PGT-A
PGT-M
Preimplantation Genetic Testing

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Introduction

Embryo biopsy

Applied technologies & Evolution

Clinical indications

Current practice
Introduction PGT-A

- Patients with genetic conditions
  - Chromosomal disorders 3.8 per 1000
  - Single-gene disorders 20 per 1000
  - Multifactorial disorders 646.4 per 1000

- Clinical genetic knowledge


https://omim.org/statistics/entry
### Biopsy strategies

<table>
<thead>
<tr>
<th>Biopsy Type</th>
<th>Day</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| Polar body biopsy      | Day 1 | - Detection of maternally inherited abnormalities: structural aberrations or monogenic diseases
                          |       | - Fresh transfer option                                                     | - No father-related information
                          |       |                                                                             | - No embryonic de novo alterations               |
| Blastomere biopsy      | Day 3 | - Study of paternal, maternal, and de novo abnormalities
                          |       | - Fresh transfer option                                                     | - No mosaicism detection                         |
| Trophoderm biopsy      | Day 5/6| - Decrease in the number of biopsied embryos
                          |       |                                                                             | - Vitrification is often needed
                          |       |                                                                             | - No detection of all types of mosaicism, such as ICM/TE |

- 1st and 2nd PB
- Cleavage-stage
- TE Blastocyst
- Blastocentesis
PGT-A: the NGS era

Embryo culture → Day-3 biopsy → Day-5/6 biopsy → SECUENCIACIÓN PGT-A/NGS → RESULTADO → female reproductive system
PGT-A: **evolution** of the technology

1995

Fluorescence
*In Situ*
Hybridization
(FISH)

- Day-3 biopsies
- 2 blastomeres
- PGT-A 1.0

≤12 chromosomes
PGT-A: evolution of the technology

- **1995**: Fluorescence In Situ Hybridization (FISH)
- **2008**: Array Comparative Genomic Hybridization (aCGH)
- **2010**: Single-nucleotide polymorphism (SNP)
- **2012**: Quantitative polymerase chain reaction (qPCR)

- **Day-3 biopsies 2 blastomeres PGT-A 1.0**
- **Blastocysts Deferred transfer PGT-A 2.0**

| ≤12 chromosomes | 24 chromosomes |
PGT-A: evolution of the technology

1995

Fluorescence In Situ Hybridization (FISH)

1995

Day-3 biopsies 2 blastomeres PGT-A 1.0

2008

Array Comparative Genomic Hybridization (aCGH)

2008

Blastocysts Deferred transfer PGT-A 2.0

2008

NGS (Illumina)

2010

Single-nucleotide polymorphism (SNP)

2010

aCGH (Illumina)

2012

Quantitative polymerase chain reaction (qPCR)

2012

NGS (Illumina)

2013

Next Generation Sequencing (NGS)

2013

PGT-A 3.0

2016-17

NGS with custom algorithm for mosaicism detection

≤12 chromosomes

24 chromosomes

Mitochondrial DNA & Mosaicism
PGT-A: evolution of the technology

1995
Fluorescence In Situ Hybridization (FISH)

Day-3 biopsies 2 blastomeres PGT-A 1.0

1995

2008
Blastocysts Deferred transfer PGT-A 2.0

Array Comparative Genomic Hybridization (aCGH)

2008

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Array Comparative Genomic Hybridization (aCGH)

Single-nucleotide polymorphism (SNP)

2010

2012
Array Comparative Genomic Hybridization (aCGH)

Quantitative polymerase chain reaction (qPCR)

2012

2013
Next Generation Sequencing (NGS)

2013

2016-17
NGS with custom algorithm for mosaicism detection

2016-17

2018-19
NGS in Spent culture media with custom algorithm

2018-19

Mitochondrial DNA & Mosaicism

≤12 chromosomes

24 chromosomes

PGT-A 3.0

niPGT-A
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 and Hunt, Nat Rev Genet, 2001)
• Female 38–41 years of age

• Higher live-birth rates using PGT-A compared to conventional morphological embryo selection
  • per first transfer (52.9% vs. 24.2%; $p = 0.0002$), and
  • per patient (36.0% vs. 21.9%; $p = 0.0309$).

• PGT-A dramatically decreases miscarriage rates compared to controls (2.7% vs. 39.0%)
• **Balanced** SR are the most frequent chr abnormalities, with a prevalence of 0.4% in prenatal samples and 0.2% in newborns.

• The most common SR are **translocations and inversions**
  - **Translocations** occur after a double break in two chromosomes and exchange of fragments between the two.
    - **Reciprocal**: breakage and exchange of distal segments between non-homologous chromosomes.
    - **Robertsonian**: fusion of two acrocentric chromosomes (chromosomes 13, 14, 15, 21, or 22) and loss of their short arms.
  - **Inversions** occur after a double intra-chromosomal break, 180° rotation of the fragment, and subsequent reinsertion.
Other

• Recurrent Miscarriage
  • Two to 3 consecutive miscarriages with a gestational age up to 14 w
  • PGT-A implantation rates: 52.63% vs 19.15% in controls ($p = 0.001$)
  • PGT-A “doubled” ongoing pregnancy rate (61.54% vs. 32.49%; $p = 0.0001$)

• Repetitive Implantation Failure
  • Three or more failed IVF attempts or failed IVF treatments after cumulative transfer of >10 good-quality embryos

• Severe Male Factor Infertility

• Previous Trisomic Pregnancy
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>32.1 ± 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 (2pn)</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</td>
<td>55 (100)</td>
<td>48 (100)</td>
<td></td>
</tr>
<tr>
<td>according to morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>assessment:</td>
<td></td>
<td></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

* by Chi-squared test
* by Fisher’s exact test
PGT-A: The NGS era

NGS analysis general steps

Library prep

WGA and fragment library construction with Ion SingleSeq Kit

Template prep: Ion Chef System

Isothermal amplification with Ion Chef System

Sequencing: S5 system

Sequence up to 96 preimplantation embryos with Ion 530 Chip

Analysis: Ion Reporter software v5.4 or higher

Data analysis and storage with Torrent Suite Software v5.4 or higher and Ion Reporter Software and server

Current practice

igenomix
PGT-A: the NGS era

Normal embryos

Normal XX

Normal XY
PGT-A: the NGS era

Embryo aneuploidy

Abnormal
Abnormal
Complex
Abnormal
Chaotic

-18, XY
+8, +10, -14, +15, -21, XY

MARD=0.105 Confidence Bar=0.1
MARD=0.113 Confidence Bar=1.0
MARD=0.275 Confidence Bar=0.1
Mosaicism

- Greco et al., 2015
  - Healthy babies
- Fragouli et al., Hum Genet 2017
  - Poor clinical outcome
  - Pregnancy 46.2% vs 15.4% p:0.003
- Further research needed
NGS: Mosaicism

30%

50%

70%

100%
Current practice PGT-A

• Clinical routine practice **to improve pregnancy rates** in sub-fertile couples

**Incidence of aneuploidy**

- Embryos screened identified as abnormal
- Maternal age:
  - Egg donors: 42.6%, 51.8%, 54.4%, 67.9%, 77.9%, 79.8%
  - <35: 95%, 91.4%
  - 35-37: 87%
  - 38-40: 73.8%
  - 41-42: 59.6%
  - >42: 26.6%

**Transfer by maternal age**

- Cases with at least one normal embryo
Ongoing pregnancy rate per ET

- <35: 65% (IVF with PGS), 49.4% (IVF without PGS)
- 35-37: 64.5% (IVF with PGS), 42.3% (IVF without PGS)
- 38-40: 61.1% (IVF with PGS), 32.9% (IVF without PGS)
- 41-42: 60.2% (IVF with PGS), 20.7% (IVF without PGS)
- >42: 53.7% (IVF with PGS), 7.8% (IVF without PGS)

*Internal IGENOMIX data based on outcomes and 2015 SART data.
**Biopsy in blastocyst stage.
Conclusions

- 24-chromosome analysis by NGS

Biopsy and transfer strategy

- Blastocyst biopsy with a trend towards deferred transfers to include day-5 and day-6 euploid blastocysts

Clinical outcome

- New RCTs with 24 chromosome analyzed showed:
  - Increased live birth rates per first attempt
  - Decreased miscarriages and multiple pregnancies
  - Decreased time to pregnancy and maybe cost

Future

- niPGT-A → No biopsy needed (ongoing research)
Introduction PGT-M (SGD)

- Patients with genetic conditions
  - Chromosomal disorders 3.8 per 1000
  - Single-gene disorders 20 per 1000
  - Multifactorial disorders 646.4 per 1000


- Clinical genetic knowledge

![OMIM Entry Statistics](https://omim.org/statistics/entry)
Biopsy D5/6

2017

- 90%
- day 3: 10%
- day 6: 90%

2018

- 98%
- day 3: 2%
- day 6: 98%

* Preliminary data (N=715 cases)
Phases

I. PRE PGT-A
   - Molecular design
   - Protocol development

II. ART
   - Ovary stimulation
   - ICSI /embryo biopsy
   - Transfer
   - Vitrification

III. Molecular biology
   - Amplification
   - Genotyping
Disease info

**Description**

Menkes disease is an X-linked recessive disorder characterized by generalized copper deficiency. The clinical features result from the dysfunction of several copper-dependent enzymes.

**Clinical Features**

In a family of English-Irish descent living in New York, Menkes et al. (1962) described an X-linked recessive disorder characterized by early retardation in growth, peculiar hair, and focal central and cerebellar degeneration. Severe neurological impairment begins within a month or two of birth and progresses rapidly to death. Few males were affected but the gene could be inferred to be inherited in 4 generations. The failure to grow and the affected infants to medical attention at the age of a few weeks and death ensued in the first or second year of life. The hair was starchy and white. Microscopically it showed twisting, varying diameter along the length of the shaft, and often fringes of the shaft at regular intervals. Rather extensive biochemical investigations showed elevated plasma glutathione as well as the only consistent abnormality. The anatomic changes in the central nervous system were described on the basis of 2 cases.

Brady (1965) observed 2 brothers who died as infants with sparse hair, anomalies, and defective hair. Blood and urine amino acids were normal. Whether this is the same disease as that in Menkes' family is uncertain. The condition described by Yoshida et al. (1964) in Japan has been described. French and Shapard (1967) presented evidence that they interpreted as indicating that the disorder may represent an immaturity of lipid metabolism.

Gene data
Linked markers
Methods

• Combined Genetic Approach
  • DIRECT: Mutation detection
  • INDIRECT: Identification of linked haplotype by informative polymorphic markers within or flanking the gene of interest.

• PCR-based methods
  • Lysis cellular
  • Multiplex PCR: all amplicons directly from biopsied cell/cells

• WGA:
  • Lysis cellular
  • Random genome pre-amplification of cell/s
  • Simplex amplicon analysis
  • NGS
• **Fragm**ent analysis (automatic): used both for direct and indirect genetic analysis (ex. Trinucleotide repeat expansion)

• **Restriction fragment length polymorphism (RFLP)** analysis: used after PCR to detect single nucleotide changes.

• **Minisequencing**: single-base extension used after PCR for mutation genotyping

• SNP Arrays
• NGS
NGS approach

TruSeq Custom Amplicon v1.5

Focus on key genomic regions of interest with this targeted resequencing solution, useful for very highplexity pools or longer amplicons.
• NM_206933.2:c.11549-5_11549-4insT

Source: Bioinformatics Dept - iGenomix
Amplification efficiency

Source: Bioinformatics Dept - iGenomix
Overlapping amplicons
RESULTS: PGT-M Historical data

PGT-M (2001-17 data)

Cases

Diseases

[Graphs showing historical data for PGT-M from 2001 to 2017]
Results
Common conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>CYCLES</th>
<th>% E TRANSFER</th>
<th>% +hCG</th>
<th>MISC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>68</td>
<td>82</td>
<td>41</td>
<td>2</td>
</tr>
<tr>
<td>CF</td>
<td>74</td>
<td>74</td>
<td>41,8</td>
<td>4</td>
</tr>
<tr>
<td>FX</td>
<td>54</td>
<td>63</td>
<td>32,3</td>
<td>3</td>
</tr>
<tr>
<td>PKD</td>
<td>109</td>
<td>79</td>
<td>39,5</td>
<td>9</td>
</tr>
<tr>
<td>HBB</td>
<td>51</td>
<td>60,7</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>DM1</td>
<td>125</td>
<td>73,6</td>
<td>38</td>
<td>9</td>
</tr>
<tr>
<td>HD</td>
<td>81</td>
<td>80</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>HEMA</td>
<td>75</td>
<td>67</td>
<td>44,8</td>
<td>6</td>
</tr>
<tr>
<td>NF1</td>
<td>48</td>
<td>89</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>CMT1A</td>
<td>37</td>
<td>73</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>DMD</td>
<td>35</td>
<td>83</td>
<td>44,8</td>
<td>3</td>
</tr>
</tbody>
</table>
Our results

Our experts are professional, reliable and approachable and are available to help guide both professionals and patients throughout the entire process. We are proud to deliver high quality results for every test, every day.

300 DIFFERENT DISEASES

2,5 K CYCLES

+20 YEARS' EXPERIENCE IN GENETICS

98% ACCURATE
## Results general overview

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>2886</td>
</tr>
<tr>
<td>Couples</td>
<td>1983</td>
</tr>
<tr>
<td>Diseases</td>
<td>377</td>
</tr>
<tr>
<td><strong>Gestation rate / transfer</strong></td>
<td><strong>45%</strong></td>
</tr>
<tr>
<td>Accuracy</td>
<td>≥98%</td>
</tr>
</tbody>
</table>

### Common
- Steinert (MD1)
- Fragile X
- Huntington
- Spinal Muscular Atrophy
- CF
- Hemophilia A

### New requests
- Lynch, Sd
- Von Hippel-Lindau, Sd
- Tuberous S (de novo)
- Neurofibromatosis 1 (de novo)
- Other (many)

80% are common disorders and 20% are less common disorders.
PGT-M (embryo Dx) for HBOC Carriers

- BRCA1 mutations in 36 couples
  - Female carrier (%): 24 (67%)
  - Male carrier (%): 12 (33%)
- BRCA2 mutations in 26 couples
  - Female carrier (%): 17 (65%)
  - Male carrier (%): 9 (35%)

62 couples

77 PGT-M cases

BRCA1

- 42 PGT-Ms (68%)

BRCA2

- 35 PGT-Ms (32%)

528 E (6.8 average)

242 E Normal (46%)
• Increase of cycles depended on the increase of new diseases
  • Open list of indications
  • Some Legal restrictions (country depended)

• Embryo biopsy at D5/6 predominates current ART with PGT

• Pregnancy rates per transfer are currently around 45% (PGT-A)
Our Team

Scientists

Carlos Simón
Al-Amar Nasser
Alonso Roberto
Blesa David
Bandeira Carla
Bover Ana
Campos Inmaculada
Cervero Ana
Chopra Rupali
Cinniglu Cengiz
Copreski Bruno
Chiu Yatfung
Diaz Antonio
Diaz Patricia
Dizon Bautista
Hervas Arantxa
Abelard
Elshaikh Noon
Gómez Eva
Gómez Carlos
Jiménez Jorge
Kayali Refik
Khajuria Rajni
Lopez Pilar
Mae Hoover Larissa
Marin Carlos
Martín Julio
Martinez José
Antonio
Más Aymara
Mateu Emilia
Milan Miguel
Mir Pere
Miravet José
Moreno Inma
Peinado Vanesa
Pinares Ania
Poo Maria Eugenia
Riboldi Marcia
Rincón-Bertolin
Alejandro
Rodrigo Lorena
Rodríguez Beatriz
Rubio Carmen
Ruiz Maria
Sánchez Maribel
Sanz Lucia
Sachdeva Kabir
Sen Gurkan
Uehara Mariane
Valbuena Diana
Vera Maria
Vilella Felipe
Whittenburg Alex

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Bermell Soledad
Centelles Vicente
Darvin Tristan
Escobedo Milagros
Eskridge Roderick
García Mirian
Gómez Maria
Martinez Asun
Martinez Lucia
Martinez Sebastian
Navarro Roser
Martinez Tantra
Mateos Pablo
Moles Sara
Morata María Jesus
Nguyen Tuan
Nieto Jessica
Peris Laura
Pozo Ana
Ravelo Kristine
Singh Vinita

[Map with locations]