Debating the **Pros** and Cons of Preimplantation Genetic Testing (PGT)

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Preimplantation genetic testing

- The analysis of the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for determining genetic or chromosomal abnormalities.

- PGT for monogenic/single gene defects (PGT-M);
- PGT for chromosomal structural rearrangements (PGT-SR);
- PGT for aneuploidies (PGT-A)

- PGT is an alternative to prenatal diagnosis: embryos obtained in vitro are tested and only disease-free embryos are transferred to the mother, to avoid the instauration of pregnancy with an affected embryo.

The ICMART in collaboration with ASRM, ESHRE, IFFS, March of Dimes, GIERAF, ASPIRE, MEFS, REDLARA and FIGO, (Zegers-Hochschild et al. F&S, HR 2017)
PGT-M and PGT-SR: State of the Art

- Safer than elective termination and more ethically and psychologically acceptable for many couples.

- Established reproductive option for couples at higher genetic risk. (ESHRE PGD consortium data, Moutou et al, HR, 2014)

- No increase of obstetric and neonatal complications following embryo biopsy (Sunkara et al., HR 2017; Desmyttere et al., HR 2009)
PGT-M, PGT-SR and PGT-A

Genetic testing vs IVF efficacy: what is the origin of this debate?

PGT for monogenic/single gene defects PGT-M

PGT for chr. structural rearrangements PGT-SR

PGT for aneuploidies PGT-A

No RCTs needed the benefit is considered self evident*

are RCTs needed?? or the benefit can be considered self evident?

RCTs are needed because the benefit is not yet considered self evident

*when prevalence is >10% of the embryos and the accuracy of the test >90%

Thornhill, ESHRE best practice guidelines, Hum Reprod 2015
Delayed childbearing and delayed marriage age have increased in developed countries in the last 20 years.

Probability of having a baby decreases by 3-5% a year after 30 and even faster after 40 years.
The prevalence of aneuploidy in human blastocyst obtained in vitro is between 30% and 85% (Franasiak et al, Fertil Steril, 2014)

The risk of spontaneous miscarriage is between 10% and 65% (Heffner, NEJM, 2004)

The risk of aneuploidy in human foetus in pre-natal diagnosis is between 0.2% and 3% (Hassold & Hunt, Nat Rev Genet, 2001)
Incidence of aneuploidy in humans

50-60% in spontaneous abortions

6% in still-births

0.6% in live-births

(Machín et al., 1974; Nielsen et al., 1975; Boué et al., 1976)
Incidence of aneuploidy in miscarriages in ART

- Histeroembryoscopy
  - Ferro et al., 2003
  - 48.7% Abnormal POCs

- Spontaneous
  - 40.6% Abnormal

- IVF Own Oocytes
  - 62.7% Abnormal

- Ovum Donation
  - 12.8% Abnormal

- <5 Mill/mL
  - 72.7%

- ≥5 Mill/mL
  - 53.6%

Campos-Galindo et al., JARG 2015
Morphology cannot be relied on to ensure the transfer of chromosomally normal embryos.

956 euploid blastocysts (mean female age 37.8)

Capalbo et al., Hum Reprod, 2014
Morphology Selection and Aneuploidy

✓ Only morphological criteria fails selecting the best embryo.

✓ The transfer of “good morphology” blastocyst not always means “chromosomally normal” embryos.  
   *(Yang et al., 2012)*

TIME-LAPSE AND ANEUPLOIDY

Type of chromosome abnormality affects embryo morphology dynamics

*(Nogales et al., 2017)*

Similar kinetics in euploid and trisomic embryos
PGT-A: Evolution of the technology

- **1995**
  - Fluorescence In Situ Hybridization (FISH)
  - PGS 1.0: Day-3 biopsies 2 blastomeres

- **2008**
  - Array Comparative Genomic Hybridization (aCGH)

- **2010**
  - Single-nucleotide polymorphism (SNP) microarray
  - Quantitative polymerase chain reaction (qPCR)
  - PGS 2.0: Blastocysts Deferred transfer

- **2012**
  - NGS (Illumina)

- **2013**
  - NGS (Life-Thermo)
  - PGS 3.0: Blastocyst and deferred transfer

- **2016-17**
  - Next Generation Sequencing (NGS) with custom algorithm

- **Mitochondrial DNA**

- ** ≤12 chromosomes**
- **24 chromosomes**

- **Mosaicism**
Why to test embryos for aneuploidies?

- Improve implantation at the first attempt
- Decrease miscarriage rates
- Decrease risk of abnormal offspring
- Decrease time to pregnancy, cost-efficiency and emotional burden

TO maximize LONG TERM treatment efficacy.

Healthy baby at home
Pro PGT Arguments

✓ Embryo aneuploidies are mostly meiotic in origin

✓ Trophoectoderm biopsy is safe and reliable (oocyte pick-up example) and very soon will not be necessary

✓ Clinical outcomes are significantly superior per transfer allowing to perform universal SET policy even in AMA patients
Aneuploidy of human preimplantation embryos now represents the most well established molecular biomarker of reproductive potential. (Gardner et al., 2015).

>98% of aneuploidies are meiotic in embryos and foetuses, present in all cells and do not self correct! (Ottini et al., Nature Genetics 2015)

Hassold & Hunt, Nature Reviews Genetics 2001

TO ERR (MEIOTICALLY) IS HUMAN: THE GENESIS OF HUMAN ANEUPLOIDY
‘The circle of desperation’

- Research funding difficult
- IVF seen as ‘an industry’
- Studies showing no effect
- Patient belief/risk of exploitation
- Poor knowledge of cause
- Patient demand
- Empirical Treatment
Trophectoderm biopsy DOES NOT affect embryo reproductive potential

Class I data from paired randomized study

Scott et al, 2013
PGT-A should elicit the same efficacy but improved efficiency compared to standard IVF
EVIDENCES FROM CLINICAL TRIALS AND OBSERVED ADVANTAGES OF PGT-A IN IVF TREATMENTS
RCT- Good Prognosis patients (SET)

Blastocyst biopsy with aCGH and SET

Women < 35 years
First IVF attempt
No previous miscarriages
(Yang et al., 2012)

Table 1 Characteristics of patients whose embryos were randomized to assessment by morphology with aCGH (Group A) and blastocyst morphology only (Group B)

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 55)</th>
<th>Group B (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>31.2 ± 2.5</td>
<td>31.5 ± 2.7</td>
</tr>
<tr>
<td>Total oocytes retrieved</td>
<td>19.5 ± 8.2</td>
<td>19.3 ± 8.1</td>
</tr>
<tr>
<td>MII (mature) oocytes</td>
<td>16.6 ± 7.8</td>
<td>16.3 ± 7.6</td>
</tr>
<tr>
<td>Oocytes fertilized (2pm)</td>
<td>13.1 ± 6.7</td>
<td>12.8 ± 6.4</td>
</tr>
<tr>
<td>Day 3 embryos</td>
<td>12.9 ± 1.8</td>
<td>12.6 ± 1.9</td>
</tr>
<tr>
<td>Day 5 blastocysts</td>
<td>8.3 ± 2.1</td>
<td>8.1 ± 2.4</td>
</tr>
</tbody>
</table>

Table 3 Comparison of laboratory findings and clinical outcome among IVF patients undergoing SET with embryo assessment by aCGH + morphology (Group A) and blastocyst morphology alone (Group B)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh blastocyst transfer according to morphology assessment:</td>
<td>55 (100)</td>
<td>48 (100)</td>
<td></td>
</tr>
<tr>
<td>Grade 5/6</td>
<td>31 (56.4)</td>
<td>28 (58.3)</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>21 (38.2)</td>
<td>19 (39.6)</td>
<td>0.677a</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (5.4)</td>
<td>1 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>39 (70.9)</td>
<td>22 (45.8)</td>
<td>0.017a</td>
</tr>
<tr>
<td>Ongoing pregnancy (≥20wks GA)</td>
<td>38 (69.1)</td>
<td>20 (41.7)</td>
<td>0.009a</td>
</tr>
<tr>
<td>Missed abortion</td>
<td>1 (2.6)</td>
<td>2 (9.1)</td>
<td>0.597b</td>
</tr>
</tbody>
</table>

Notes: All data reported as n (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age

a by Chi-squared test
b by Fisher’s exact test
RCT- All patients (SET)

Blastocyst biopsy with aCGH and SET

Women 21-42 years
First IVF attempt
No previous miscarriages
(Scott et al., FS 2013)

Women <43 years
AMH ≥1.2 ng/ml
FSH <12 IU/L
(Forman et al., FS 2013)

![Graph showing outcomes for CCS and control groups.]

Outcome per treatment cycle: Delivery rates are statistically significantly increased in treatment cycles in which embryos undergo comprehensive chromosome screening (P=.03). The initial chemical and clinical pregnancy rates were not different.


![Graph showing pregnancy outcomes per randomized patient.]

A

Pregnancy Outcome Per Randomized Patient (Intention-to-Treat)
- Single euploid blastocyst transfer (N=89)
- Untested 2-blastocyst transfer (N=86)

Clinical Pregnancy:
- CCS: 69%
- Control: 81%

Ongoing Pregnancy:
- CCS: 61%
- Control: 65%
In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study

Carmen Rubio, Ph.D., José Bellver, M.D., Lorena Rodrigo, Ph.D., Gema Castillón, M.D., Alfredo Guillén, M.D., Carmina Vidal, M.D., Juan Giles, M.D., Marcos Ferrando, M.D., Sergio Cabanillas, M.D., José Remohí, M.D., Antonio Pellicer, M.D., and Carlos Simón, M.D.

Igenomix Valencia/INCLIVA, Valencia; Instituto Valenciano de Infertilidad, Valencia University, Valencia; Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, Valencia University, Valencia; Instituto Valenciano de Infertilidad, Barcelona; Instituto Valenciano de Infertilidad, Madrid, Universidad Juan Carlos I, Madrid; Instituto Valenciano de Infertilidad, Bilbao; and Instituto de Investigación Sanitaria La Fe, Valencia, Spain
Flow-chart

- 326 patients informed

- 205 cycles included

- 105 patients Blastocyst

- 100 patients PGT-A

- 48 refused to participate

- 73 did not meet inclusion criteria: 35 in blastocyst and 38 in PGD-A group (mostly due to ovarian response)

Rubio F&S, 2017
Results

Clinical outcome after the first attempt: fresh transfer

<table>
<thead>
<tr>
<th></th>
<th>PGT-A</th>
<th>Non PGT-A</th>
<th>p-value</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles performed</td>
<td>100</td>
<td>105</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>% of cycles with transfer</td>
<td>68.0</td>
<td>90.5</td>
<td>0.0001</td>
<td>0.22 (0.10-0.48)</td>
</tr>
<tr>
<td>Mean Embryos/transfer (SD)</td>
<td>1.3 (0.5)</td>
<td>1.8 (0.4)</td>
<td>&lt;0.0001</td>
<td>CI: 0.35-0.65</td>
</tr>
<tr>
<td>Implantation Rate (IR)</td>
<td>52.8</td>
<td>27.6</td>
<td>&lt;0.0001</td>
<td>2.94 (1.72-5.0)</td>
</tr>
<tr>
<td>Clinical PR/ transfer</td>
<td>54.4</td>
<td>43.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy rate/ patient</td>
<td>37.0</td>
<td>39.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>2.7</td>
<td>39.0*</td>
<td>0.0007</td>
<td>0.06 (0.008-0.48)</td>
</tr>
<tr>
<td>Ectopics rate</td>
<td>0</td>
<td>4.9</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing IR</td>
<td>49.4</td>
<td>14.9</td>
<td>&lt;0.0001</td>
<td>5.57 (3.09-10.03)</td>
</tr>
<tr>
<td>Delivery rate/transfer</td>
<td>52.9</td>
<td>24.2</td>
<td>0.0002</td>
<td>3.52 (1.80-6.87)</td>
</tr>
<tr>
<td>Delivery rate/patient</td>
<td>36.0</td>
<td>21.9</td>
<td>0.0309</td>
<td>2.00 (1.08-3.71)</td>
</tr>
</tbody>
</table>

ClinicalTrials.gov NCT01571076; Two-side Fishers' test; * One fetal loss with Down syndrome

Rubio F&S, 2017
# Results

## Cumulative clinical outcome after transfer of cryopreserved embryos

<table>
<thead>
<tr>
<th></th>
<th>PGT-A</th>
<th>Non PGT-A</th>
<th>p-value</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles performed</td>
<td>100</td>
<td>105</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>No. of cryo-transfers</td>
<td>1</td>
<td>35</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total of transfers</td>
<td>69</td>
<td>130</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total embryos transferred</td>
<td>90</td>
<td>226</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cumulative PR/ patient</td>
<td>38.0</td>
<td>55.2</td>
<td>0.0172</td>
<td>0.50 (2.28-0.87)</td>
</tr>
<tr>
<td>Cumulative MR</td>
<td>2.6</td>
<td>36.2</td>
<td>&lt;0.0001</td>
<td>0.05 (0.01-0.37)</td>
</tr>
<tr>
<td>Ectopics rate</td>
<td>0</td>
<td>3.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative delivery rate/patient</td>
<td>37.0</td>
<td>33.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No. of livebirths/patient (%)</td>
<td>45 (45.0)</td>
<td>39 (37.1)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

ClinicalTrials.gov NCT01571076; Two-side Fishers’ test; * One fetal loss with Down syndrome
RCT- Advanced Maternal Age

Time to pregnancy           No. Transfers to a live birth

---

ClinicalTrials.gov NCT01571076 Igenomix-IVI
Rubio et al. F&S, 2017
## Results

### Cost-effectiveness estimation per baby at home

<table>
<thead>
<tr>
<th></th>
<th>PGT-A</th>
<th>Non PGT-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>IVF lab cost</td>
<td>5490x100 (549,000)</td>
<td>5490x105 (576,450)</td>
</tr>
<tr>
<td>Drug cost</td>
<td>1200x100 (120,000)</td>
<td>1200x105 (126,000)</td>
</tr>
<tr>
<td>Vitrification cost</td>
<td>1100x13 (14,300)</td>
<td>1100x55 (60,500)</td>
</tr>
<tr>
<td>Cost of additional transfers</td>
<td>1950x1 (1950)</td>
<td>1950x35 (68,250)</td>
</tr>
<tr>
<td>Cost of PGD-A + day-3 embryo biopsy</td>
<td>3890x100 (389,000)</td>
<td>---</td>
</tr>
<tr>
<td>Cost of D&amp;C+POC</td>
<td>1023x1 (1023)</td>
<td>1023x21 (21,483)</td>
</tr>
<tr>
<td>Cost of medical treatment of ectopic</td>
<td>---</td>
<td>2040x2 (4080)</td>
</tr>
<tr>
<td>Mean cost/baby day-3</td>
<td>1075,273/45 babies (23,895)</td>
<td>856,763/39 babies (21,968)</td>
</tr>
<tr>
<td>Estimated cost (€)/baby blastocyst</td>
<td>19,250</td>
<td>21,968</td>
</tr>
<tr>
<td>Estimated cost ($)/baby USA</td>
<td>36,098</td>
<td>40,211</td>
</tr>
</tbody>
</table>
RCT- Advanced Maternal Age

Cost-effectiveness estimation per baby at home

Day-3 biopsy
Blastocyst (Spain)
Blastocyst (USA)

PGT-A
Control

ClinicalTrials.gov NCT01571076 Igenomix-IVI
Rubio et al. F&S, 2017
Conclusions

**Clinical Outcome**

1st ET: significant increase in delivery rates, drastic decrease in MR.

Cumulative cryo-transfers: similar results in both arms.

**Time to pregnancy**

Number of transfers: significant decrease in the number of attempts in the PGT-A.

Theoretical model: lower number of transfers, miscarriages and time needed for a live-birth.

**Cost-efficiency ($/€ per baby)**

Similar cost than blastocyst transfer
## Clinical outcome after the first attempt: fresh transfer

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PGT-A</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>50</td>
<td>51</td>
<td>---</td>
</tr>
<tr>
<td>Female mean age ±SD</td>
<td>32.8 ±3.4</td>
<td>33.2 ±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>% Patients with fresh transfer</td>
<td>94.0</td>
<td>80.4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean embryos/transfer ±SD</td>
<td>1.7±0.4</td>
<td>1.5±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy rate/ transfer</td>
<td>40.4</td>
<td>73.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Pregnancy rate/ patient</td>
<td>38.0</td>
<td>58.8</td>
<td>0.059</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>26.3</td>
<td>6.6</td>
<td>0.054</td>
</tr>
<tr>
<td>Ongoing pregnancy rate/transfer</td>
<td>29.8</td>
<td>65.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Ongoing pregnancy rate/patient</td>
<td>28.0</td>
<td>52.9</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Ongoing pregnancies >22 weeks. *Two-side Fishers’ test*

ClinicalTrials.gov NCT01571076 Igenomix-IVI
Rubio et al al. F&S
Submitted
### RCT- Severe Male Factor (Interim analysis)

Cumulative clinical outcome after cryotransfers

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PGT-A</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>50</td>
<td>51</td>
<td>---</td>
</tr>
<tr>
<td><strong>Fresh+Frozen transfers</strong></td>
<td>47+20</td>
<td>41+3</td>
<td>---</td>
</tr>
<tr>
<td><strong>Mean embryos/transfer ± SD</strong></td>
<td>1.7 ± 0.4</td>
<td>1.5 ± 0.5</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>Cumulative PR/transfer</strong></td>
<td>41.8</td>
<td>72.7</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Cumulative PR/patient</strong></td>
<td>56.0</td>
<td>62.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Miscarriage rate</strong></td>
<td>28.6</td>
<td>9.4</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Ongoing cumulative PR rate/transfer</strong></td>
<td>29.8</td>
<td>65.9</td>
<td>0.0004</td>
</tr>
<tr>
<td><strong>Ongoing cumulative PR rate/patient</strong></td>
<td>40.0</td>
<td>56.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ongoing cumulative implantation rate</strong></td>
<td>17.9 (21)</td>
<td>52.9 (36)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Ongoing pregnancies >22 weeks. *Two-side Fishers’ test*

ClinicalTrials.gov NCT01571076 Igenomix-IVI
Rubio et al al. F&S
Submitted
Meta-Analysis on PGt-A for 24 chromosomes

Can Comprehensive Chromosome Screening Technology Improve IVF/ICSI Outcomes? A Meta-Analysis

Minghao Chen¹*, Shiyou Wei²*, Junyan Hu³*, Song Quan¹*

1232 articles were identified through database searching
1235 articles for title and abstract review
1212 articles were excluded after initial assessment
23 articles for full-text review
12 full-text articles were excluded with following reasons:
5 no suitable control group
4 with polar body biopsy
1 included chromosome abnormal patients
1 did not report implantation rate
1 used clinical outcomes reported previously
11 articles were included in meta-analysis
4 articles were RCTs
7 articles were cohort studies

Chen et al., PLoS One, 2015
Meta-Analysis on PGT-A for 24 chromosomes

Can Comprehensive Chromosome Screening Technology Improve IVF/ICSI Outcomes? A Meta-Analysis

Minghao Chen*+, Shiyou Wei*+, Junyan Hu**, Song Quan**

RESEARCH ARTICLE

No benefit of PGT-A on CLB

Fig 5. Forest plots showing the results of meta-analysis on live birth comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI. (a) Forest plot of pooled RR on live birth of RCTs; (b) Forest plot of pooled RR on live birth of cohort studies.

Chen et al., PLoS One, 2015
Meta-Analysis on PGT-A for 24 chromosomes

Decreased miscarriage with PGT-A

Fig 6. Forest plots showing the results of meta-analysis on miscarriage comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI. (a) Forest plot of pooled RR on miscarriage of RCTs; (b) Forest plot of pooled RR on miscarriage of cohort studies.
Meta-Analysis on PGT-A for 24 chromosomes

Can Comprehensive Chromosome Screening Technology Improve IVF/ICSI Outcomes? A Meta-Analysis
Minghao Chen*, Shiyou Wei**, Junyan Hu**, Song Quan**

**Fig 7. Forest plots showing the results of meta-analysis on multiple pregnancy comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI. (a) Forest plot of pooled RR on multiple pregnancy of RCTs; (b) Forest plot of pooled RR on multiple pregnancy of cohort studies.

Chen et al., PLoS One, 2015
Meta-Analysis on PGT-A for 24 chromosomes

**RCTs**

**Sustained implantation rate (> 20 weeks gestation)**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>PGS-CCS Events</th>
<th>Total</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al. 2012</td>
<td>38</td>
<td>55</td>
<td>20</td>
<td>48</td>
<td>14.5%</td>
<td>1.65 [1.14, 2.42]</td>
</tr>
<tr>
<td>Forman et al. 2013</td>
<td>54</td>
<td>87</td>
<td>83</td>
<td>172</td>
<td>37.8%</td>
<td>1.29 [1.03, 1.61]</td>
</tr>
<tr>
<td>Scott et al. 2013</td>
<td>89</td>
<td>134</td>
<td>78</td>
<td>163</td>
<td>47.7%</td>
<td>1.39 [1.14, 1.70]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>276</td>
<td>383</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>1.39 [1.21, 1.60]</td>
</tr>
<tr>
<td>Total events</td>
<td>181</td>
<td>181</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: $\chi^2 = 1.29$, df = 2 (P = 0.53); $I^2 = 0%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $Z = 4.61$ (P &lt; 0.00001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Observational**

**Sustained implantation rate (> 20 weeks gestation)**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>PGS-CCS Events</th>
<th>Total</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sher et al. 2009</td>
<td>34</td>
<td>94</td>
<td>39</td>
<td>111</td>
<td>14.9%</td>
<td>2.88 [1.94, 4.29]</td>
</tr>
<tr>
<td>Forman et al. 2012</td>
<td>77</td>
<td>140</td>
<td>76</td>
<td>182</td>
<td>54.6%</td>
<td>1.32 [1.05, 1.65]</td>
</tr>
<tr>
<td>Lee et al. 2015</td>
<td>25</td>
<td>55</td>
<td>12</td>
<td>63</td>
<td>9.2%</td>
<td>2.39 [1.33, 4.29]</td>
</tr>
<tr>
<td>Feichtinger et al. 2015</td>
<td>29</td>
<td>110</td>
<td>60</td>
<td>403</td>
<td>21.2%</td>
<td>1.77 [1.20, 2.62]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>399</td>
<td>959</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>1.75 [1.46, 2.07]</td>
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<tr>
<td>Total events</td>
<td>165</td>
<td>187</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: $\chi^2 = 13.10$, df = 3 (P = 0.004); $I^2 = 77%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Test for overall effect: $Z = 6.48$ (P &lt; 0.00001)</td>
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</tr>
</tbody>
</table>

Dahdouh et al, F&S, 2015
Blastocyst biopsies and NGS cycles performed in 2017
>100,000 trophoectoderm biopsies analysed worldwide

PGT-A: the NGS era

Ongoing pregnancy rate per embryo transfer

*Internal IGENOMIX data based on outcomes and 2015 SART data. ** Biopsy in blastocyst stage.
What are the evidences against PGT-A?

✓ **No RCTs or clinical studies** showing lack of effect or detrimental impact of PGT-A performed on blastocysts with 24-chr testing platforms

✓ Many **reviews and opinion papers** against the application of PGT-A

✓ **Only 1 descriptive study** *(Gleicher et al Reprod Biol Endocrinol. 2016)*
  - 11 blastocysts with multiple TE biopsies and inconsistent results
  - 10 ET of “aneuploid” blastocysts with 5 live births

No raw data from PGT-A shown or made publically available

No DNA fingerprinting was performed to confirm genetic identity between embryos and the foetuses
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

SNP array: Of the 99 embryos assigned aneuploid, 4 (4%) sustained implantation

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

<table>
<thead>
<tr>
<th>Positive Predictive Value (Euploid embryos resulting in sustained implantation)</th>
<th>Negative Predictive Value (Aneuploid embryos resulting in sustained implantation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59.7%</td>
</tr>
</tbody>
</table>
Comparison PGT-A vs Prenatal Diagnosis

✓ Mosaicism and imperfect clinical predictive value have to be discussed based on up-to-date data and included in consent forms as for any diagnostic method

✓ Requires experienced IVF and PGT laboratory and careful implementation in the clinical practice

<table>
<thead>
<tr>
<th>PGT-A</th>
<th>PRENATAL DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Invasiveness: none or extremely low</td>
<td>✓ Invasiveness 0.2-1% Abortion risk</td>
</tr>
<tr>
<td>✓ Prevalence (Chromosomal risk) 20-90%</td>
<td>✓ Chromosomal risk (prevalence) 0.1-4%</td>
</tr>
<tr>
<td>✓ No result rate: ~1%</td>
<td>✓ No result rate: ~1%</td>
</tr>
<tr>
<td>✓ Mosaicism: present 6%</td>
<td>✓ Mosaicism: present 1-2% CVS</td>
</tr>
<tr>
<td>✓ Accuracy: 98-99%</td>
<td>✓ Accuracy: 98-99%</td>
</tr>
</tbody>
</table>

Gk, dia + gnosis, knowledge
niPGT-A: previous experience

✓ Non-invasive studies based on spent culture medium in comparison to trophectoderm

Shamonki et al., F&S 2016 → 3.5% concordance (N=57)
Feichtinger et al., RBMonline 2017 → 27% concordance (N=22)
Xu et al., PNAS 2016 → 85.7% concordance (N=42)

Low number of concordance studies

High variability between results

- Embryo mosaicism
- Maternal DNA contamination

Hammond et al., FS 2017
niPGT-A: our previous results

Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development

Vera-Rodriguez et al., Hum Reprod 2017
niPGT-A: our previous results

Day-5 blastocyst

- Traphectoderm biopsy (4–6 cells)
  - N = 56
- Spent culture media
  - N = 56

WGA

- Partial maternal contamination
  - Concordant 30.4%
- Full maternal contamination
  - 30.4%
- Non-informative
  - 8.9%

NGS

- 17 embryos

niPGT-A results summary:

- Concordant: 30.4%
- Partial maternal contamination: 30.4%
- Full maternal contamination: 30.4%
- Non-informative: 8.9%
niPGT-A: optimization of the protocol

Summary Pilot Study ni PGT-A (Igenomix-Genera)

Conventional Incubator

NO Hatching on D3

Drop volume: 10μl

Media from D4 to D6/7

Trophectoderm biopsy (4-6 cells)
N=115

Spent culture medium (10ul)
N=115

WGA

WGA

NGS

Trophectoderm DNA versus embryo-free DNA

Concordances
Partial maternal contamination
Full maternal contamination
Non-informative

Rubio et al., ESHRE 2018
niPGT-A: Igenomix/Genera Pilot Study

NGS profiles of trophectoderm biopsies and spent culture media

**Medium:** 47, XY, -7+14+17

**Trophectoderm biopsy:** 47, XY, -7+14+17
niPGT-A: Igenomix/Genera Pilot Study

NGS profiles of trophectoderm biopsies and spent culture media

Medium: 46, XX

Trophectoderm biopsy: 46, XX
## niPGT-A: optimization of the protocol

### Summary Pilot Study ni PGT-A (Igenomix-Genera)

<table>
<thead>
<tr>
<th>RESULTS</th>
<th>Day 5</th>
<th>Day 6/7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Informative results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Trophectoderm</td>
<td>0.0</td>
<td>3.7</td>
<td>2.6</td>
</tr>
<tr>
<td>% Spent Culture Media</td>
<td>18.2</td>
<td>0.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Embryo concordances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Tropho and media results</td>
<td>81.8</td>
<td>96.3</td>
<td>92.2</td>
</tr>
<tr>
<td>Embryo concordance</td>
<td>63.0</td>
<td>83.5</td>
<td>78.3</td>
</tr>
<tr>
<td>Autosome concordance</td>
<td>66.7</td>
<td>87.3</td>
<td>82.1</td>
</tr>
<tr>
<td>Total chromosome concordance</td>
<td>40.7</td>
<td>72.2</td>
<td>64.2</td>
</tr>
<tr>
<td>Embryo discordances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive</td>
<td>29.6</td>
<td>8.9</td>
<td>14.2</td>
</tr>
<tr>
<td>False positive (chaotic profile media)</td>
<td>14.8</td>
<td>5.1</td>
<td>7.5</td>
</tr>
<tr>
<td>False negative</td>
<td>3.7</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Only sex discordance euploid</td>
<td>3.7</td>
<td>3.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

**ESHRE 2018 SELECTED ORAL PRESENTATION**
niPGT-A: Multicenter study

Study flowchart

Recruitment
- Selection criteria
- Informed consent signature

PGT-A cycle
- Biopsy and Spent Culture Media collection (Day 5/6/7)

NGS analysis of the samples
- Trophectoderm Biopsies
- Spent Culture Media

SET fresh or deferred
- ET of euploid embryos
- Blinded analysis of the Spent Culture Media

Clinical follow-up
- Ongoing pregnancy
- Miscarriage (POC analysis)
- Concordant with PGT-A
- Discordant with PGT-A*

*In discordant results, blastocyst reanalysis in some centres.

Study population
Embryos from IVF patients undergoing PGT-A with SET for any medical indication between 20 and 44 years old with own or donated oocytes.

Estimated sample size: N=3245 samples
**Pro PGT Argument**

IVF should aim at maximizing LONG TERM treatment efficacy. **Healthy baby at home**

- Embryo aneuploidies are mostly meiotic in origin
- Trophoectoderm biopsy is safe and reliable (oocyte pick-up example) and very soon will not be necessary
- Clinical outcomes are significantly superior per transfer allowing to perform universal SET policy even in AMA patients where is more needed

*From Standard IVF to Preimplantation Genetic Testing IVF.*

- Increase implantation and pregnancy rates at the first cycle
- Reduce time to pregnancy
- Reduce multiple pregnancies
- Reduce miscarriages
- Reduce chromosomal abnormal newborns.
- Cost-effective
Conclusion

ART should not aim at maximizing **SHORT TERM** treatment “efficacy” irrespective of adverse events, such as miscarriage, multiples, or chromosomal abnormal newborns. This is against all ethical and medical basic principles.

ART should aim at maximizing **LONG TERM** treatment efficacy.

Healthy baby at home
Acknowledgements - PGT-A Team -

Montreal, CANADA: Gaahazl Haghi, Anca Bojinescu

Los Angeles, USA: Vi Nguyen, Derek Shaibi, Gurkan Sen, Tristan Darwin, Quang Pham, Kenney Tuyen, Refik Kayali, Abelard Bautista, Sue Robles

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MARTINEZ FERNANDEZ, MARIA  
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MARTINEZ MERINO, LUCIA  
MATEOS GREGORIO, PABLO  
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UPADHYAY, DIVYESH  

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**Collaborators:**

Unión Europea  
Fondo Europeo de Desarrollo Regional  
"Una manera de hacer Europa"  

University of Valencia  
Stanford University  
IVI  
Instituto de Investigación Sanitaria  
BCM  
Instituto de Salud Carlos III  

---

"Unas manos que hacen Europa"
Summary of up-to-date data from preclinical and clinical studies on PGT-A

✓ **Demonstrated advantages**
  - Increase implantation rate per ET
  - Decrease miscarriage rate
  - Decrease abnormal pregnancies
  - Decrease in the use of invasive and non-invasive prenatal diagnosis
  - Decrease time to pregnancy
  - Potential for being cost-effective

✓ **Potential disadvantages**
  - Potential for minimal loss of embryos
  - Needs expertise

No improvement of CLBR because all what you have is what you get, but demonstrated advantages are clear
Best Ethical Practice for Clinicians

Medical providers offering genetic test should:

- Offer all women the opportunity to receive reliable, medically relevant prenatal tests that have demonstrated safety and effectiveness in their demographic.

- Work with third-party to help all patients access, if medically appropriate. Structure the informed consent process so that it is comprehensive (…).

- Ensure that patients are offered genetic counselling both before and after testing.

- Give patients clear opportunities to decline testing.

- Encourage patients to make clear choices about which results they wish to receive before testing is undergone.

Desperation is expensive: one patients bill

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prontogest</td>
<td>£760.00</td>
</tr>
<tr>
<td>Intralipids</td>
<td>£300.00</td>
</tr>
<tr>
<td>Full Blood Count (FBC)</td>
<td>£40.00</td>
</tr>
<tr>
<td>Progesterone (Prog)</td>
<td>£30.00</td>
</tr>
<tr>
<td>HCG &amp; Prog</td>
<td>£70.00</td>
</tr>
<tr>
<td>NK Assay</td>
<td>£310.00</td>
</tr>
<tr>
<td>HCG &amp; Prog</td>
<td>£70.00</td>
</tr>
<tr>
<td>HCG &amp; Prog</td>
<td>£70.00</td>
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<td>9+0 Scan</td>
<td>£110.00</td>
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<td>10+0 Scan</td>
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<tr>
<td>12+4 Scan (FMC)</td>
<td>£230.00</td>
</tr>
<tr>
<td>Blood Tests (HIV &amp; Hep)</td>
<td>£200.00</td>
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<tr>
<td>Hormone Profile</td>
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<tr>
<td>Rubella</td>
<td>£45.00</td>
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<tr>
<td>Full Immune Blood Test</td>
<td>£805.00</td>
</tr>
<tr>
<td>E2</td>
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</tr>
<tr>
<td>Progesterone (Prog)</td>
<td>£30.00</td>
</tr>
<tr>
<td>E2 &amp; LH</td>
<td>£60.00</td>
</tr>
<tr>
<td>E2 &amp; LH</td>
<td>£60.00</td>
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<td>E2, LH, FSH &amp; Prog</td>
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<td>E2, LH &amp; FSH</td>
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<tr>
<td>E2 (x2), LH, FSH &amp; Prog</td>
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<tr>
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<tr>
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<td>E2 (x2), LH, FSH &amp; Prog</td>
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<tr>
<td>E2 (x2), LH, FSH &amp; Prog</td>
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<td>IVIG</td>
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**TOTAL** £13,271

*Slide from Nick Macklon*