tr>
<td>Capalbo</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2.8%</td>
</tr>
<tr>
<td>Johnson</td>
<td>29</td>
<td>4</td>
<td>19</td>
<td>41</td>
<td>51.7%</td>
</tr>
<tr>
<td>Percent of Total</td>
<td>13</td>
<td>6</td>
<td>49</td>
<td>9</td>
<td>42.8%</td>
</tr>
</tbody>
</table>

Legend
- **Green**: euploid
- **Red**: aneuploid

Capalbo et al., Under review
Incidence of chromosome mosaicism in blastocysts and CVS specimens from spontaneous and IVF derived pregnancies

- Spontaneous Pregnancies (4656): 1.2%
- IVF pregnancies (681): 1.3%
- Blastocysts (384): 5.4%

Data from Huang et al., 2009 for CVS samples, Capalbo 2014, Jhonson 2010, Fragouli 2011, Northrop 2010 for blastocysts
False positive mosaicism detection

10% False Positive error rate x test

40% FP mosaicism detection \((1 - 0.9^6)\)

70% FP mosaicism detection \((1 - 0.9^{10})\)
Predicting blastocyst mosaicism

Strength of evidence for true mosaicism

Legend
- euploid
- trisomy
- monosomy
- untested

Intermediate log2 ratio-one multicell biopsy

Evidence used to report mosaicism diagnosis

Capalbo et al., Under review
How to manage mosaicism in clinical practice

- No method of CCS can give a correct diagnosis when testing a mosaic embryo; by definition there will always be a sampling error;

- No method can distinguish between technical fluctuations of log2 ratios and the genuine presence of mosaicism in a single multicellular TE biopsy;

- Deviant log2 ratios have not to be reported or at least use to suggest the presence of a pattern “consistent” with mosaicism.

- Mosaicism has to be acknowledged in the consent form as a biological limitation of the approach considering that it will have not a major impact on clinical outcome due to the observed low incidence in human blastocysts and pregnancies.
Non-selection design to determine the positive and negative clinical predictive value

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study

Richard T. Scott, M.D.,* Kathleen Ferry, B.S.,* Billy Hu, M.S.,* Ann Tani, M.S.,* Katherine Scott, M.S.,* 
and Nathan A. Topl, M.D.**

* Reproductive Medical Associates of New Jersey, Montclair, New Jersey, and ** Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey

24-chromosome blinded analysis
Non-selection design to determine the positive and negative clinical predictive value

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study

Dick T. Scott Jr., M.D.,*† Kathleen Ferry, B.S.,* Jing Su, M.S.,* Xin Tao, M.S.,* Katherine Scott, M.S.,* and Nathan R. Treff, Ph.D.,*†

WGA-SNP array: of the 99 embryos assigned aneuploid, 4 (4%) sustained implantation

Targeted-NGS: of the 41 embryos assigned aneuploid, 0 sustained implantation

POSITIVE PREDICTIVE VALUE (EUPLOID EMBRYOS RESULTING IN SUSTAINED IMPLANTATION) 59.7%

NEGATIVE PREDICTIVE VALUE (ANEUPLOID EMBRYOS RESULTING IN SUSTAINED IMPLANTATION) 2.8%

Total
PGS RE-INVENTED: CLINICAL EVIDENCES

Blastocyst biopsy and comprehensive chromosomol testing
CCS-based PGD-A – results of a meta-analysis

Sustained implantation rate (> 20 weeks gestation)

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>PGS-CCS</th>
<th>Control</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Heterogeneity: Chi² =</th>
<th>Test for overall effect: Z =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al. 2012</td>
<td>38</td>
<td>20</td>
<td>14.5%</td>
<td>1.66 [1.14, 2.42]</td>
<td>1.29, df = 2 (P = 0.53); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Forman et al. 2013</td>
<td>54</td>
<td>83</td>
<td>37.8%</td>
<td>1.29 [1.03, 1.61]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Scott et al. 2013</td>
<td>89</td>
<td>78</td>
<td>47.7%</td>
<td>1.39 [1.14, 1.70]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>276</td>
<td>383</td>
<td>100.0%</td>
<td>1.39 [1.21, 1.60]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
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Sustained implantation rate (> 20 weeks gestation)

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<td>Sher et al. 2009</td>
<td>34</td>
<td>39</td>
<td>14.9%</td>
<td>2.88 [1.94, 4.29]</td>
<td>13.10, df = 3 (P = 0.004); I² = 77%</td>
<td>6.48 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Forman et al. 2012</td>
<td>77</td>
<td>76</td>
<td>54.6%</td>
<td>1.32 [1.05, 1.65]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Lee et al. 2015</td>
<td>25</td>
<td>12</td>
<td>9.2%</td>
<td>2.39 [1.33, 4.29]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
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<tr>
<td>Feichtinger et al.2015</td>
<td>29</td>
<td>60</td>
<td>21.2%</td>
<td>1.77 [1.20, 2.62]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>399</td>
<td>959</td>
<td>100.0%</td>
<td>1.75 [1.48, 2.07]</td>
<td>1.39, df = 2 (P = 0.31); I² = 0%</td>
<td>4.61 (P &lt; 0.00001)</td>
</tr>
</tbody>
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Dahdouh et al, F&S, 2015
Moving toward eSET for all patients and in all IVF settings

1 ≥ 2

Forman EJ et al. HR 2013
Euploid embryo transfer results in a significantly lower miscarriage rate: results of a meta-analysis

Chen et al., Plos one 2015
Cost-effectiveness of the approach ----> some evidences already suggest the costs for health system can be reduced by minimization of miscarriages and multiples

Wrong advertisement to patients ---> nothing to deal with the clinical effectiveness of the method and not all reproductive specialists provide a biased counselling on PGS!

“all embryos in an IVF cycle can be cryopreserved and transferred in subsequent cycles without impairing the cumulative pregnancy rate of that IVF cycle”

• eSET without improved selection doesn’t work in most clinical settings,
• miscarriages cannot be avoided without excluding aneuploid embryos,
• TIME-TO PREGNANCY is important to minimize drop-out!
Blastocyst biopsy and qPCR based CCS is effective in AMA patients

Reduction of multiple pregnancies in the advanced maternal age population after implementation of an elective single embryo transfer policy coupled with enhanced embryo selection: pre- and post-intervention study

Filippo Maria Ubaldi¹,*, Antonio Capalbo¹,², Silvia Colamaria¹, Susanna Ferrero¹, Roberta Maggiulli¹, Gábor Vajta³, Fabio Sapienza¹, Danilo Cimadomo¹,², Maddalena Giuliani¹, Enrica Gravotta¹, Alberto Vaiarelli¹, and Laura Rienzi¹

Ubaldi et al., Human Reproduction 2015
Mean female age 39.6

Introduction of eSET policy combined with blastocyst culture, PGS and freeze all – AMA population
Implementing PGD/S in IVF clinics, what are the key challenges? Methodological pre-requisites

1. Efficient **blastocyst stage culture** and frozen-thawed **eSET** policy

   ![Blastocyst Stage Culture](image1)

2. Safe and effective **Blastocyst stage TE biopsy**

   ![Blastocyst Stage TE Biopsy](image2)

3. Validated, accurate and effective genetic technologies

   ![46,XX qPCR](image3)
WGA and Targeted approaches for PGS

WGA

SNP array

gCG

qPCR

tNGS

WGA-NGS

Decreasing costs!!
Acknowledgments

**GENERA**, Centers for Reproductive Medicine

Clinical Director: *Filippo Maria Ubaldi*

IVF Laboratory Director: *Laura Francesca Rienzi*

**GENETYX**, molecular genetics laboratory

Laboratory director: *Antonio Capalbo*

Molecular biology: *Cristina Patassini, Danilo Cimadomo, Emiliano Scepi, Valeria Romanelli, Anna Cecchele, Adriano Giancami, Laura Girardi.*