

# THE FUTURE OF IVF: SAFE AND INNOVATIVE



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Medical Director  
IVI GCC, Abu Dhabi

# Infertility

- ✓ 48 million Infertile couples in 2010 worldwide.

Mascarenhas et al, PLoS Med. 2012



- ✓ More than 5 million babies have been born worldwide.
- ✓ 2 – 6% live births in Europe by ART.

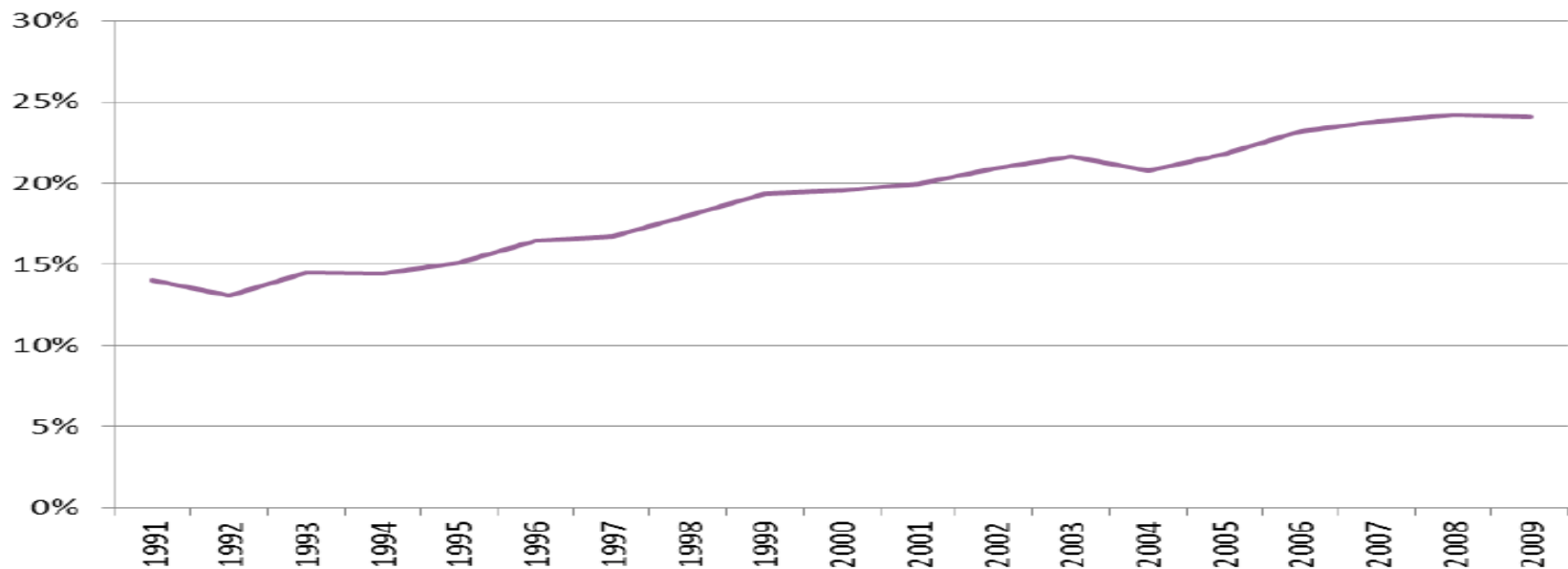
Mansour et al, Hum Reprod. 2006



- ✓ 1,5 million IVF cycles/year worldwide.

- 1991- 2015: pubmed 19000 publications on IVF, however:

Live birth rate, per cycle started



HFEA, 2011

# Why?

- Still limited knowledge
- Focus oocyte/embryo
  - Neglect the endometrium?

# Zondek et al. 1930

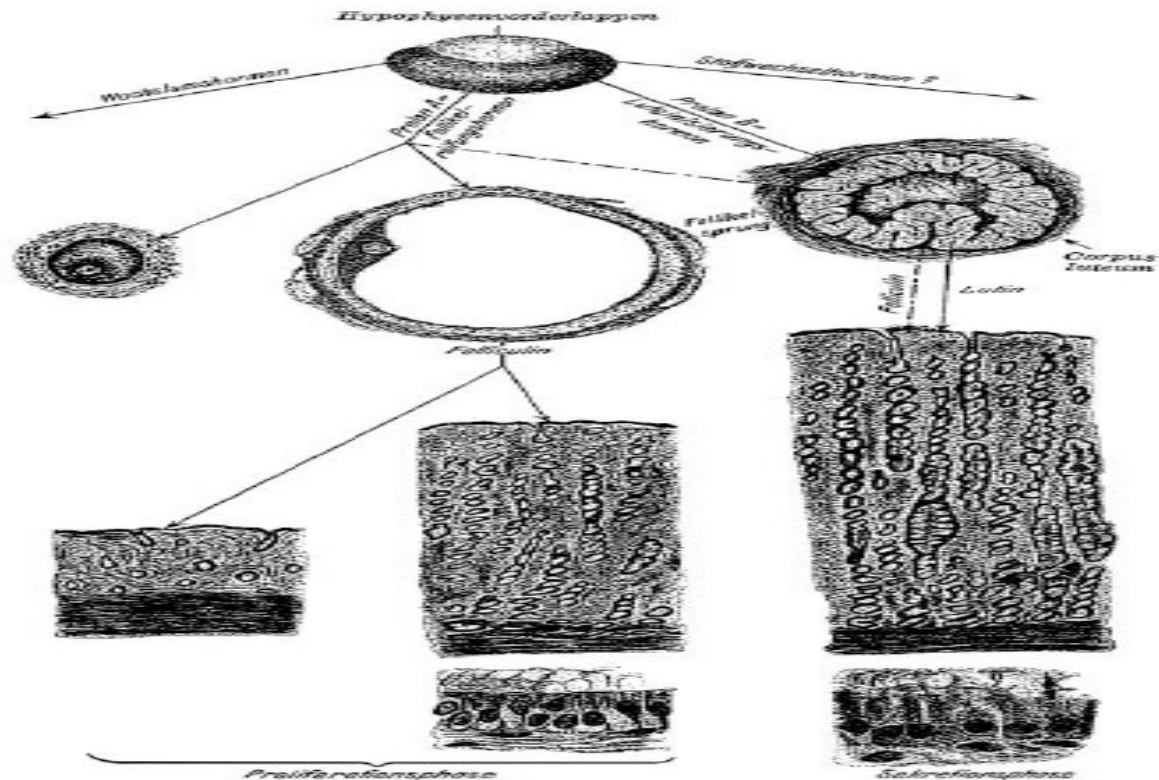
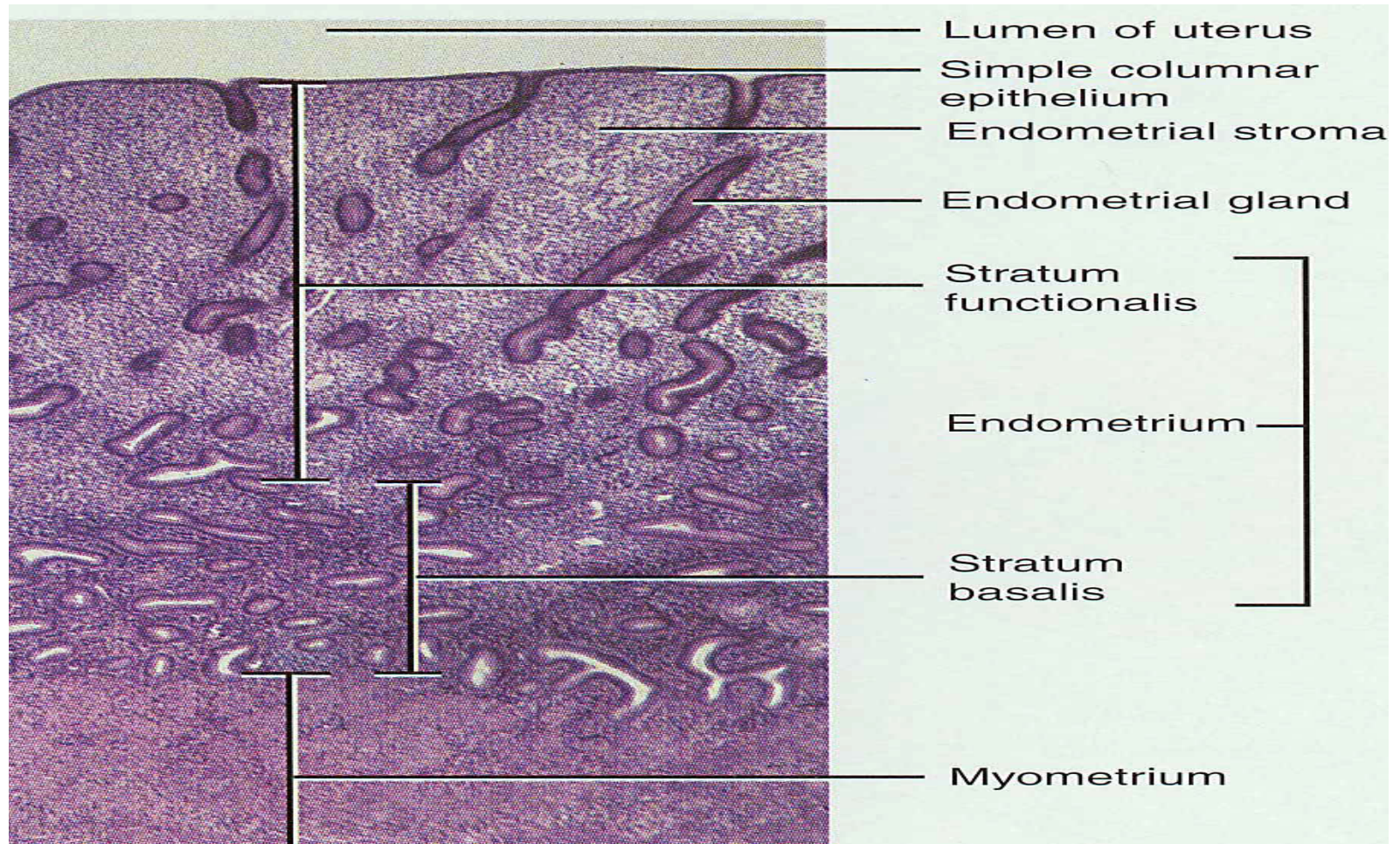


Abb. 1. Hypophysenvorderlappen und Genitalfunktion des Weibes.

**Figure 1.** Zondek's illustration of the relationship between the hypothalamus, pituitary, ovaries, and endometrium (Zondek, 1930). Reprinted by permission from Zondek (1930), Ueber die Hormones des Hypophysenvorderlappens. Klin Wochenschrift 9,245-248, Copyright Springer Verlag.

# Endometrial Histology: Noyes et al 1950

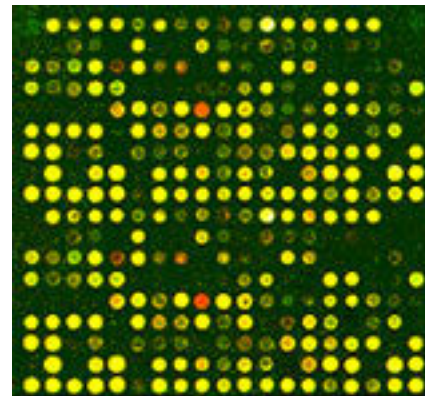


# Endometrial receptivity

## ERA®



1950's



21<sup>st</sup> century

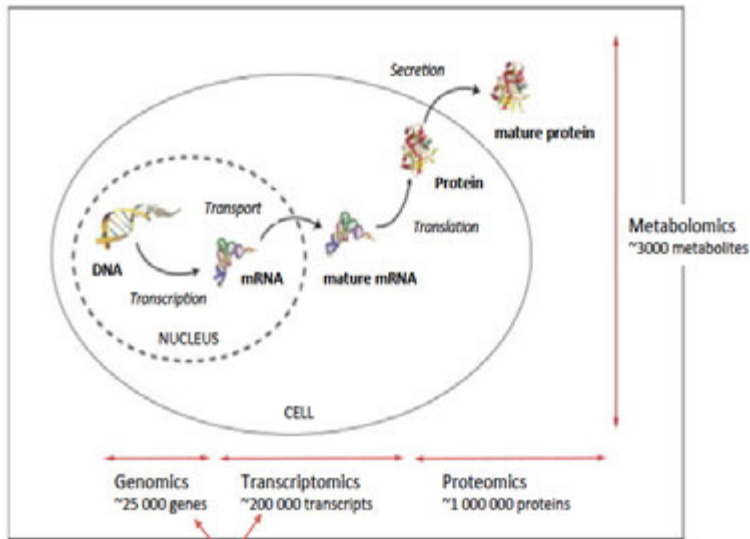
238 customized genes  
related to the WOI

Receptive vs Non Receptive

# What about Omics?

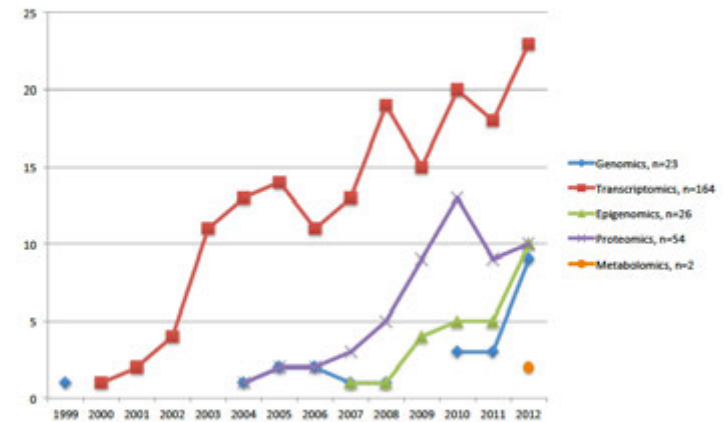
G-E-T-P-M

## 'Omics' levels



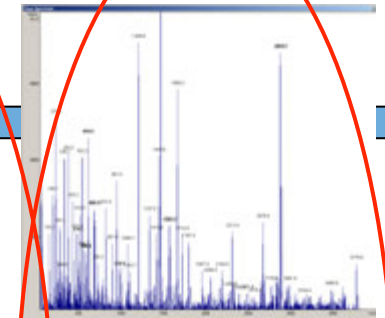
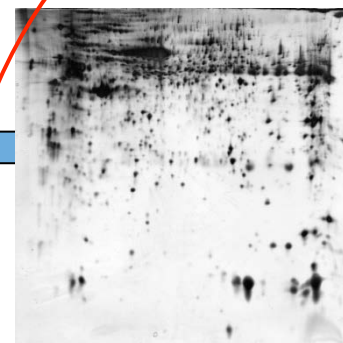
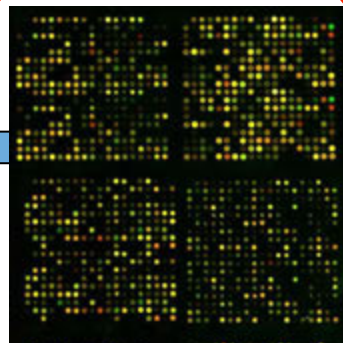
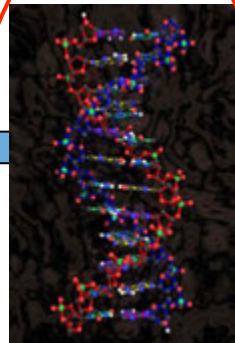
- Epigenomics  
Changes in gene activity that are not caused by changes in DNA
- DNA methylation
  - Histone modifications
  - RNA interference (microRNA)

## 'Omics' studies in human endometrium



Altmäe S et al. Hum Reprod Update 2014;20:12–28.

# The age of -OMICS



Transcriptome

Proteome

Metabolome

TRANSCRIPTION

TRANSDUCTION

DNA

RNA

mRNA

Protein

METABOLITES

Transcription regulation

Alternative splicing

Transduction regulation

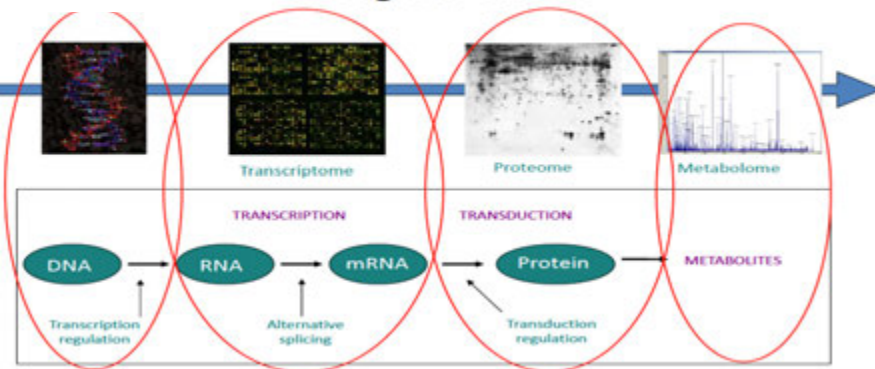
# ERA

## The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

Maria Ruiz-Alonso, M.Sc.,<sup>b</sup> David Blesa, Ph.D.,<sup>a,b</sup> Patricia Díaz-Gimeno, Ph.D.,<sup>a,c</sup> Eva Gómez, M.Sc.,<sup>a</sup> Manuel Fernández-Sánchez, M.D.,<sup>d</sup> Francisco Carranza, M.D.,<sup>d</sup> Joan Carrera, M.D.,<sup>e</sup> Felip Vilella, Ph.D.,<sup>a</sup> Antonio Pellicer, M.D., Ph.D.,<sup>a,b</sup> and Carlos Simón, M.D., Ph.D.<sup>a,b</sup>

<sup>a</sup> Fundación Instituto Valenciano de Infertilidad, and Instituto Universitario IVI/Incliva, Valencia University, Valencia; <sup>b</sup> Niomics, Paterna; <sup>c</sup> Computational Medicine Institute, Centro de Investigación Príncipe Felipe, Valencia; <sup>d</sup> Instituto Valenciano de Infertilidad Sevilla, Seville; and <sup>e</sup> Clínica Girona Unidad de Reproducción Humana, Girona, Spain

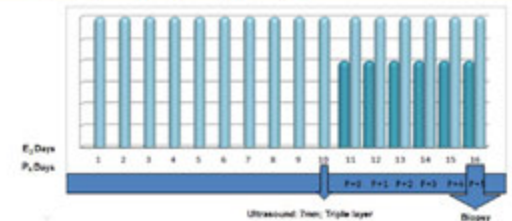
### The age of -OMICS



### HORMONE REPLACEMENT THERAPY CYCLE

Endometrial biopsy must be taken on day P+5, after proper E<sub>2</sub> priming

- E<sub>2</sub>: 6 mg/day
- P<sub>4</sub>: 800 mg/day

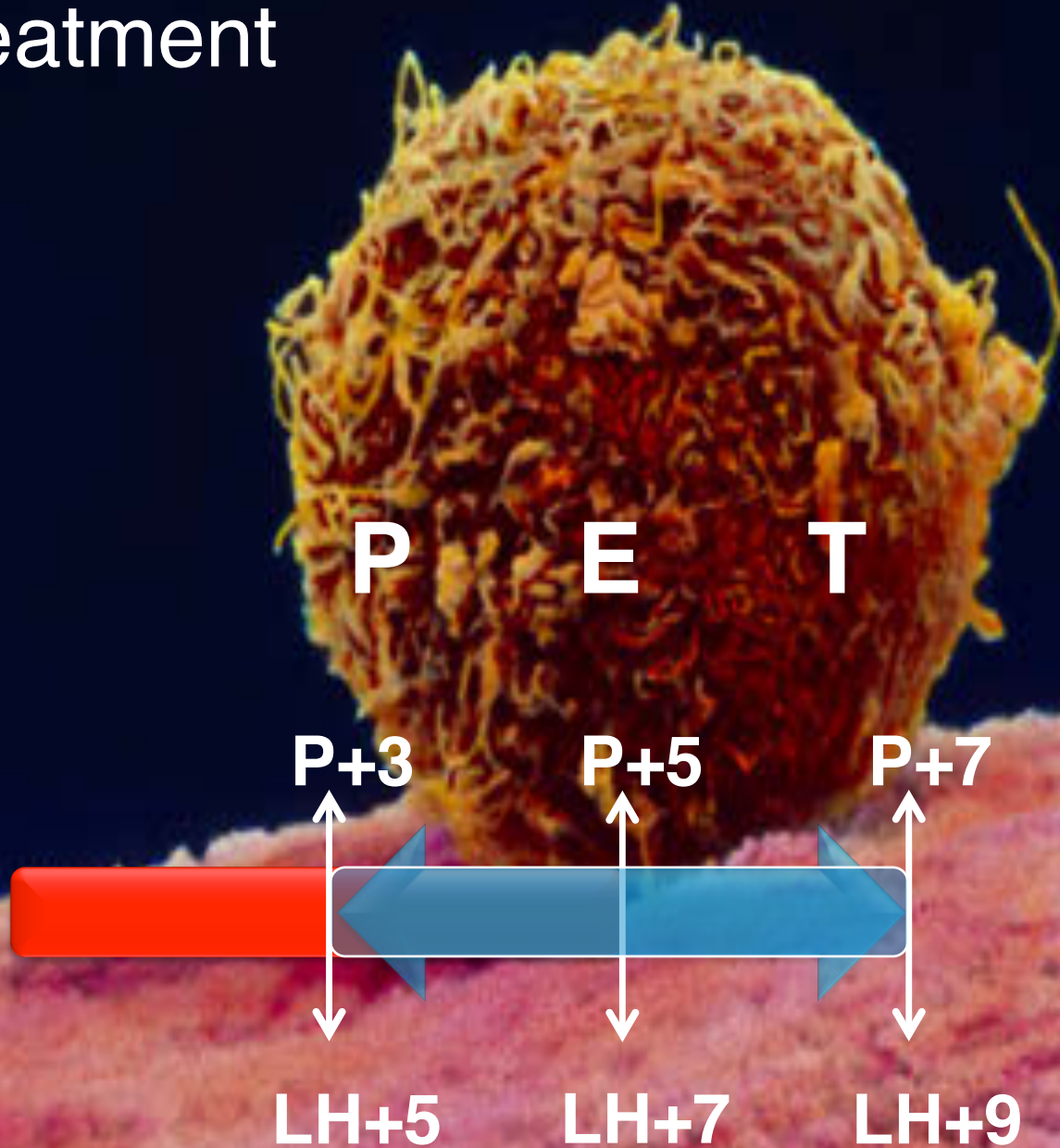


*Hum Reprod.* 2014 Sep;29(9):1957-67. doi: 10.1093/humrep/deu171. Epub 2014 Aug 8.

**Deciphering the proteomic signature of human endometrial receptivity.**

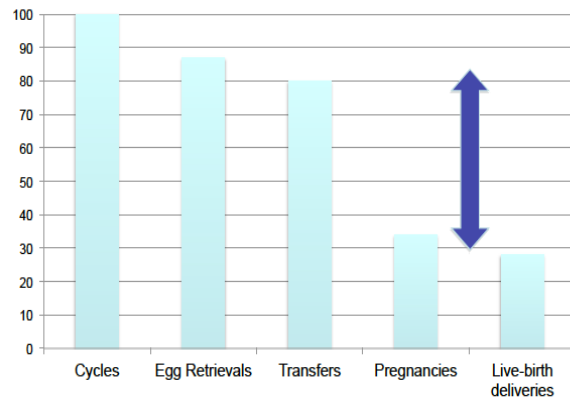
Garrido-Gómez T<sup>1</sup>, Quifonero A<sup>2</sup>, Antúñez O<sup>3</sup>, Díaz-Gimeno P<sup>2</sup>, Bellver J<sup>2</sup>, Simón C<sup>4</sup>, Domínguez F<sup>5</sup>.

# Personalized embryo transfer (pET) as a treatment



# Human fertility efficiency

## Implantation is rate-limiting step in in vitro fertilisation (IVF)

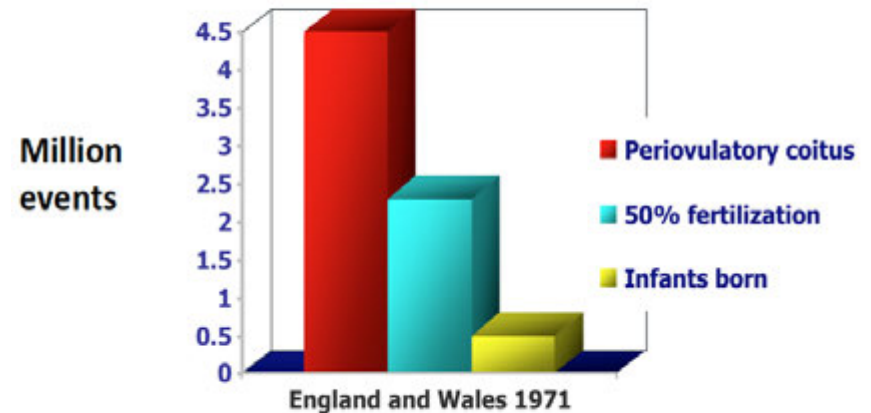


CDC (Center for disease control and prevention) [www.cdc.gov](http://www.cdc.gov)

## 'Where have all the conceptions gone?'

Estimated fetal loss

Roberts and Lowe, Lancet 1975;i:498



Percentage loss

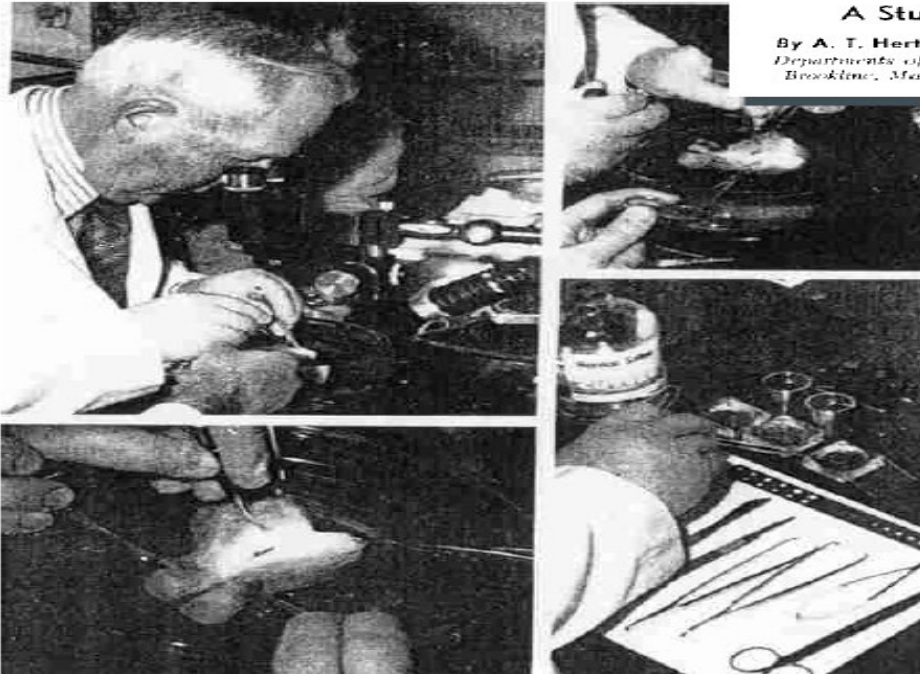
78%

## Opening the box

THIRTY-FOUR FERTILIZED HUMAN OVA, GOOD, BAD AND  
INDIFFERENT, RECOVERED FROM 210 WOMEN  
OF KNOWN FERTILITY

A Study of Biologic Wastage in Early Human Pregnancy

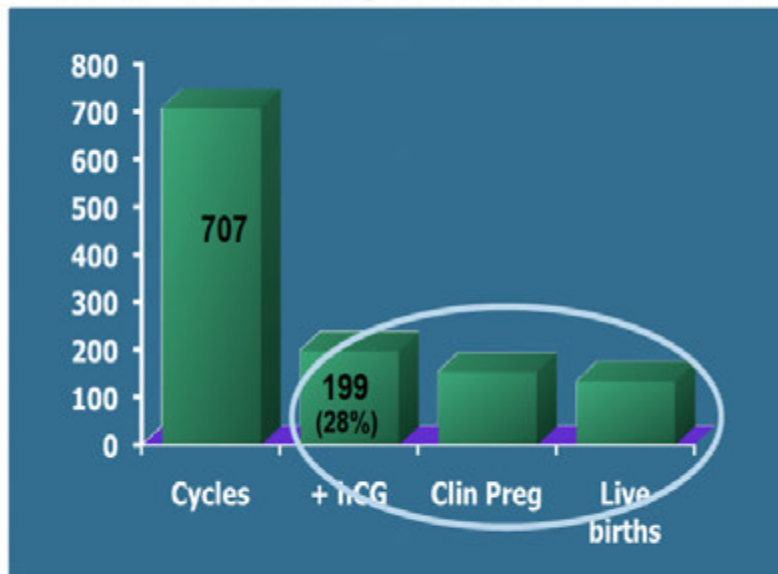
By A. T. Hertig, M.D., John Rock, M.D., E. C. Adams, M.D., and M. C. Menkin, M.D.  
Departments of Pathology and Gynecology, Harvard Medical School, Peter Hospital for Women,  
Brookline, Massachusetts, and Department of Embryology, Carnegie Institution of Washington



*Hertig et al, Pediatrics 1959; 23:202*

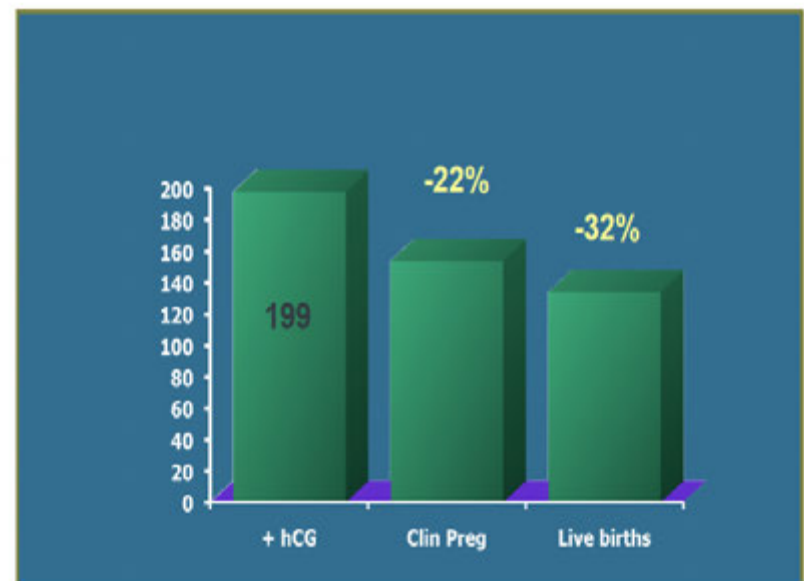
- 107 'optimal fertility' uteri
- 34 contained a conceptus
- 10 abnormal
- Others: failure of fertilization?  
: disintegration?  
: failure to detect?

## Incidence of implantation failures



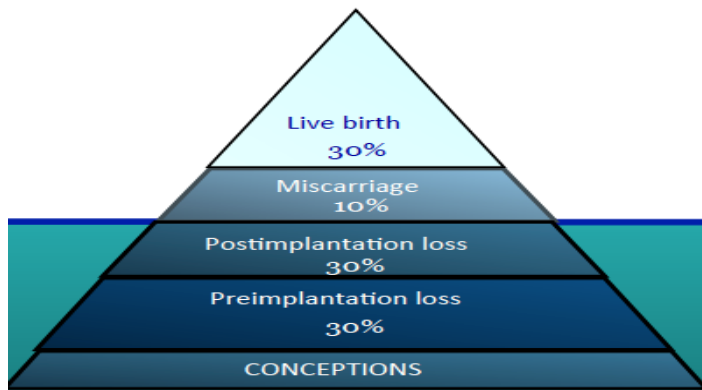
Wilcox et al. *N Engl J Med* 1988;319:189-94

## A closer look...

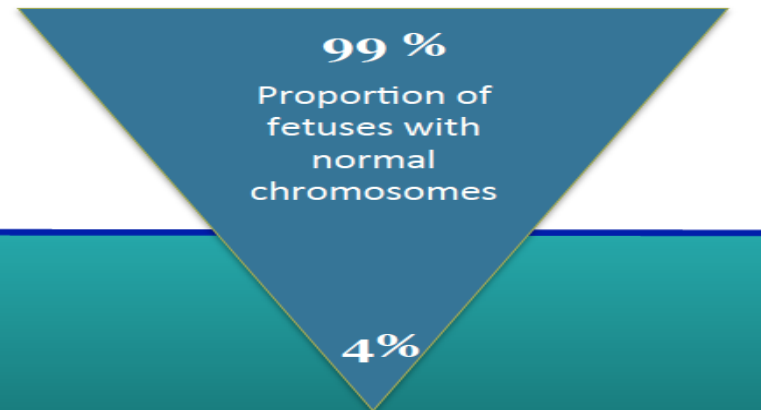


Wilcox et al. *N Engl J Med* 1988;319:189-94

# The pregnancy loss iceberg



(Macklon et al, Hum Reprod Update, 2002)

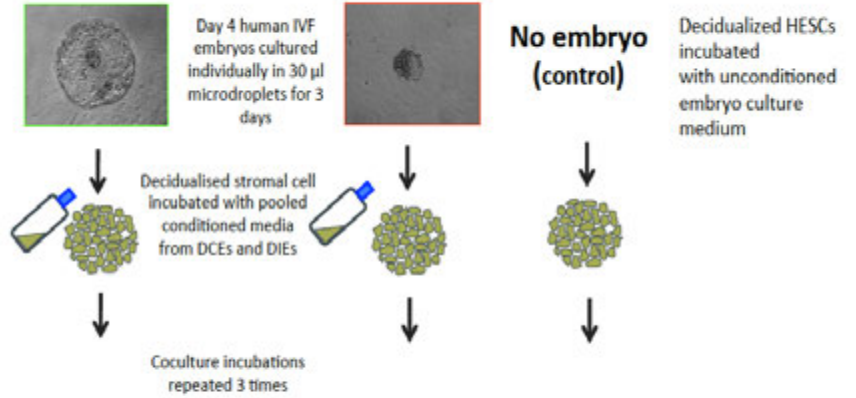


(Vanneste et al, Nat Med, 2009)

- What about IVF?

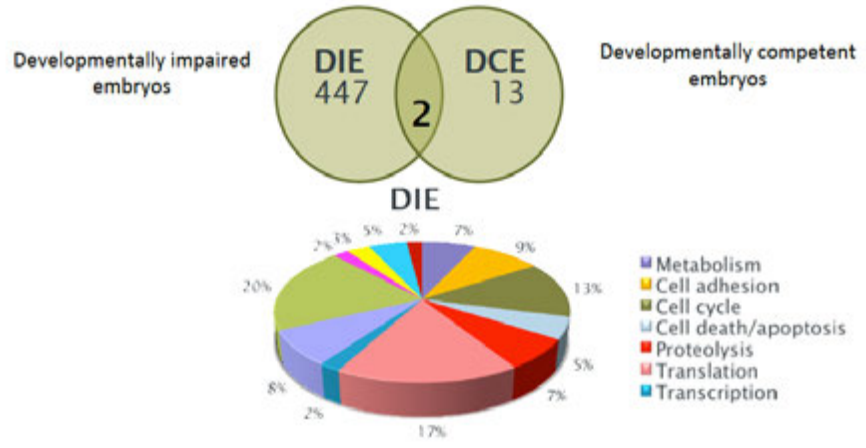
# Endometrium and embryo communication

## Do embryos trigger a selective transcriptional response in decidualizing endometrial stromal cells?



DCE, developmentally competent embryo; DIE, developmentally incompetent embryo; HESC, human embryonic stem cell

## DIEs trigger far greater transcriptional response than DCEs

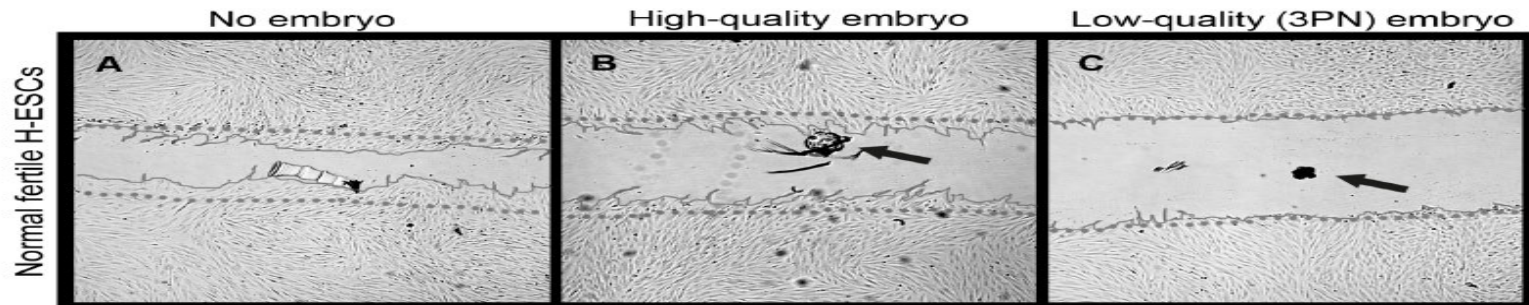


PCR, polymerase chain reaction

Brosens et al 2014 SCIENCE Report

# Endometrium and embryo communication

## Stromal cells don't migrate to low quality embryos



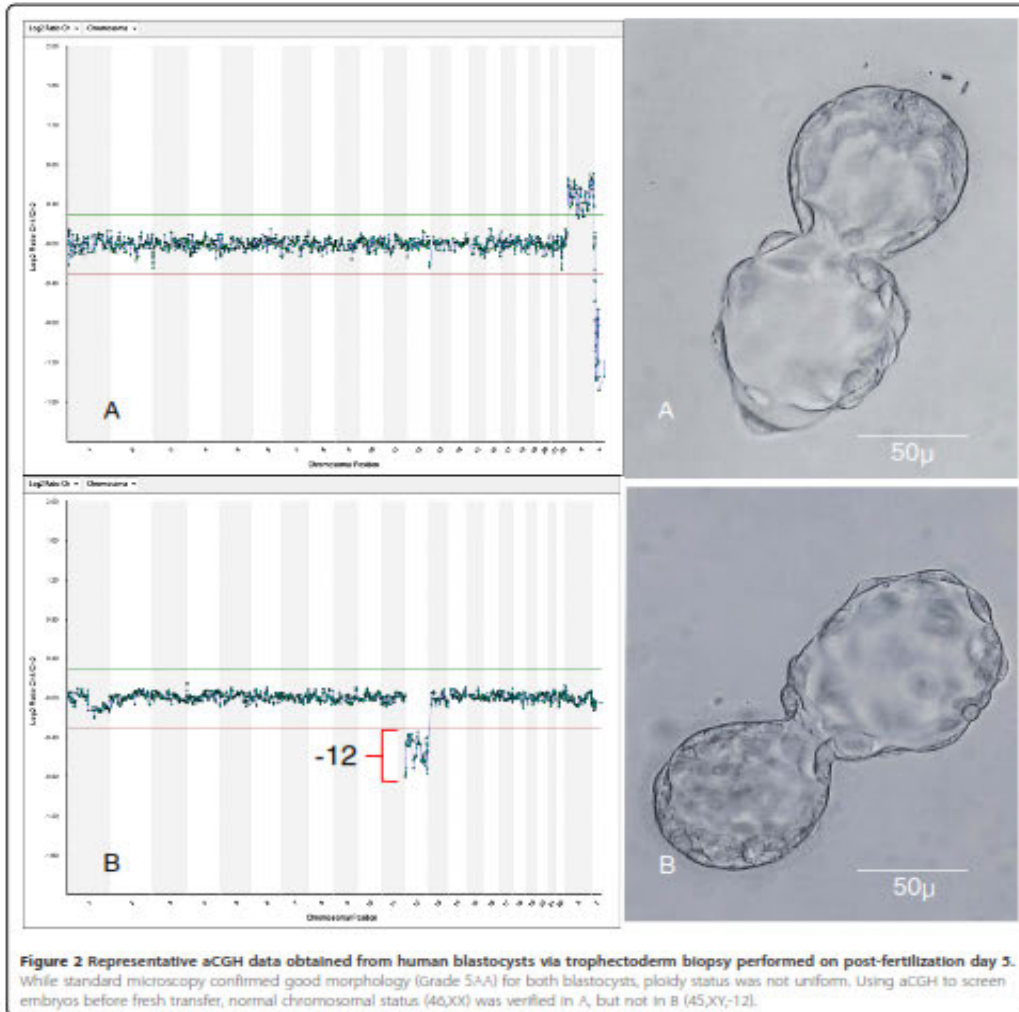
*Weimar C et al. PLoS ONE: July 2012; e41424*

# Clinical outcome by aCGH according to maternal age (Dec 2012)

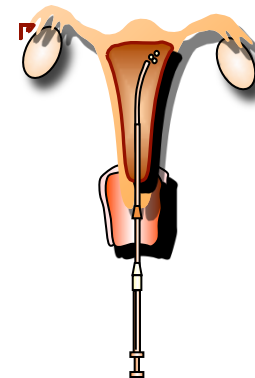
	<u>≤35 yrs</u>	<u>36-37 yrs</u>	<u>38-39 yrs</u>	<u>40-41 yrs</u>	<u>42-43 yrs</u>	<u>44-46 yrs</u>
No. of cycles	163	137	225	383	213	76
% of embryo transfers	85.3	78.1	68.4	49.9	31.4	14.5
Mean embryos tr. (SD)	1.6 (0.5)	1.6 (0.6)	1.5 (0.5)	1.4 (0.5)	1.3 (0.4)	1.2 (0.5)
% of abnormal embryos	60.4	66.5	75.3	81.8	88.9	93.9
PR/ transfer	59.0	57.0	50.6	44.5	58.2	54.5
% of miscarriages	7.3	3.2	11.5	6.2	7.7	16.7
Implantation rate	50.0	47.9	47.7	40.3	57.5	53.8

**Ongoing pregnancies: two of them in 44 yrs old women, two in 45 yrs and one in 46 yrs**

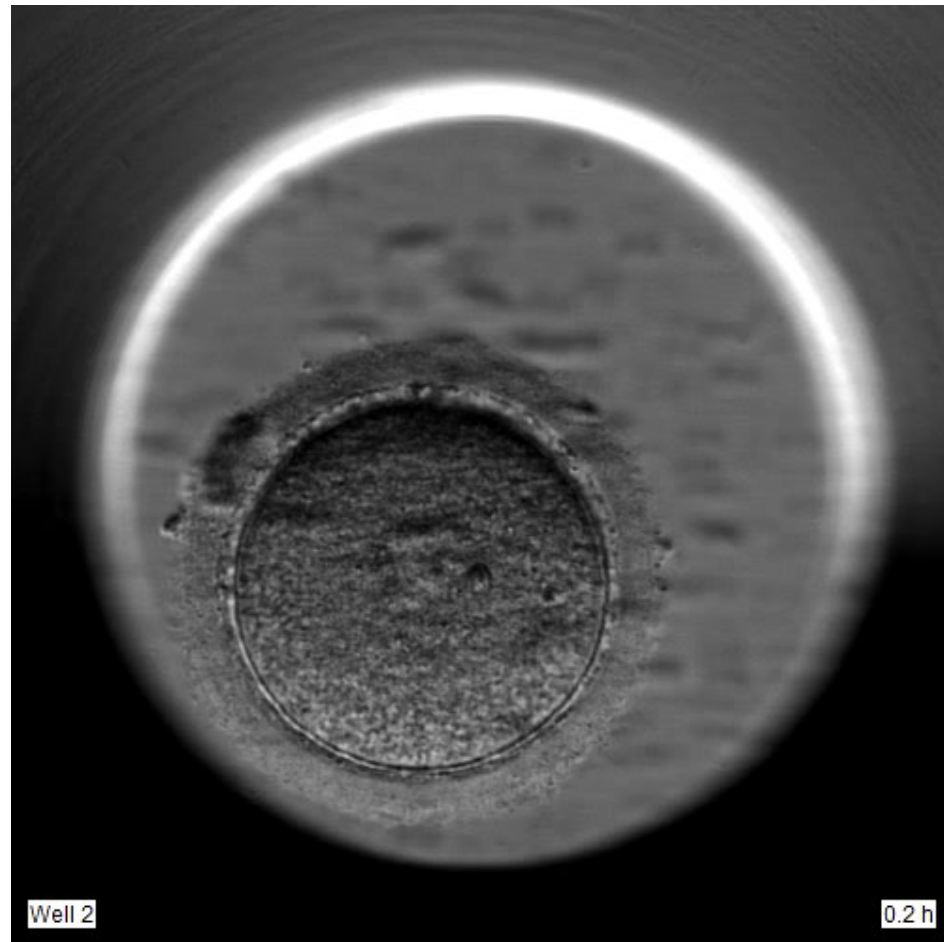
# χ Preimplantation Genetic Screening



- Only morphological criteria fails selecting the best embryo.
- Transference of “good morphology” embryos not always means “good quality” embryos.



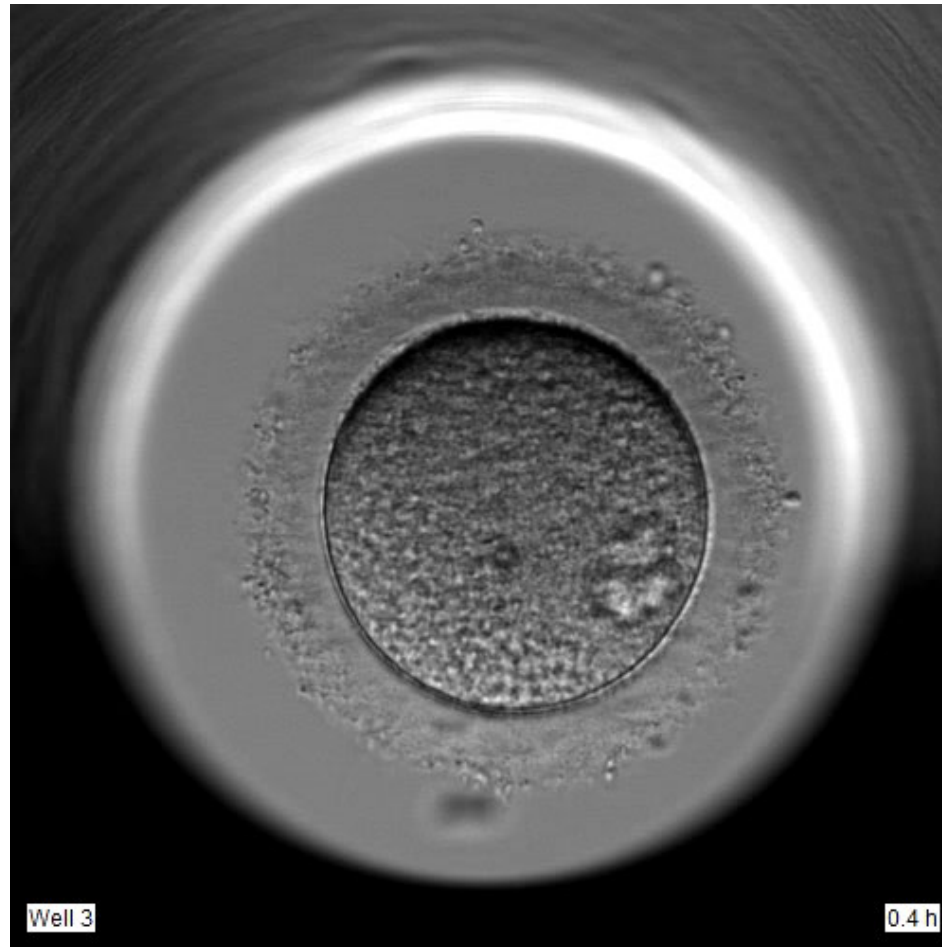
# Good embryo development



# PGS result:

- Abnormal Female
  - +14, +17, +18, +21

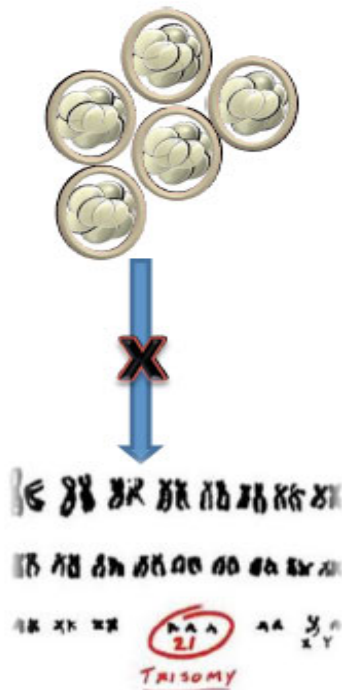
# Limited embryo development



- 46 XX,
  - Normal embryo!

## Aims of Preimplantation Genetic Screening

Expected advantages in IVF



Increase implantation rate



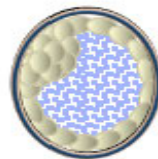
Decrease in abortion rate



Less abnormal pregnancies



Single ET



Decrease time to pregnancy



Increase the cost-effectiveness



# Preimplantation Genetic Screening

## Assesment of embryo viability:

- ✓ Improve implantation
- ✓ Decrease miscarriage rates
- ✓ Decrease risk of abnormal offspring



## Indications

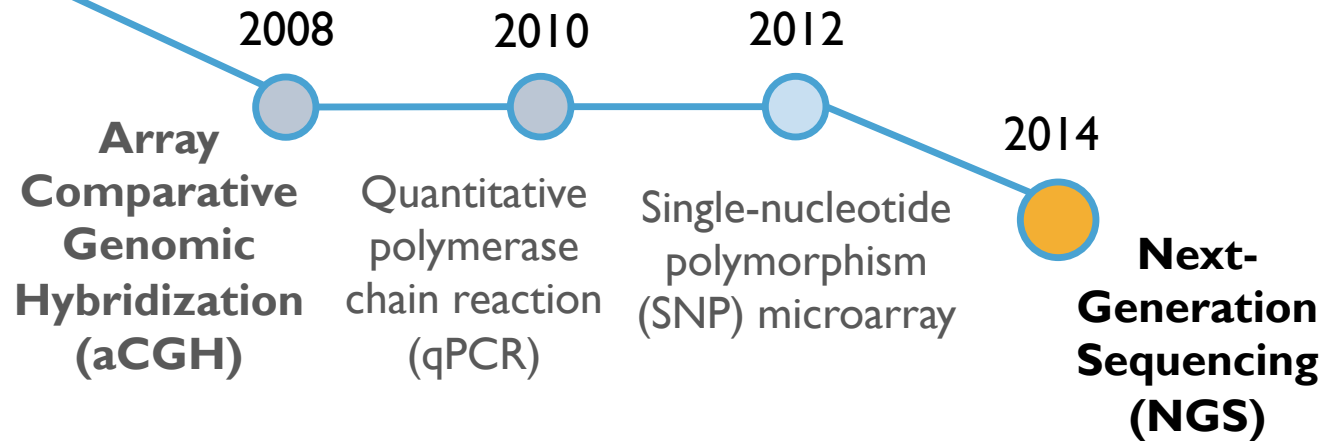
- Advanced maternal age ( $\geq 38$  years)
- Prior pregnancy /child chromosomally abnormal
- Multiple implantation failures ( $\geq 2$  failed IVF)
- Recurrent miscarriage ( $\geq 2$  miscarriages)
- Severe male factor (low sperm count)

# PGS: How ?

1991-1995

PCR

FISH



Analysis of a identified single gene disease

2-12 chromosomes

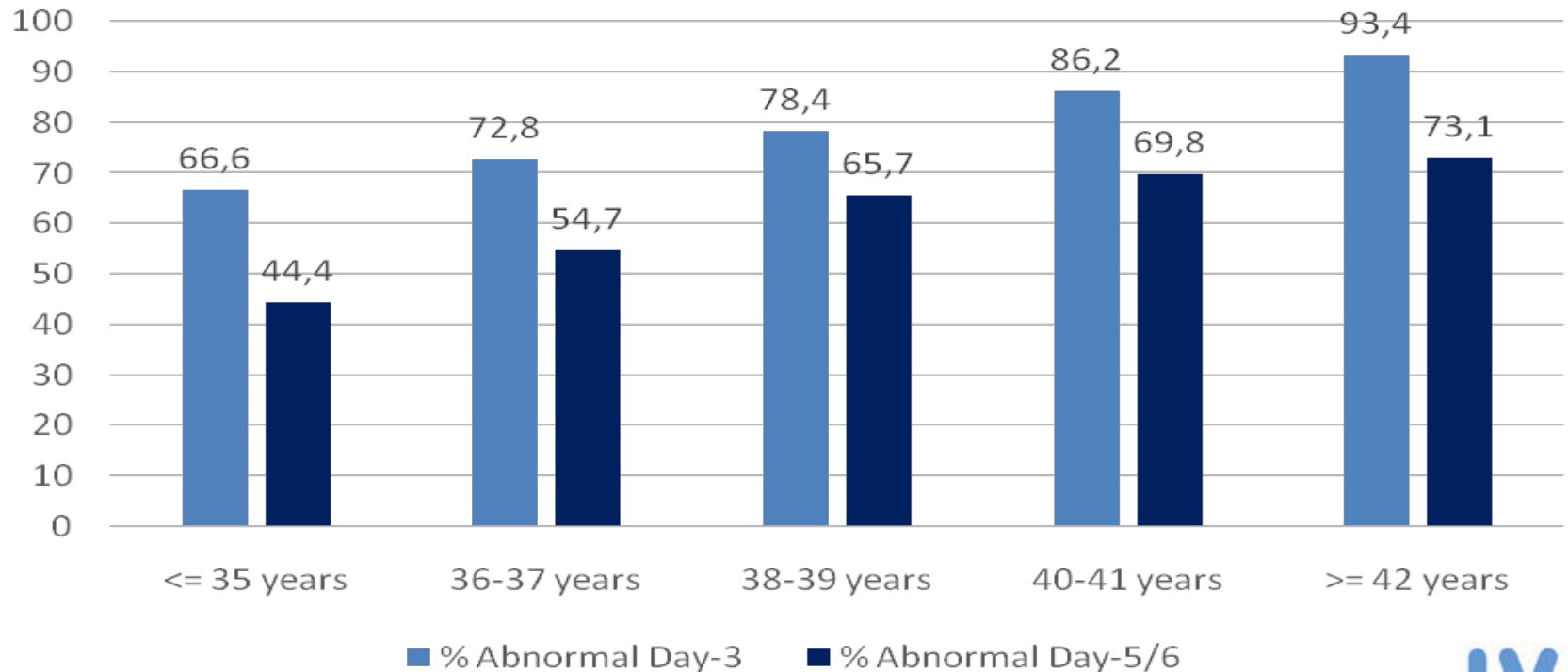
24 chromosomes

24 chromosomes  
+  
Mito DNA; mutations

# PGS: When ?

## 2011-2014 percentage of abnormal embryos (5115 cycles)

4918 cycles with day-3 biopsies  
197 cycles with day-5/6 biopsies



# PGS: To whom ?

## RCT, blastocyst biopsy with single embryo transfer (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)**

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

**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)**

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

Notes: All data reported as n (%). SET = single embryo transfer; aCGH = array comparative genomic hybridization; GA = gestational age <sup>a</sup> by Chi-squared test <sup>b</sup> by Fisher's exact test

# PGS: To whom ?

## Single embryo transfer (SET)

**Women 21-42 years**

**First IVF attempt**

**No previous miscarriages**

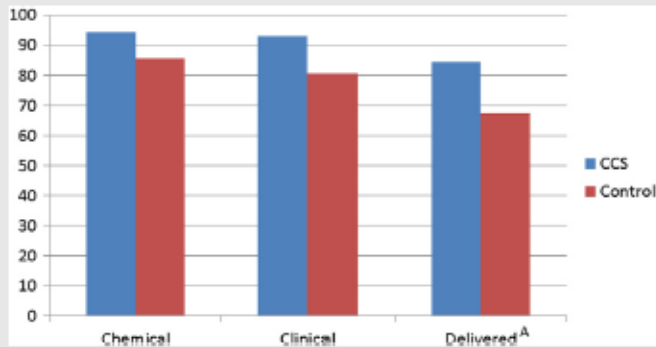
*(Scott et al., FS 2013)*

**Women <43 years**

**AMH  $\geq 1.2$  ng/ml**

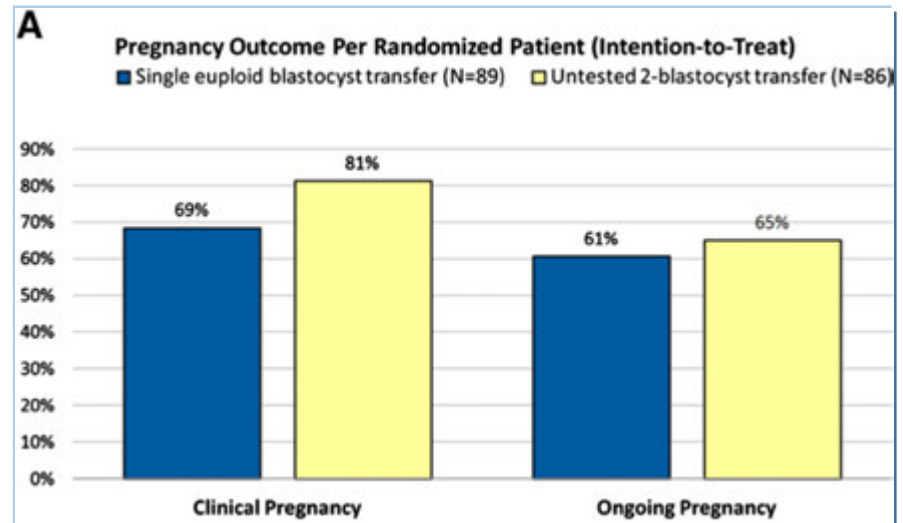
**FSH <12 IU/L**

*(Forman et al., FS 2013)*



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.

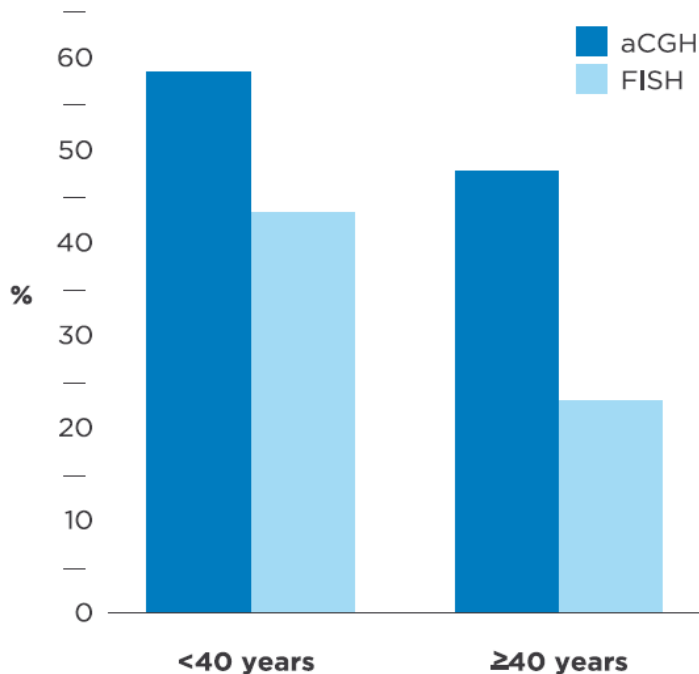
*Scott. RCT showing CCS improves delivery rates. Fertil Steril 2013.*



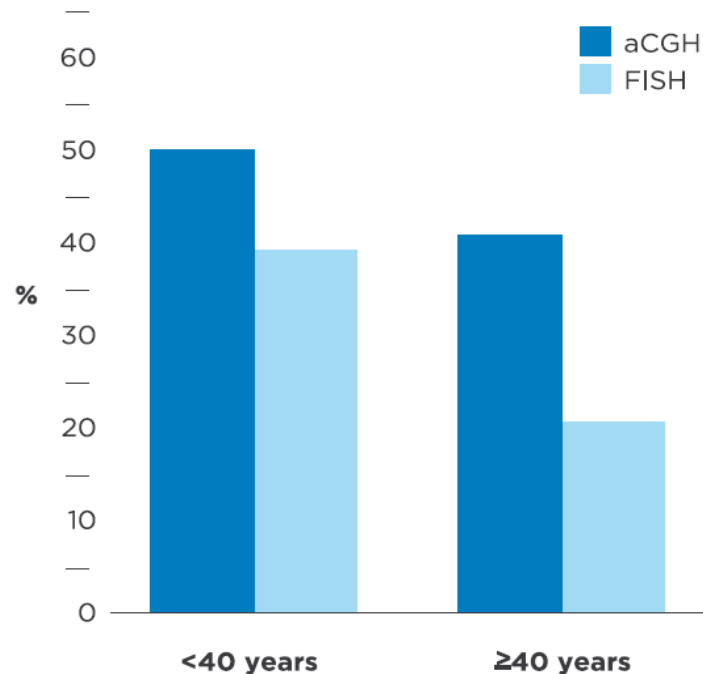
# PGS: How ?

PGS with aCGH achieves better reproductive outcome than FISH (24-chr. vs 9 chr. Panel)

### Pregnancy Rate/transfer



### Implantation Rate



N° cycles-AMA: 880, IF:187, RM:204, MF:116  
Pregnancy of 12 weeks gestation or more

# Preimplantation Genetic Screening

## PGS

### PGS: How ?

- 24-chromosome analysis by Array-CGH → NGS mitDNA

### PGS: When ?

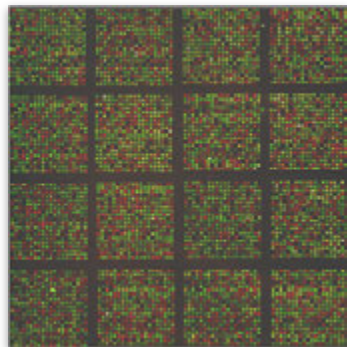
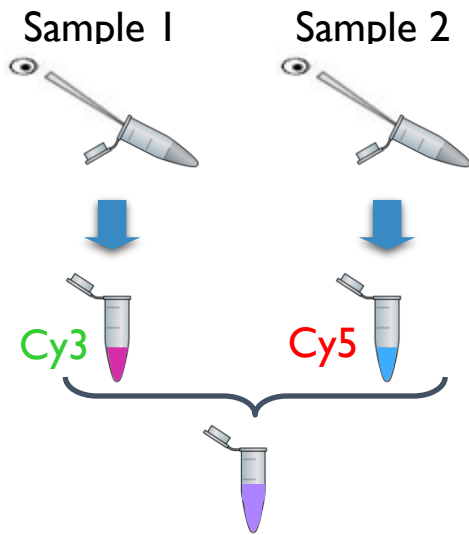
- Day-3 or trophectoderm biopsies offer similar results in similar good prognosis population.
- For AMA and low ovarian response day-3 could be more informative.

### To whom ?

- High aneuploidy risk: AMA, RM, RIF, MF, PTP
- Young good prognosis patients
- Single embryo transfer
- PGD +PGS

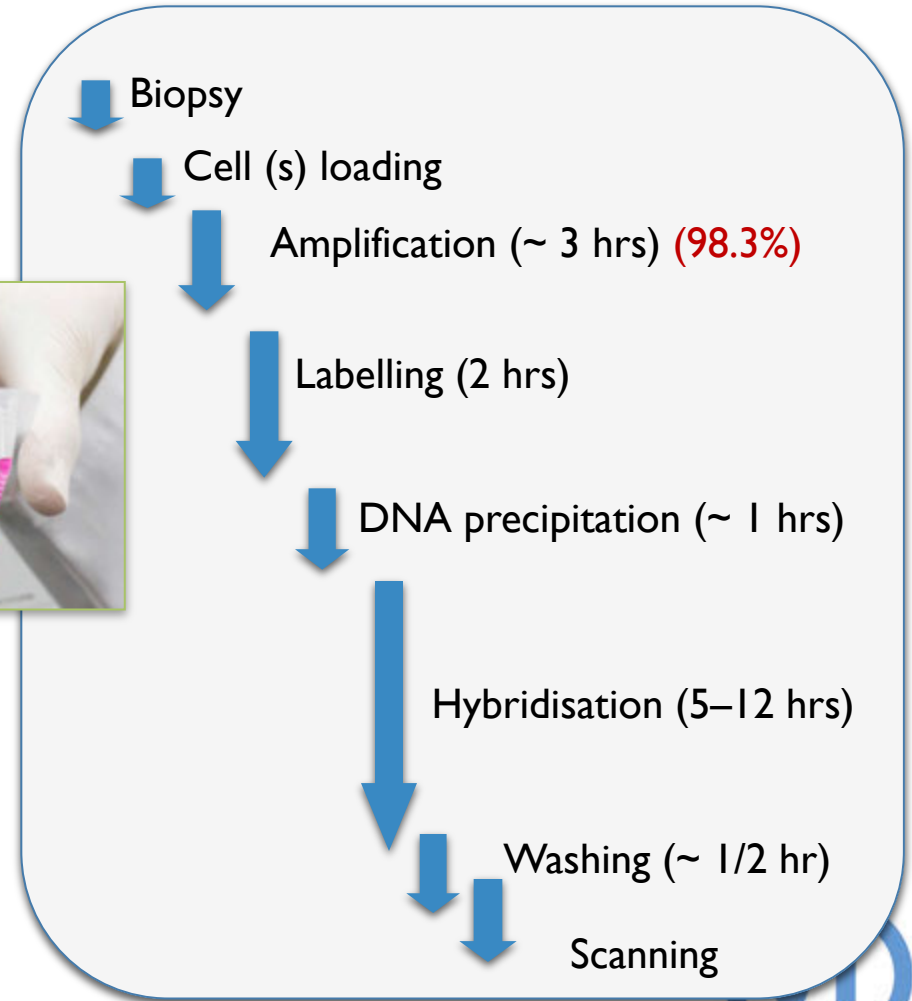
# PGS: How ?

## Array CGH Work-flow



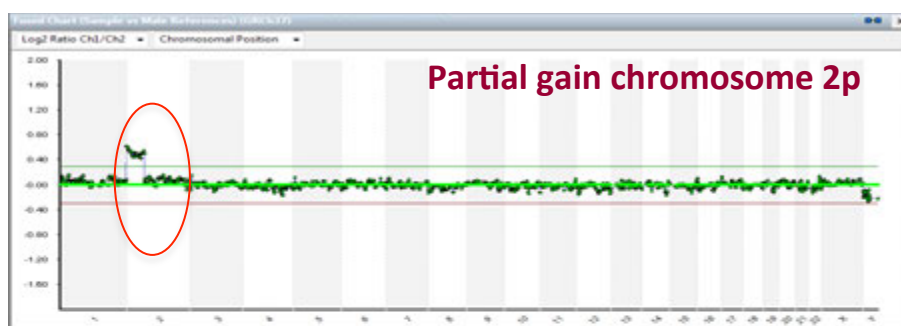
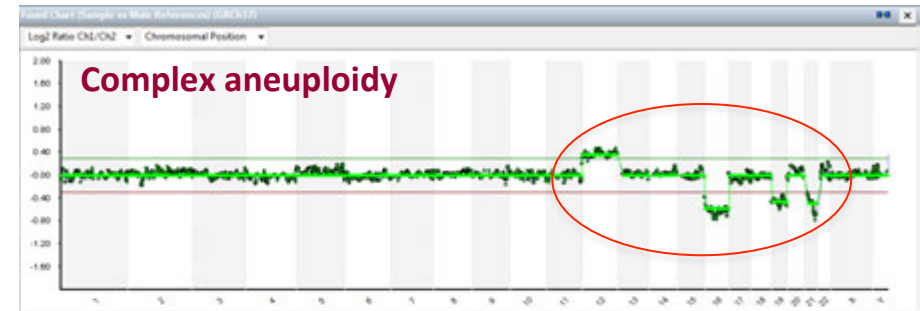
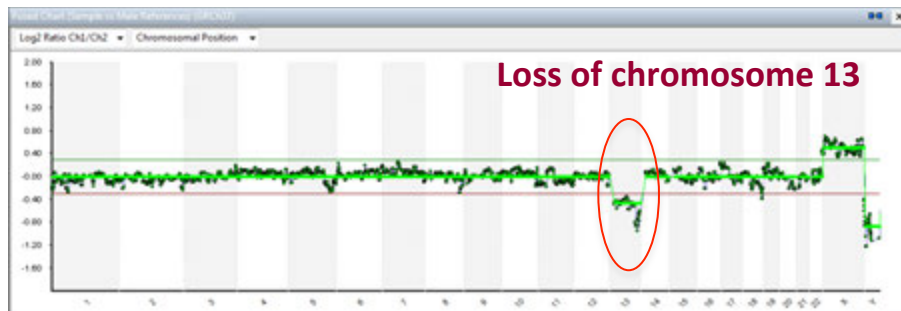
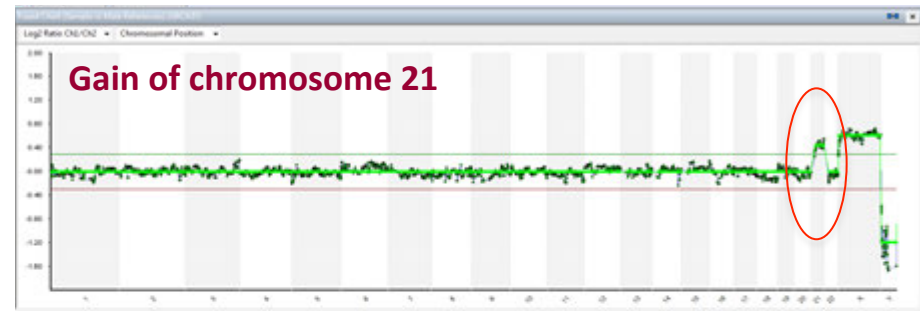
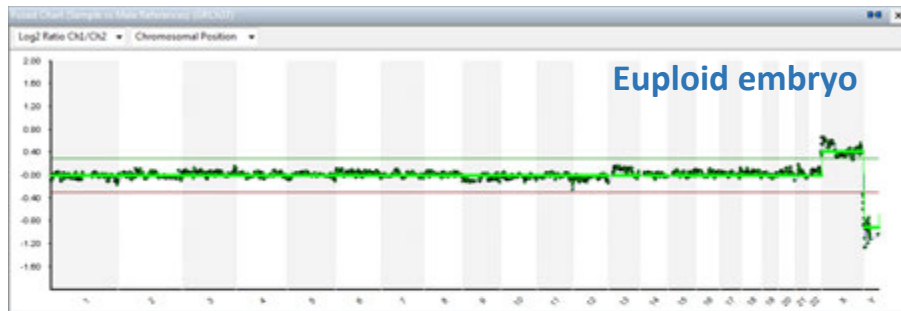
24sure  
BlueGnome  
2684 clones  
1Mb coverage

BlueFuse Multi  
software



# PGS: How ?

## Array CGH profiles



# PGS: How ?

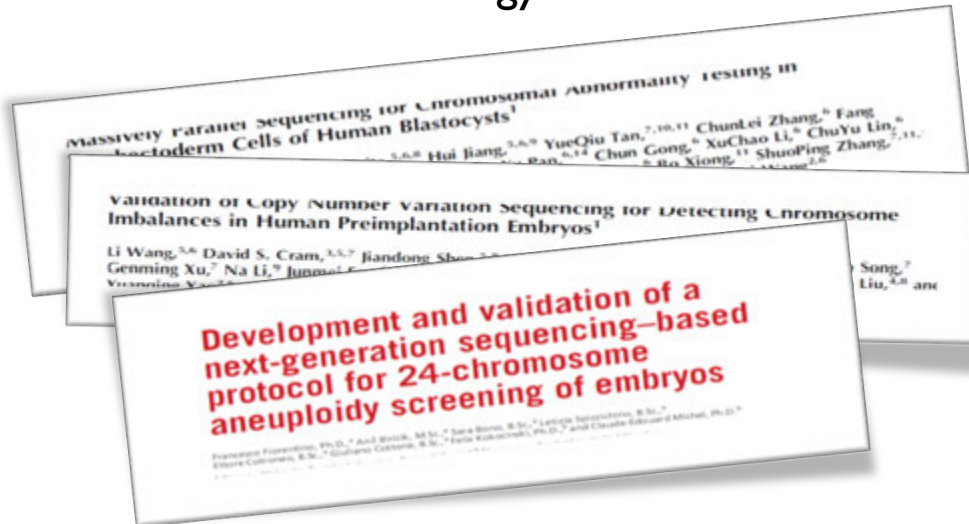
## NGS

### Where are we?

- ✓ For blastomere and trophectoderm samples
- ✓ For whole-chr and segmental aneuploidies
- ✓ Validated technology

### Where we go?

- ✓ Mitochondrial DNA analysis
- ✓ Embryo viability markers
- ✓ Single gene disorders

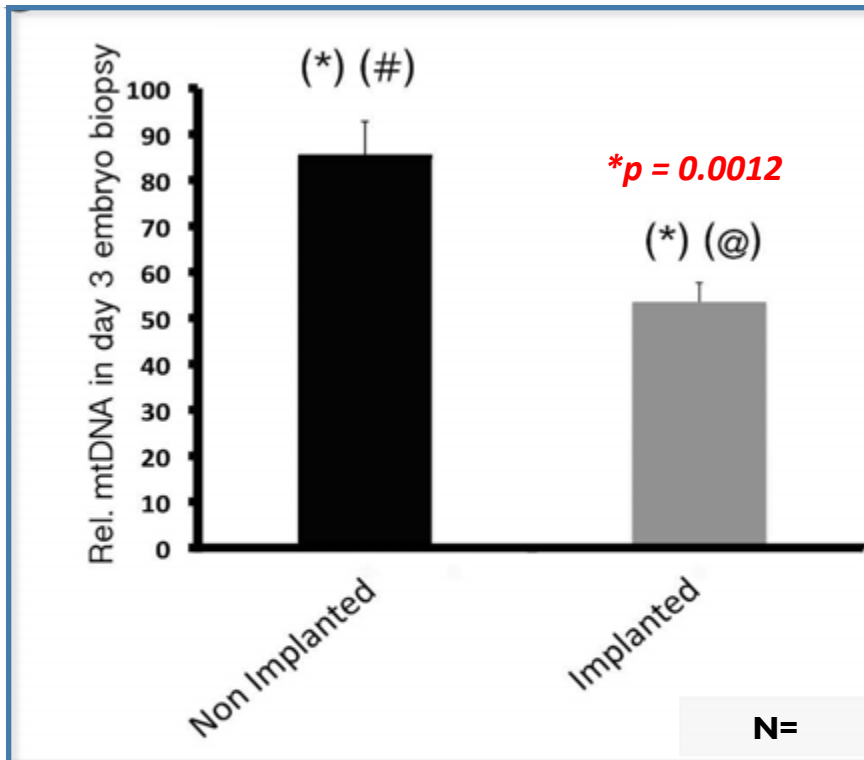


Personalized  
Reproductive  
Medicine

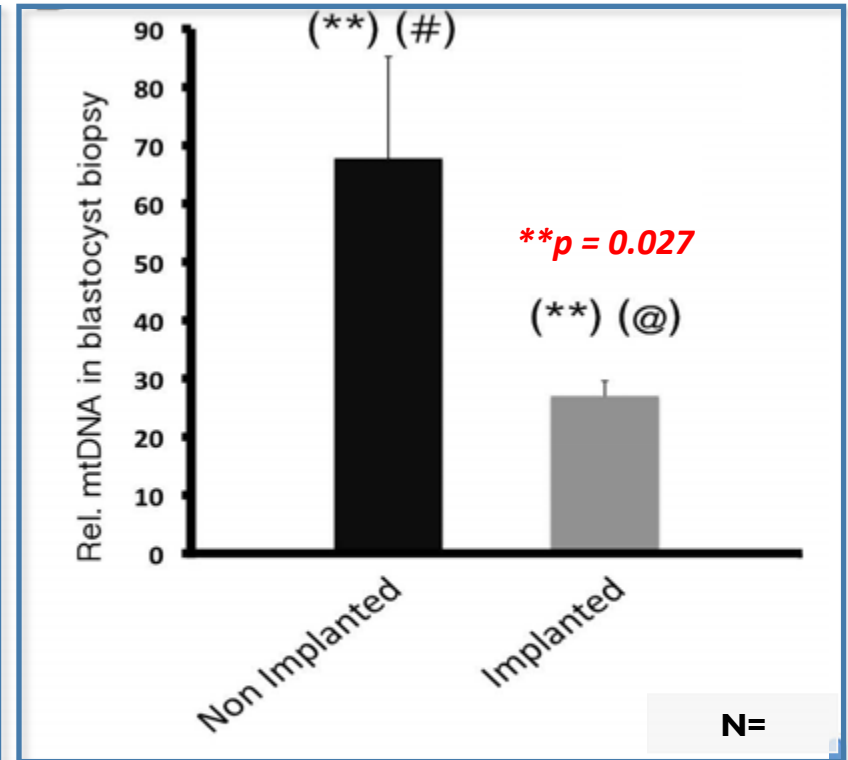
# PGS: How ?

## Mitochondrial DNA cuantification

### Day-3 biopsies



### Trophectoderm



## Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates

E.J. Forman<sup>1,2,\*</sup>, X. Tao<sup>1</sup>, K.M. Ferry<sup>1</sup>, D. Taylor<sup>1,2,3</sup>, N.R. Treff<sup>1,2,3</sup>,  
and R.T. Scott Jr<sup>1,2</sup>

**Table III** Pregnancy outcomes by treatment group.

	Control SET	CCS-SET
Chemical pregnancy rate	108/182 (59.3%)	96/140 (68.6%)
Ongoing pregnancy rate	76/182 (41.8%)	77/140 (55.0%)
Clinical miscarriage rate	25/101 (24.8%)	9/86 (10.5%)
Monozygotic twin rate	0/76 (0%)	1/77 (1.3%)
Gestational age at delivery (weeks $\pm$ SD)	38.9 $\pm$ 1.4	38.6 $\pm$ 2.0
Birthweight (grams $\pm$ SD)	3286 $\pm$ 522	3281 $\pm$ 525

\*Chi-square.

†Fisher's exact test.

‡t-test.

ADVERTISEMENT

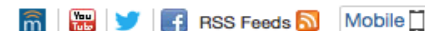
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and Sterility.**

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## Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles

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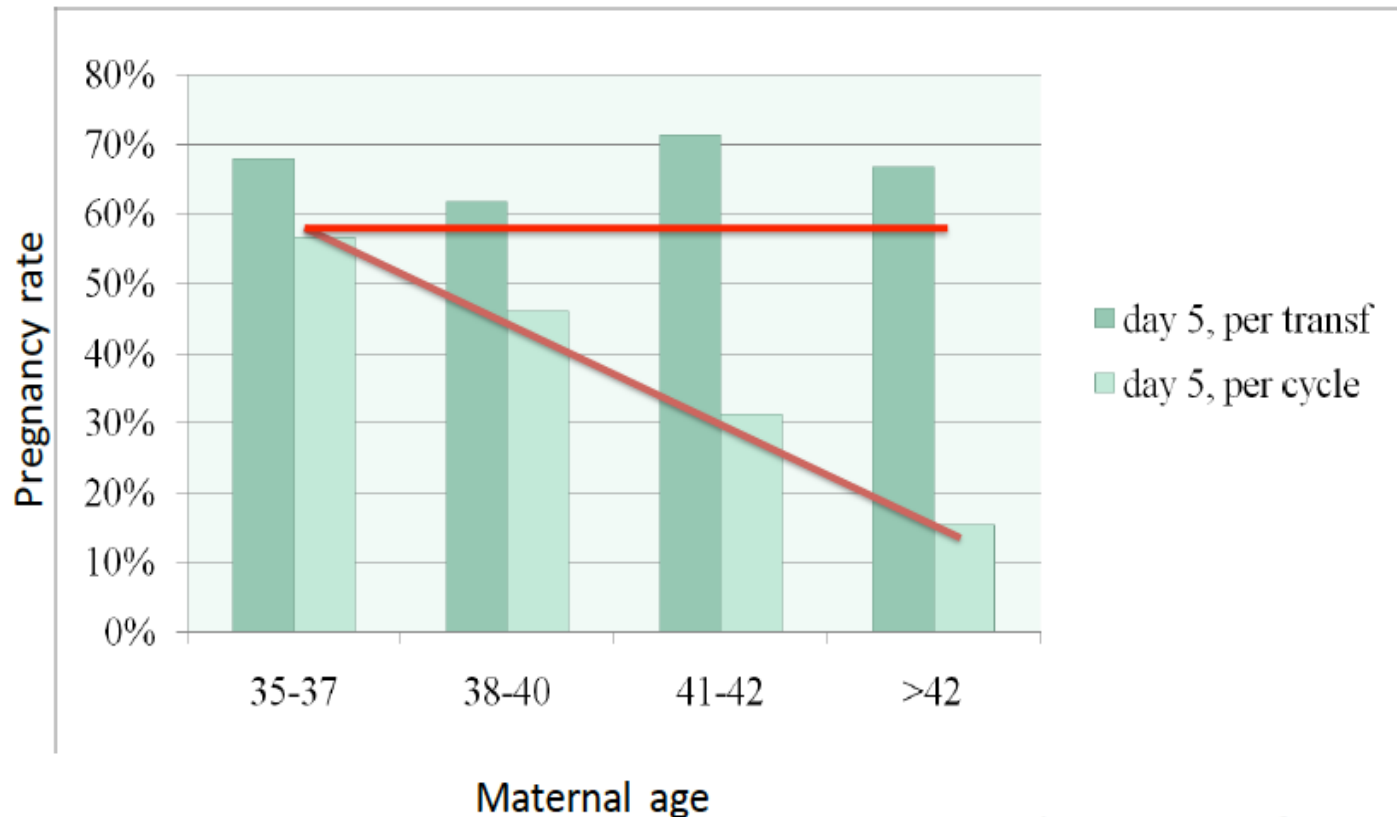
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### Article Tools

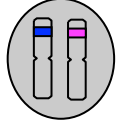
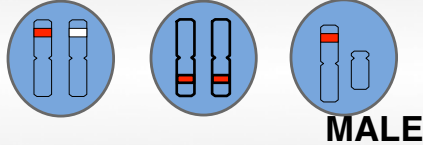

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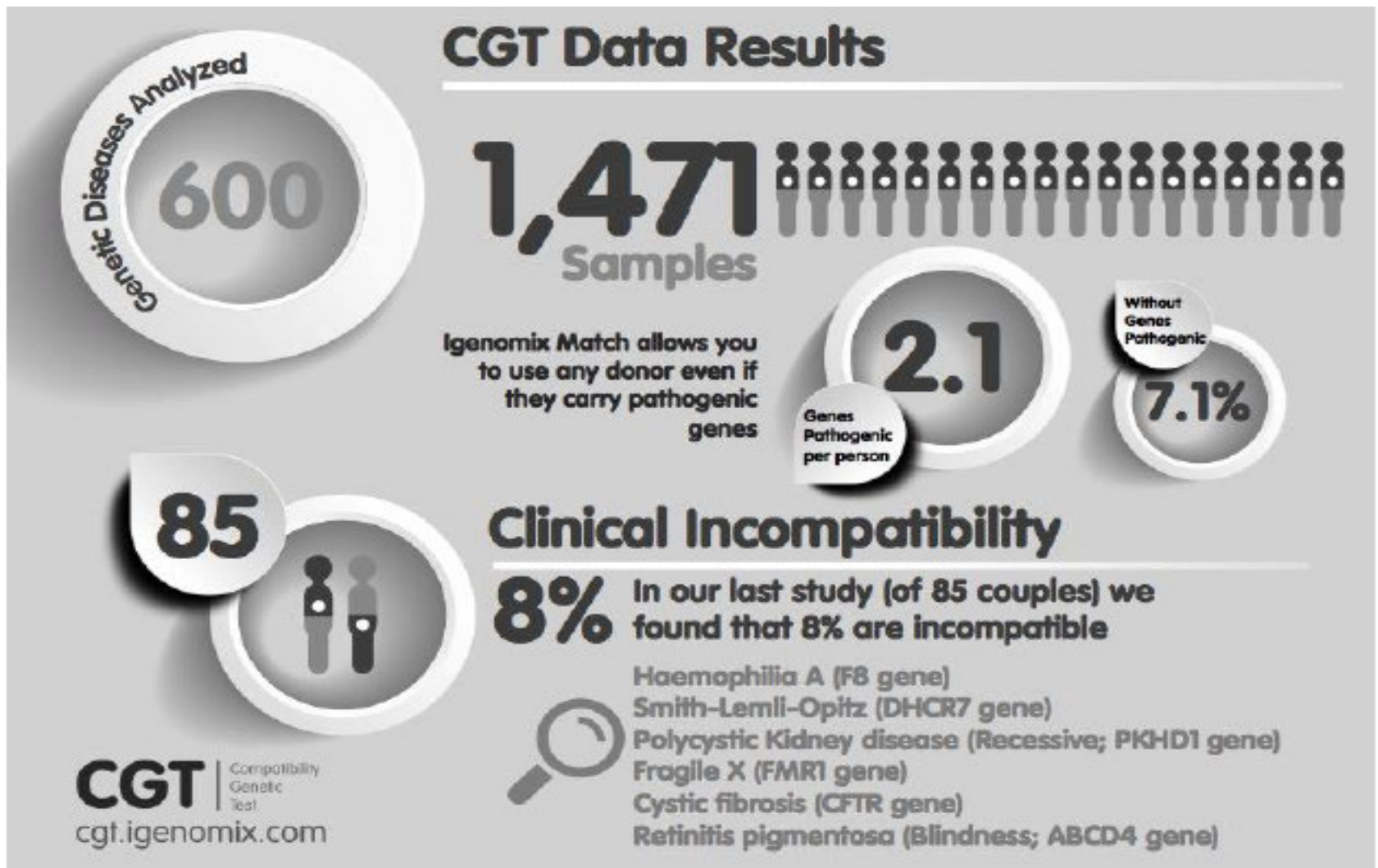
The PR per transfer was higher in the PGS group (84.4% vs. 41.5%).

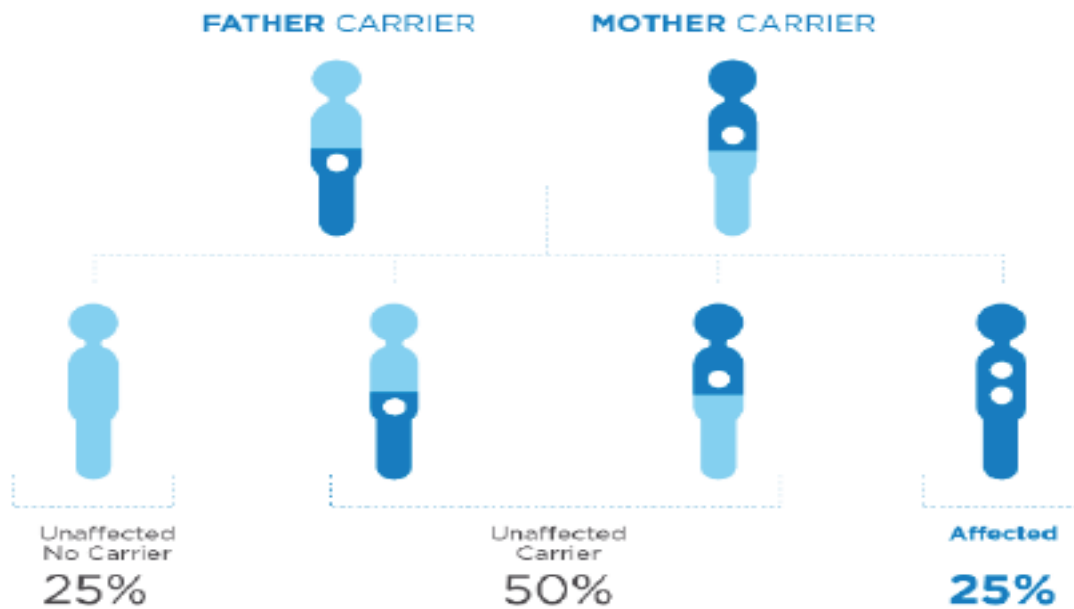
# Blastocyst biopsy and aCGH appear to limit the effect of age when performed on Day 5



# Genetic Conditions- CLASSIFICATION

- Multifactorial  + Environment factors
- **Monogenic** 
- Chromosomal 
- Mitochondrial
- Somatic mutations (cancer)\*





# Single Gene Disorders

**18%**

**Pediatric Hospitalizations**

**Infant mortality  
(developed  
countries)**

**20%**

# Goal of the Carrier Genetic Test (CGT)

- ✓ To promote health and prevent inherited single gene diseases
  - *Autosomal Recessive*
  - *X-linked*
- ✓ To offer family-planning reproductive choices to patients
  - *Natural conception and Prenatal Diagnosis*
  - *ART and Preimplantation Genetic Diagnosis*
  - *ART and Gamete Donation*

# How often mutations are found?

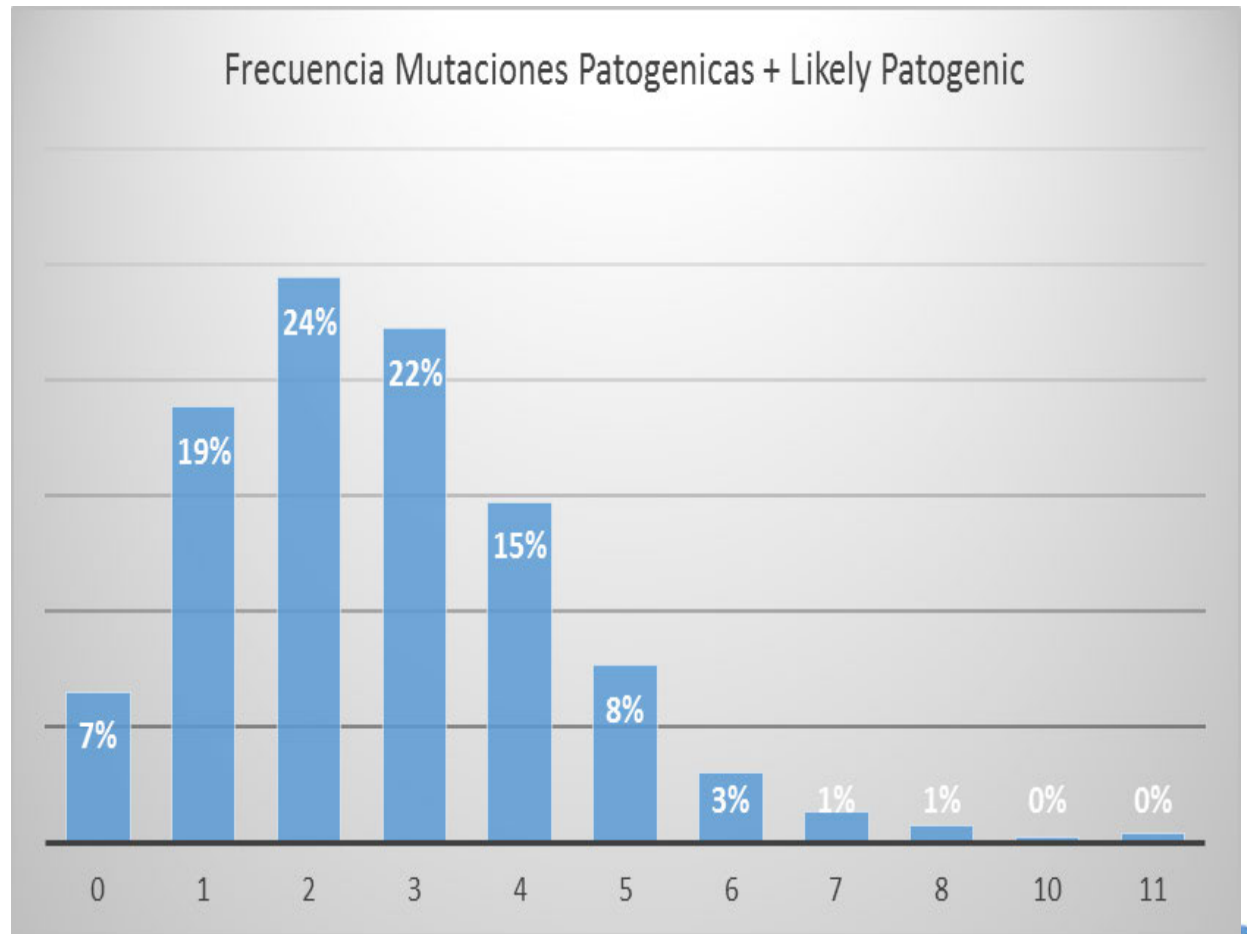
1.584



2.1

Pathogenic  
or likely  
pathog

**Positive: 1471 (92,9%)**  
**Negative: 113 (7.1%)**



# How Many Couples have shared risk?

- The majority of genetic mutation carriers don't have a family history of these disorders

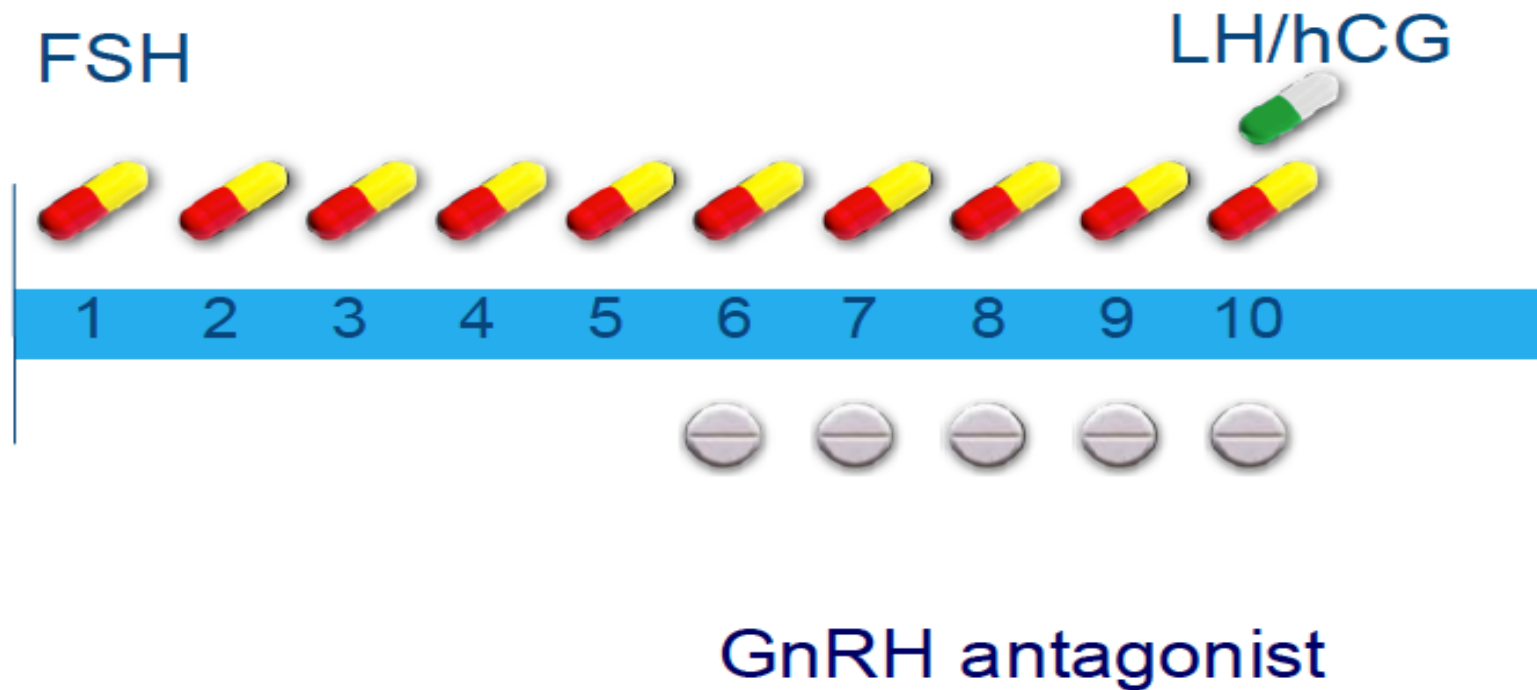
85 couples  
170 CGTs



8%

Haemophilia A (F8 gene): 1  
Smith-Lemli-Opitz (DHCR7 gene): 1  
Polycystic Kidney disease (Recessive; PKHD1 gene): 2  
Fragile X (FMR1 gene): 1  
Cystic fibrosis (CFTR gene): 1  
Retinitis pigmentosa (Blindness; ABCD4 gene): 1

# Still far away Future:



# Future of IVF: Conclusions

- Endometrial assessment: ERA
- Chromosomal screening: PGS
  - Avoiding multiples: SET
- CGT: PGD
- Oral stimulation: Still far away