The Future of Reproductive Medicine

Carlos Simón M.D., Ph.D.
Professor Ob/Gyn, University of Valencia.
Scientific Director Fundación IVI & Igenomix
Adjunct Clinical Professor Ob/Gyn, Stanford University, USA

Disclosure

CS reports to be a coinventor of some diagnostic tests and recipient of honoraria for lectures and unrestricted research grants from Merck, Ferring and MSD.
Reproductive Medicine. The present

- More than 7 million babies have been born worldwide.

- 2 – 6% live births in Europe by ART. (Mansour et al, Hum Reprod. 2014)


- 1,5 million IVF cycles/year worldwide.

Reproductive Medicine. Our Mission


That our patients achieve their dreams ONE HEALTHY BABY AT A TIME
**Breakthrough in Science. Genetics and Stem Cells**

- Design and synthesis of a minimal bacterial genome. *(Hutchison III, et al. Science 2016)*

![Diagram of Design and Synthesis Process](image)

- The new frontier of genome editing with CRISPR-Cas9. *(Doudna JA & Charpentier E. Science 2014)*

![CRISPR-Cas9 Diagram](image)


![Embryos Culture](image)

---

**Reproductive Medicine. Building the Future**

- **EXPANDING GENETIC CARRIER SCREENING**

- **THE EMBRYO**

- **THE ENDOMETRIUM**

- **FUTURE REPRODUCTIVE MEDICINE**
Genetic risk when planning to conceive

**Until now...**
- Learning about family’s health history to prevent inherited diseases
- Testing gene by gene (few)

**Now...** to prevent inherited diseases in the child, you must
first understand the personal risk in the couple

Expanded Genetic Carrier Testing

- Testing individuals to identify heterozygote carriers of severe Mendelian disorders

**Genotyping (Known mutations)**
- Gene by Gene
  - Targeted mutations
  - Population at risk
- Gene Panel by ARRAY
  - Targeted mutations
  - Large N of mutations

**Sequencing (All)**
- Gene panel by NGS
  - Sequencing all coding exons
  - Large N of genes

Early 70s, AJ population (Tay-Sachs) 2010 – 13 – Lazarin et al.
2011 - Bell et al.
2015 - Martin et al.
2016 - Abulí et al.

**NEEDS**
- Development of a curated-data repository of variants and associated phenotypes

**FUTURE**
- Whole exome sequencing and/or whole genome sequencing
Reproductive Medicine. Building the Future

- EXPANDING GENETIC CARRIER SCREENING
- THE EMBRYO
- THE ENDOMETRIUM
- FUTURE REPRODUCTIVE MEDICINE

From Invasive to Non-invasive diagnosis

- **Ploidy**
  - RSH: Griffin et al., 1991, Rubio et al., 2005
  - NGS: Yin et al., 2013

- **Morphology**
  - Cummins et al., 1986
  - Puissant et al., 1987

- **Visuals**
  - Wong et al., 2010
  - Meseguer et al., 2011

- **Omics**
  - Transcriptomics: Zhang et al., 2009, Shaw et al., 2013
  - Metabolomics: Conaghan et al., 1993, Gardner et al., 2001
  - Proteomics: Katz-Jaffe et al., 2005, Dominguez et al., 2008

- **Energy**
  - Mitochondrial DNA: Diez-Juan et al., 2015, Fragouli et al., 2015

- **Morphokinetics**
  - Rodrigo et al., 2014

- **aCGH**
  - Rubio et al., 2013
  - Rodrigo et al., 2014

- **FISH**
  - Griffin et al., 1991
  - Rubio et al., 2005

- **NGS**
  - Yin et al., 2013
  - Fiorentino et al., 2014
PGS: How?

- Mostly day-3 biopsies
- 2 blastomeres learning curves

FISH

1991–1995

Array comparative genomic hybridization (aCGH)

Quantitative polymerase chain reaction (qPCR)

Single-nucleotide polymorphism (SNP) microarray

2008

2010

2012

Next-generation sequencing (NGS)

Blastocyst biopsy

Deferred transfer

Good prognosis

PGS: When?

PGS: To whom?

- General Population

<table>
<thead>
<tr>
<th>Study</th>
<th>Oocytes retrieved (PGS/control)</th>
<th>Blastocysts obtained (PGS/control)</th>
<th>Embryo transfer (PGS / control)</th>
<th>Main clinical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al., 2012</td>
<td>19.5 ± 8.2/19.3 ± 8.1</td>
<td>8.3 ± 2.1/8.1 ± 2.4</td>
<td>Elective SET/elective SET Fresh</td>
<td>PGS-CCS improves clinical pregnancy rates and ongoing pregnancy rates (≥20 weeks)</td>
</tr>
<tr>
<td>Forman et al., 2013</td>
<td>16.9 ± 8.4/15.7 ± 7.1</td>
<td>5.8 ± 3.6/5.3 ± 3.0</td>
<td>Elective SET/DET Fresh or Frozen</td>
<td>Same ongoing pregnancy rates (≥24 weeks); PGS-CCS reduce sharply multiple pregnancy rates (0%).</td>
</tr>
<tr>
<td>Scott et al., 2013a</td>
<td>17.2 ± 0.9/17.1 ± 0.9</td>
<td>8.0 ± 0.7/7.9 ± 0.7</td>
<td>DET/DET Fresh</td>
<td>PGS-CCS improves sustained implantation rates and delivery rates.</td>
</tr>
</tbody>
</table>

Dahdouh et al, RBMonline 2015

- High aneuploidy risk: AMA, MF, RM, RIF, PTP. (Rubio et al, ESHRE and ASRM 2014)
Cryopreservation has modified Reproductive Medicine

- An OHSS-free clinic by segmentation of IVF treatment.  
  *(Devroey et al, Hum Reprod. 2011)*


- Oocyte vitrification as an efficient option for elective fertility preservation.  
  *(Cobo et al, Fertil Steril. 2016)*

- **Are we ready to eliminate the transfer of fresh embryos in IVF?**  
  *(Barnhart et al, Fertil Steril. 2016)*
Reproductive Medicine. Building the Future

• EXPANDING GENETIC CARRIER SCREENING
• THE EMBRYO
• THE ENDOMETRIUM
• FUTURE REPRODUCTIVE MEDICINE

Why an Euploid, Morphologically Normal Embryo DOES NOT IMPLANT?

TIMING IS KEY

ARE WE DELIVERING THE EMBRYO AT THE WRONG TIME?
Window of endometrial receptivity

Endometrial receptivity analysis
Endometrial receptivity analysis + NGS

238 genes
Bioinformatic analysis of data
Classification and prediction from gene expression

Patented in 2009: PCT/ES 2009/000386

Diaz-Gimeno et al, Fertil Steril. 2011
Conclusions

- The best day for embryo transfer from the uterine perspective depends on the patient.
- The transcriptomic signature of endometrial receptivity reveals that the endometrial factor is responsible for 25% of cases of patients with RIF.
- Personalized Embryo Transfer (pET) normalize clinical results.
- A multicenter RCT is underway.

Predictive Implantation Test

**PGE_2** and **PGF_2α** Concentrations in Human Endometrial Fluid as Biomarkers for Embryonic Implantation

F. Vilella,* L. Ramirez,* O. Berlanga, S. Martínez, P. Alamá, M. Meseguer, A. Pellicer, and C. Simón

Fundación Instituto Valenciano de Infertilidad (F.V., L.R., O.B., S.M., C.S.), Valencia University and Instituto Universitario IVIINCLIVA, Valencia University, 46980 Valencia, Spain; Instituto Valenciano de Infertilidad (I.V.) (P.A., M.M., A.P., C.S.), Valencia University, 46013 Valencia, Spain; and Department of Obst/Gyn (C.S.), Stanford University School of Medicine, Stanford California 94305

Vilella et al., JCEM 2013
Regenerative Medicine in Reproductive Medicine

**Asherman’s Syndrome**

**Treatment:** **PRACTICE**
(Prevention, Anticipation, Comprehensive Therapy, Timely Surveillance of subsequent Pregnancies, Investigation and Continuous Education)

**Endometrial Atrophy (<5mm)**

**Treatment:**
- High doses of E2 Pentoxifylline 800mg/day + Vitamin E (1000 IU/day) during 6 months (Takasaki, 2010)
- Sildenafil 100mg vaginally (Takasaki, 2010)
- 6mg L-arginine daily (Acharya, 2009)
- Intrauterine perfusion of 300mg of G-CSG (Gleicher, 2011; Barad 2014)

**PHASE I. Completed**
16 patients with AS or EA
Reproductive Medicine. Building the Future

- EXPANDING GENETIC CARRIER SCREENING
- THE EMBRYO
- THE ENDOMETRIUM
- FUTURE REPRODUCTIVE MEDICINE

In 2000, 32% of the IVF cycles required the donation of either sperm or eggs (Adamson et al, 2006).

However, even though donation of gametes results in very high pregnancy rates, there are ethical, legal and personal concerns to consider.

Generation of functional sperm (Hayashi et al, Cell 2011), and functional eggs (Hayashi et al, Science 2012) from mESCs that give rise to healthy and fertile offspring.

In vitro only generation of artificial gametes from mESCs to functional spermatids. (Zhou et al, Cell Stem Cells 2016)
Direct reprogramming of fibroblasts into different cell fates have been reported.

Advances in reprogramming from somatic cells

- Hematopoietic progenitors (Szabo et al, 2010)
- Neurons (Kim et al, 2011; Vierbuchen et al, 2010)
- Cardiomyocytes (Ieda et al, 2010)
- Sertoli cells (Buganim et al, 2012)

Reprogramming strategy with 6 selected factors

- LIN28A
- SYCP3
- DAZL
- PRDM14
- VASA

Germ Cell Medium

Human somatic cells (46, XY)

+GDNF
+P4gf
+SCF
+LIF
+RA

Induced Germ cell-like cell clumps

Analysis tools:

- ICC analysis
- Genetic analysis
- Epigenetic analysis
- Meiotic progression assay
- Haploid cell formation assay
- Xenotransplant assay

Medrano et al., Scientific Reports 2016
1st Revolution

ORAL CONTRACEPTIVE PILLS

Reproduction dissociates from Sex

Preventing unwanted pregnancies

2nd Revolution

FERTILITY PRESERVATION

Sex dissociates from Reproduction

Conception of healthy babies

Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

(Marie Curie, 1867-1934)
References


References


References


