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


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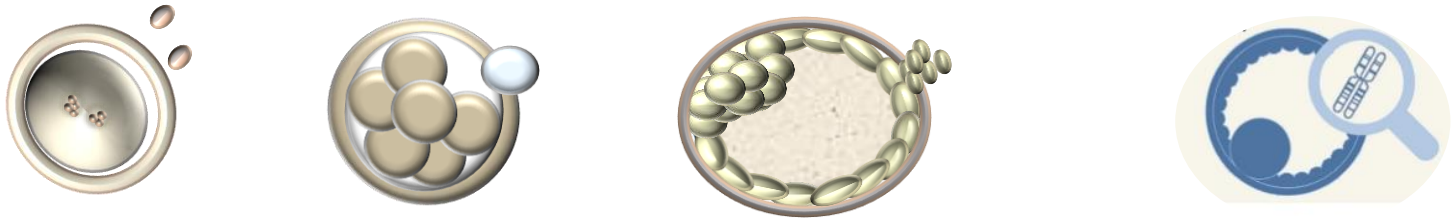
# Disclosure

**Carlos Simon**

Scientific Director of Igenomix SL

# 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)

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 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**.

# 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

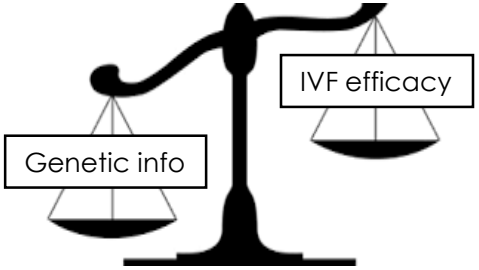
## Genetic testing vs IVF efficacy: what is the origin of this debate?

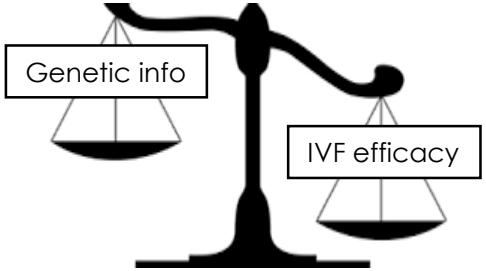
PGT for monogenic/single gene defects PGT-M

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?

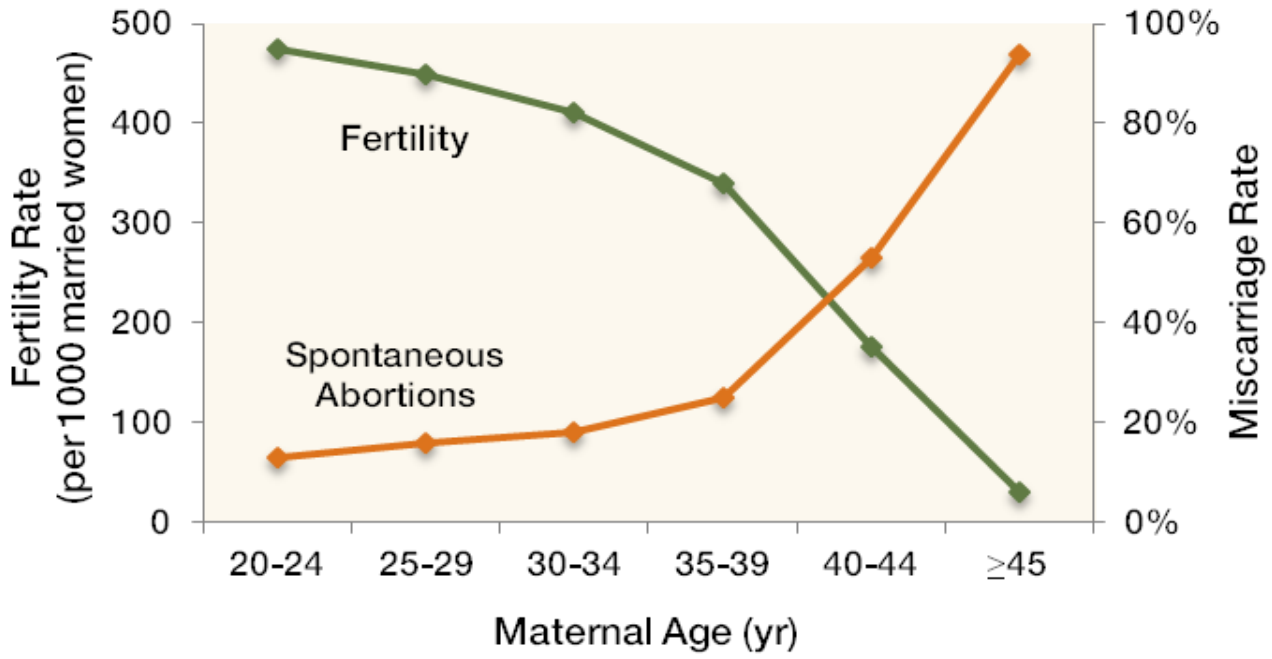
RCTs are needed because the benefit is not yet considered self evident

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

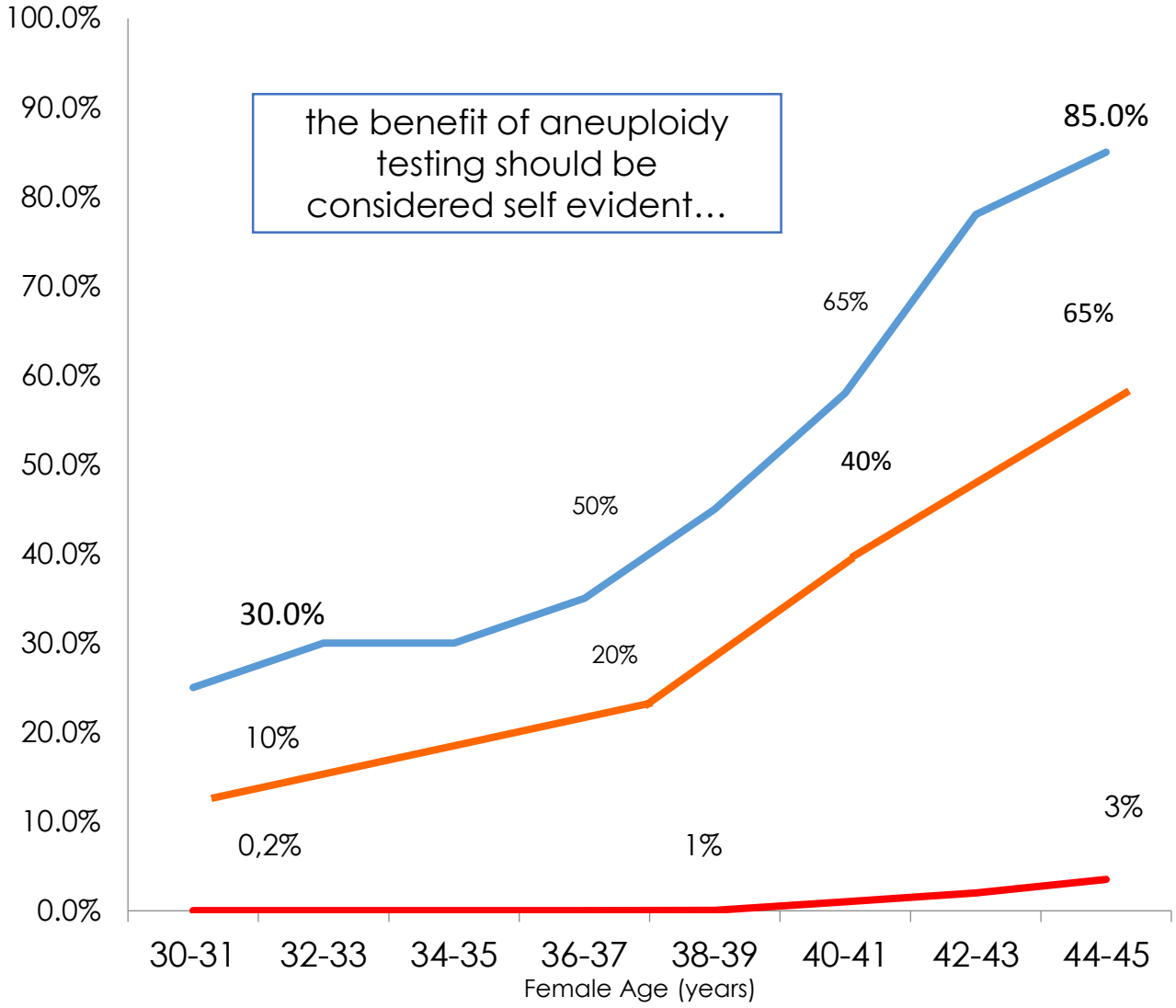

*Thornhill, ESHRE best practice guidelines, Hum Reprod 2015*

# Female Age and Aneuploidy

- ✓ 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.



# Female Age and Aneuploidy




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)

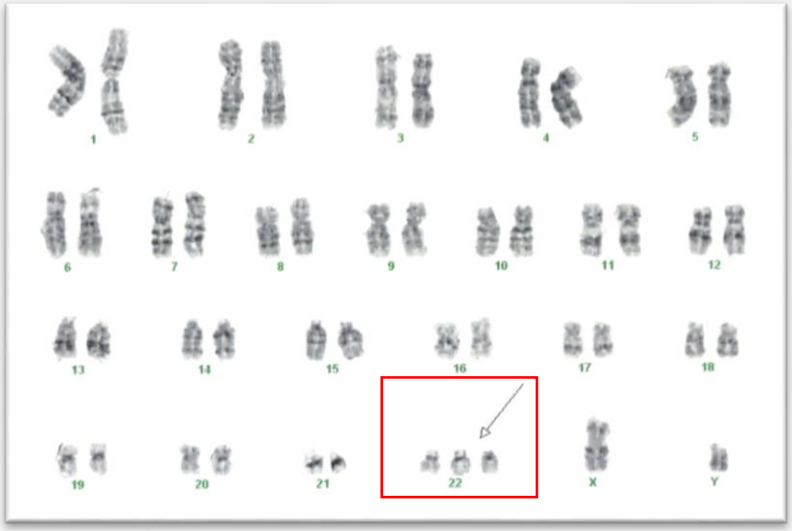
The risk of **aneuploidy** in human foetus **in pre-natal diagnosis** is between 0.2% and 3% (Hassold &Hunt, Nat Rev Genet, 2001)

# 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 | IVF Own Oocytes | Ovum Donation |
|---|---|---|
| 40.6 % Abnormal | 62.7 % Abnormal | 12.8 % Abnormal |

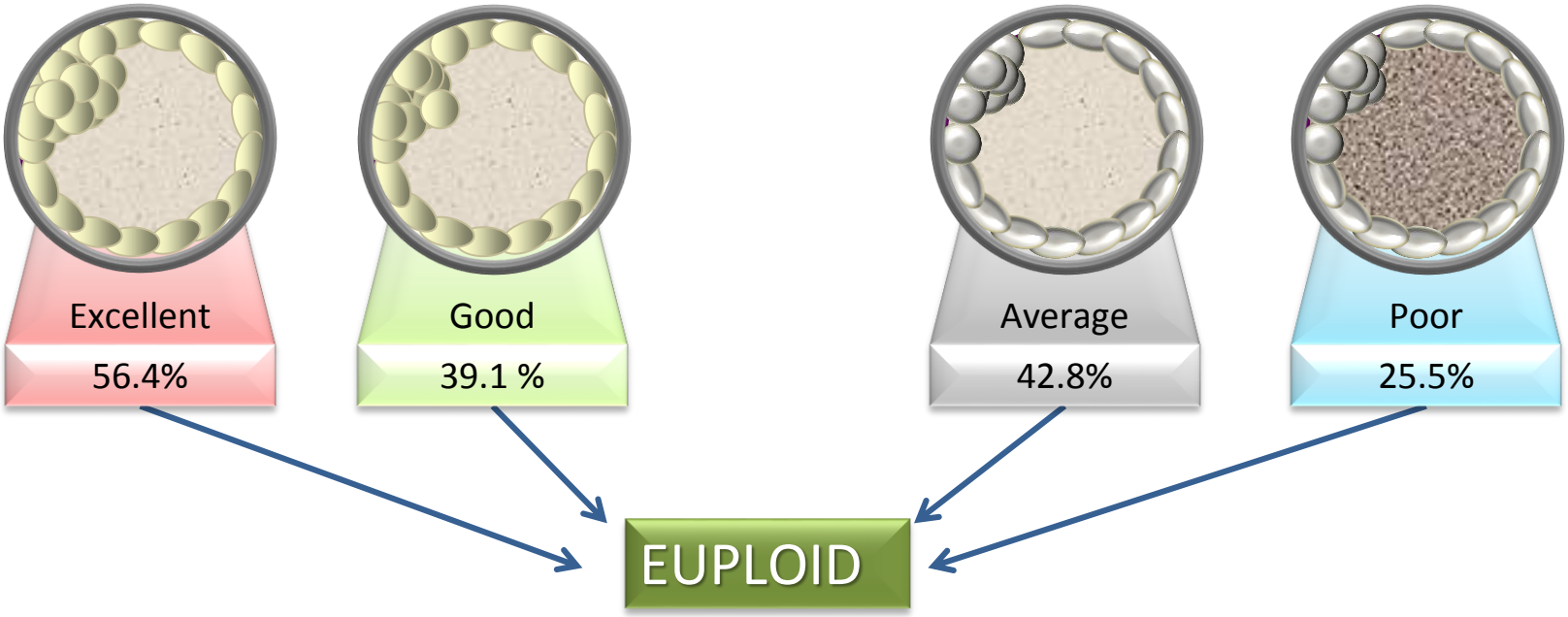
| <5 Mill/mL | ≥5 Mill/mL |
|---|---|
| 72.7 % | 53.6 % |

*Campos-Galindo et al., JARG 2015*

# Morphology Selection and Aneuploidy

## 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

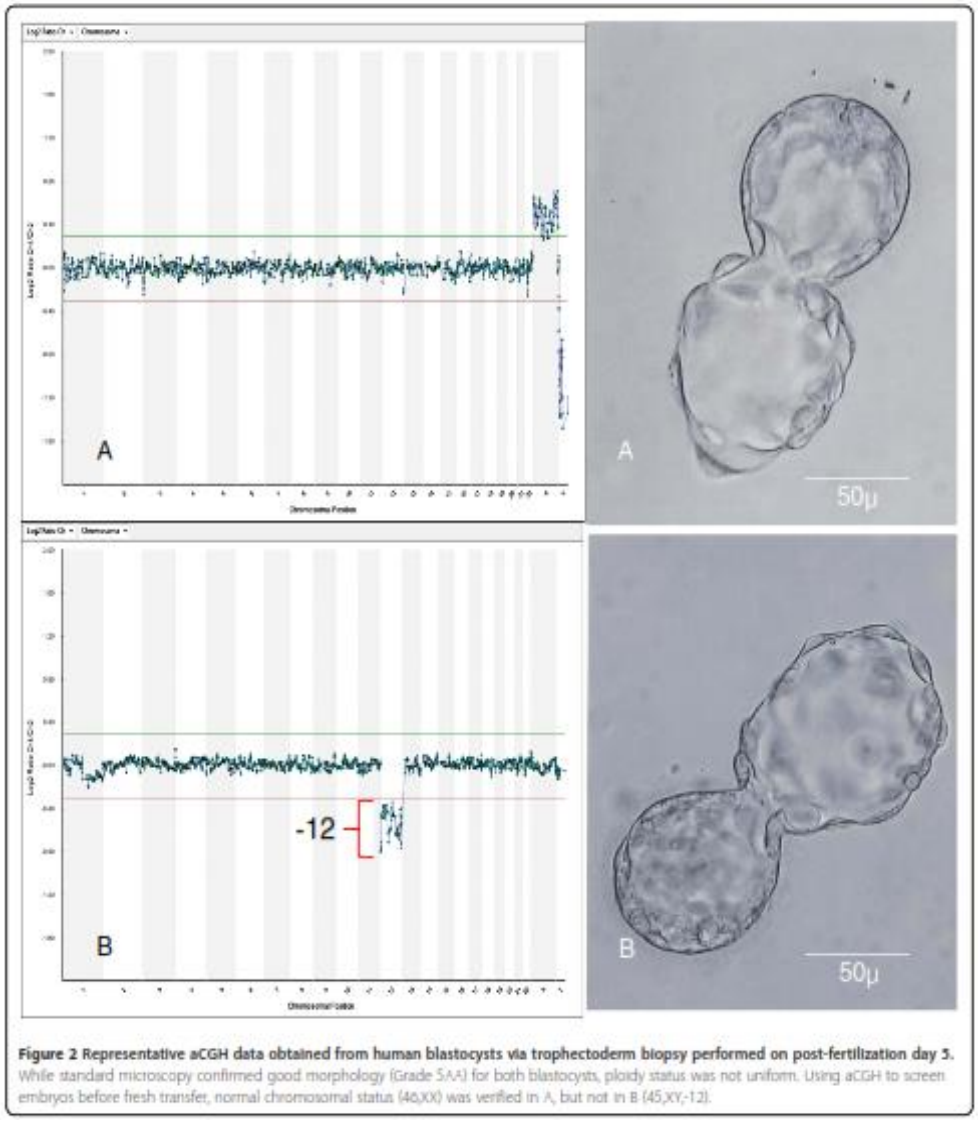



**Figure 2** Representative aCGH data obtained from human blastocysts via trophectoderm biopsy performed on post-fertilization day 5. While standard microscopy confirmed good morphology (Grade 5:AA) for both blastocysts, ploidy status was not uniform. Using aCGH to screen embryos before fresh transfer, normal chromosomal status (46,XX) was verified in A, but not in B (45,XY,-12).

- ✓ **Only morphological criteria fails selecting the best embryo.**
- ✓ **The transfer of "good morphology" blastocyst not always means "chromosomally normal" embryos.**

*(Yang et al., 2012)*

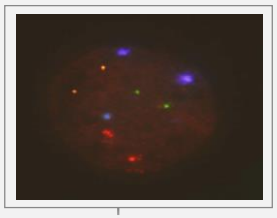
TIME-LAPSE AND ANEUPLOIDY

**Type of chromosome abnormality affects embryo morphology dynamics**

*(Nogales et al., 2017)*

Similar kinetics in euploid and trisomic embryos

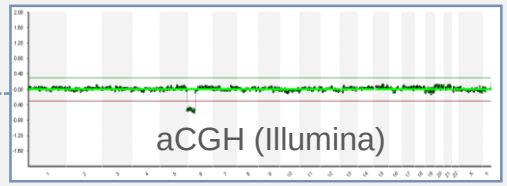
# PGT-A: Evolution of the technology



1995

**Fluorescence *In Situ* Hybridization (FISH)**

PGS 1.0
Day-3 biopsies
2 blastomeres




PGS 2.0
Blastocysts
Deferred transfer

2008

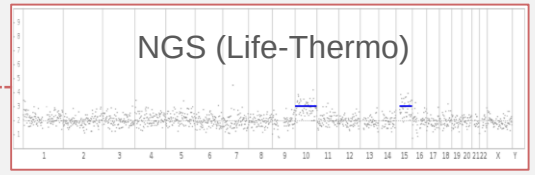
**Array Comparative Genomic Hybridization (aCGH)**

2010

Single-nucleotide polymorphism (SNP) microarray

2012

Quantitative polymerase chain reaction (qPCR)


PGS 3.0
Blastocyst and deferred transfer

2013

**Next Generation Sequencing (NGS)**

2016-17

Next Generation Sequencing (NGS) with custom algorithm

≤12 chromosomes

24 chromosomes

Mitochondrial DNA

Mosaicism

# Why to test embryos for aneuploidies ?

TO maximize LONG TERM treatment efficacy.

Healthy baby at home

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

# 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

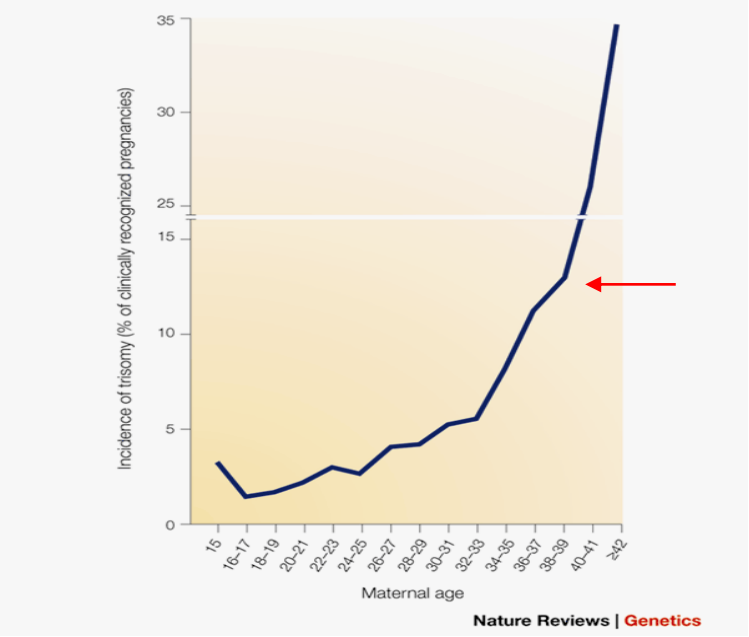
# Human Embryo Aneuploidy

✓ Aneuploidy of human preimplantation embryos now represents the most well established molecular biomarker of reproductive potential. *(Gardner et al.,2015).*

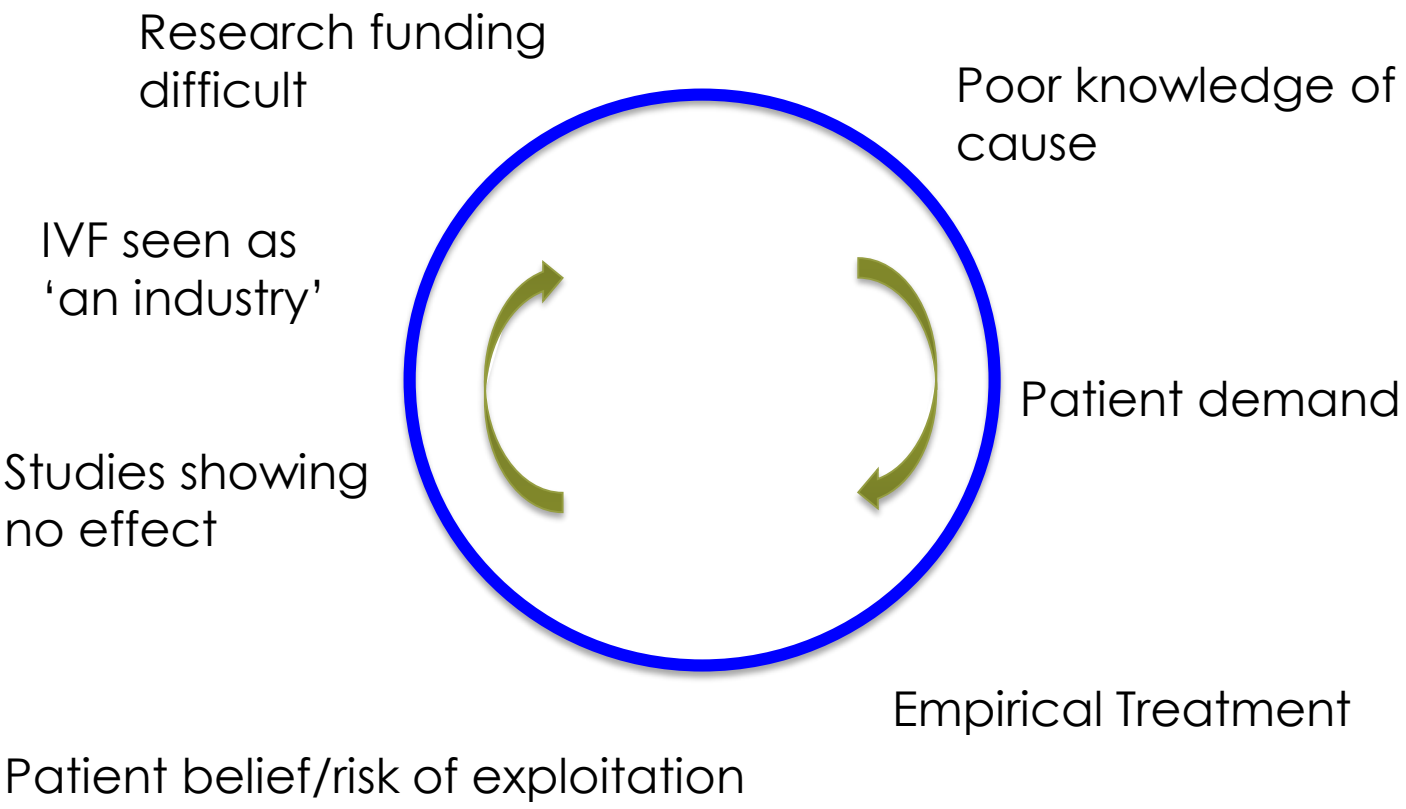
✓ >98% of aneuploidies are meiotic in embryos and foetuses, present in all cells and **do not self correct**!
*(Ottini et al., Nature Genetics 2015)*

Hassold & Hunt, *Nature Reviews Genetics* 2001

**TO ERR (MEIOTICALLY) IS HUMAN: THE GENESIS OF HUMAN ANEUPLOIDY**

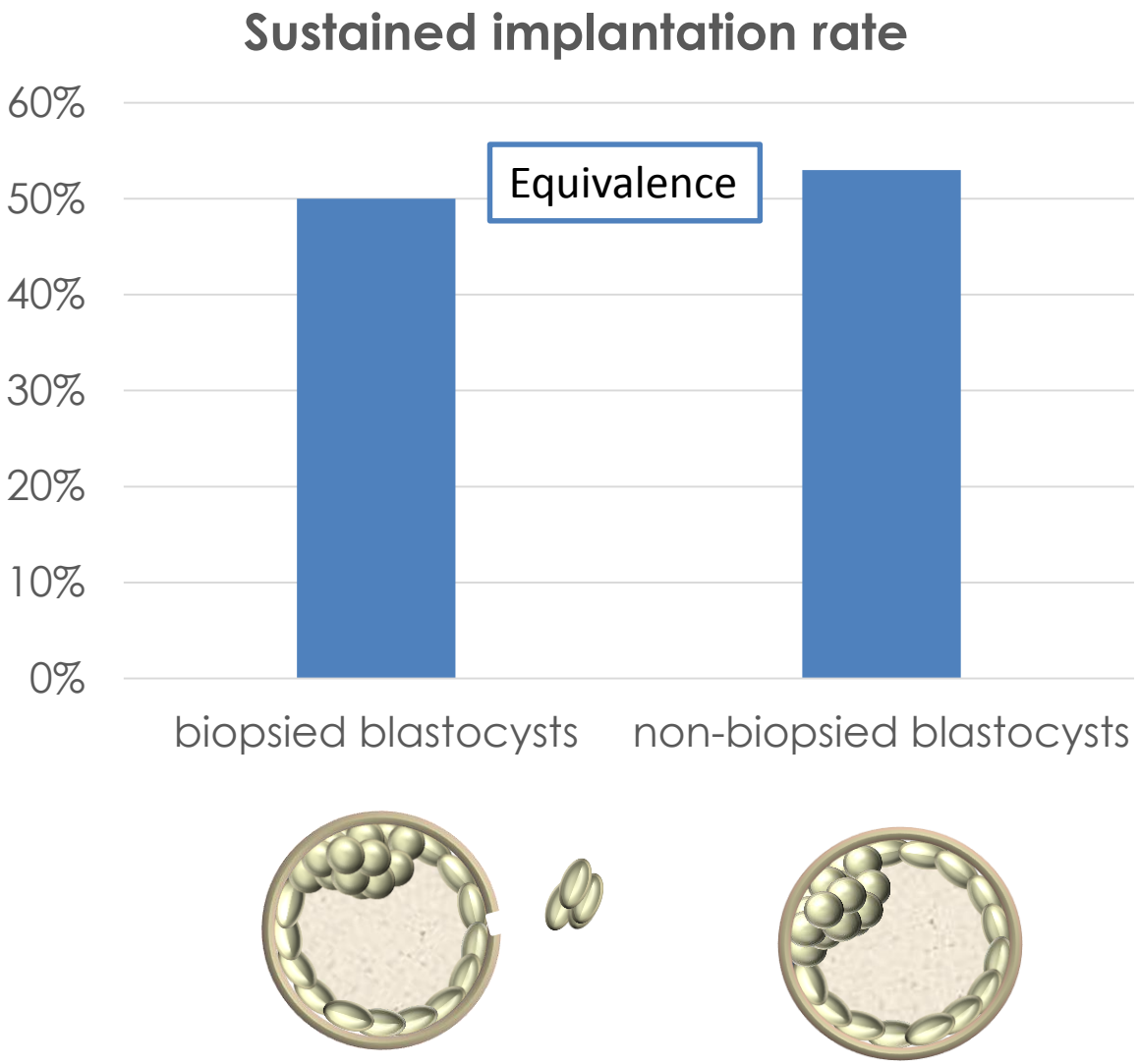


# ‘The circle of desperation’



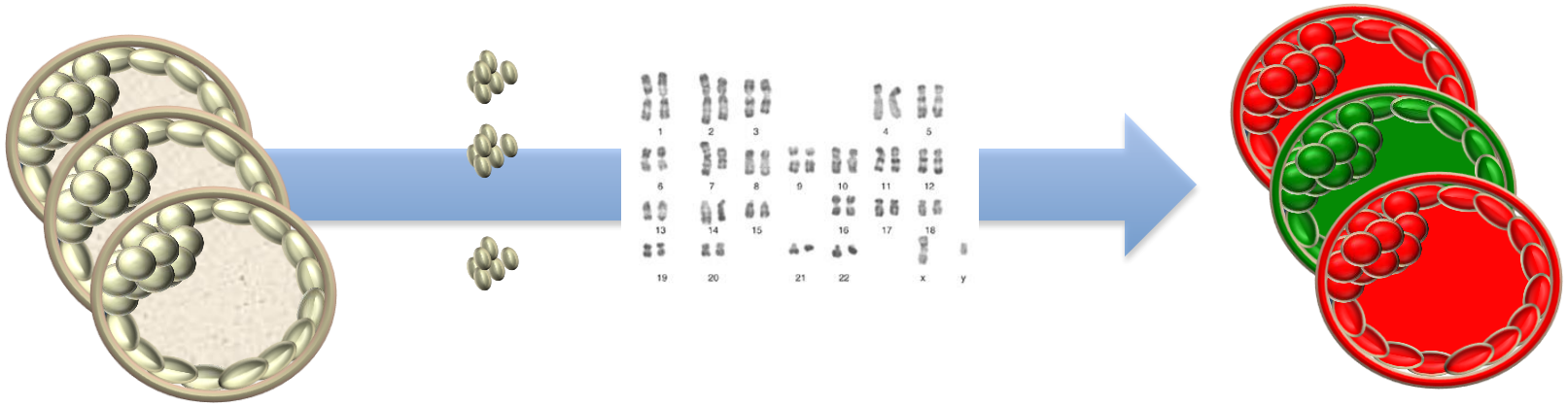
# Trophectoderm biopsy DOES NOT affect embryo reproductive potential

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 attemp**

**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 (2*pn*) | 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[a] |
| *Grade 3* | 3 (5.4) | 1 (2.1) | |
| Clinical pregnancy | 39 (70.9) | 22 (45.8) | 0.017[a] |
| Ongoing pregnancy (≥20wks GA) | 38 (69.1) | 20 (41.7) | 0.009[a] |
| Missed abortion | 1 (2.6) | 2 (9.1) | 0.597[b] |

*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**

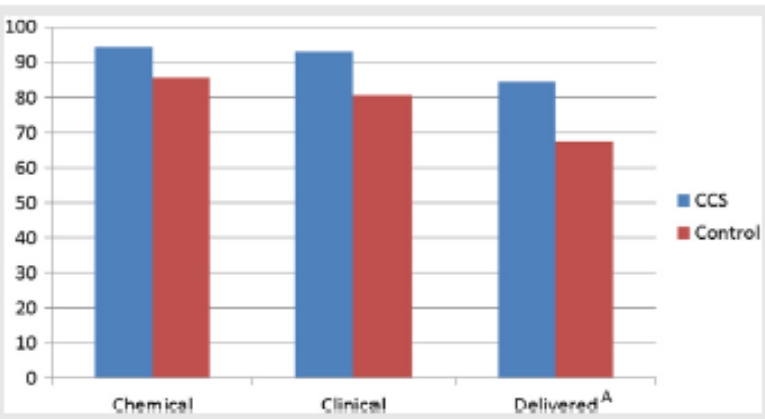
*(Scott et al., FS 2013)*

**Women <43 years**

**AMH ≥1.2 ng/ml**

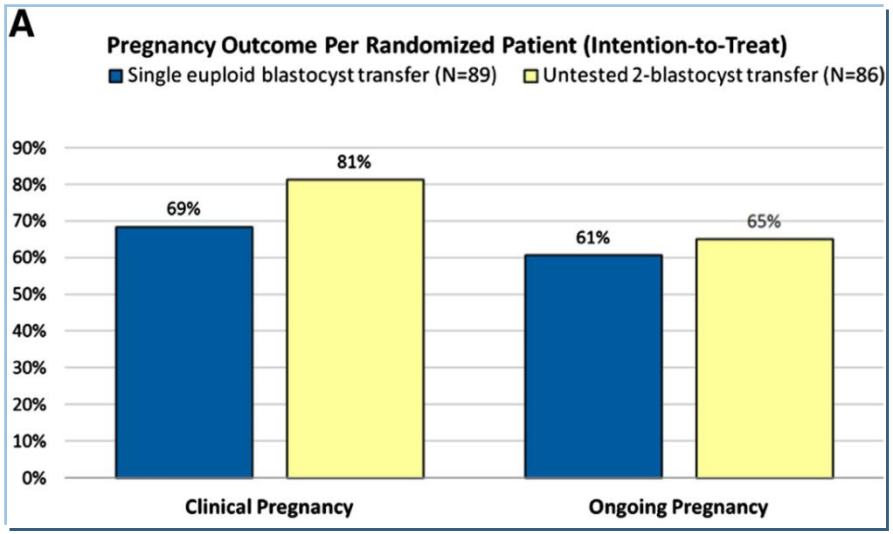
**FSH <12 IU/L**

*(Forman et al., FS 2013)*




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.*



# Advanced Maternal Age Patients

SEMINAL CONTRIBUTION

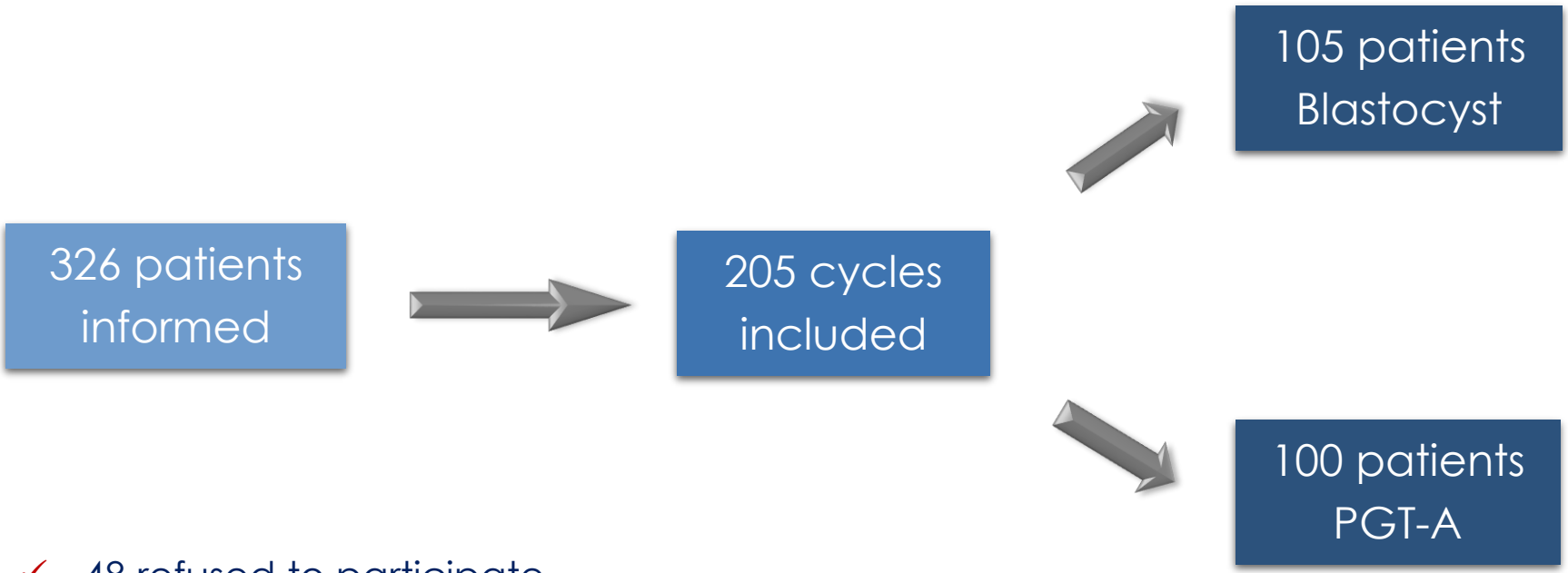
Fertility and Sterility®

# In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study

Carmen Rubio, Ph.D.,[a] José Bellver, M.D.,[b,c] Lorena Rodrigo, Ph.D.,[a] Gema Castillón, M.D.,[d] Alfredo Guillén, M.D.,[e] Carmina Vidal, M.D.,[b] Juan Giles, M.D.,[b] Marcos Ferrando, M.D.,[f] Sergio Cabanillas, M.D.,[b] José Remohí, M.D., Ph.D.,[b,c] Antonio Pellicer, M.D., Ph.D.,[b,c,g] and Carlos Simón, M.D., Ph.D.[a,b,c]

[a] Igenomix Valencia/INCLIVA, Valencia; [b] Instituto Valenciano de Infertilidad, Valencia University, Valencia; [c] Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, Valencia University, Valencia; [d] Instituto Valenciano de Infertilidad, Barcelona; [e] Instituto Valenciano de Infertilidad, Madrid, Universidad Juan Carlos I, Madrid; [f] Instituto Valenciano de Infertilidad, Bilbao; and [g] Instituto de Investigación Sanitaria La Fe, Valencia, Spain

# Flow-chart




- ✓ 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*

*ClinicalTrials.gov NCT01571076*

# Results

## Clinical outcome after the first attempt: fresh transfer

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

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

*Rubio F&S, 2017*

# Results

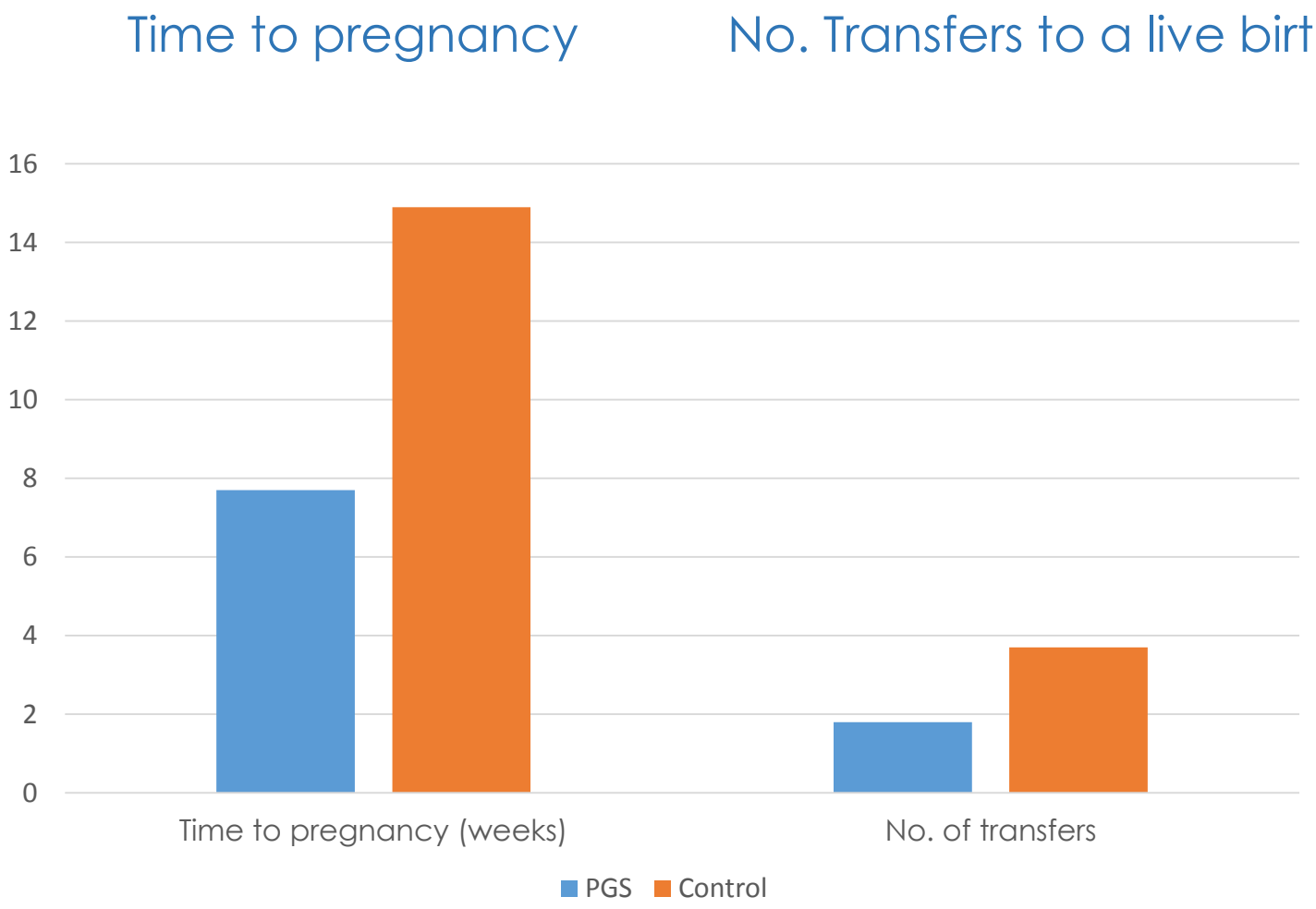
## Cumulative clinical outcome after transfer of cryopreserved embryos

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

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

*Rubio F&S, 2017*

# RCT- Advanced Maternal Age




*ClinicalTrials.gov NCT01571076 Igenomix-IVI*

*Rubio et al. F&S, 2017*

# Results

## Cost-effectiveness estimation per baby at home

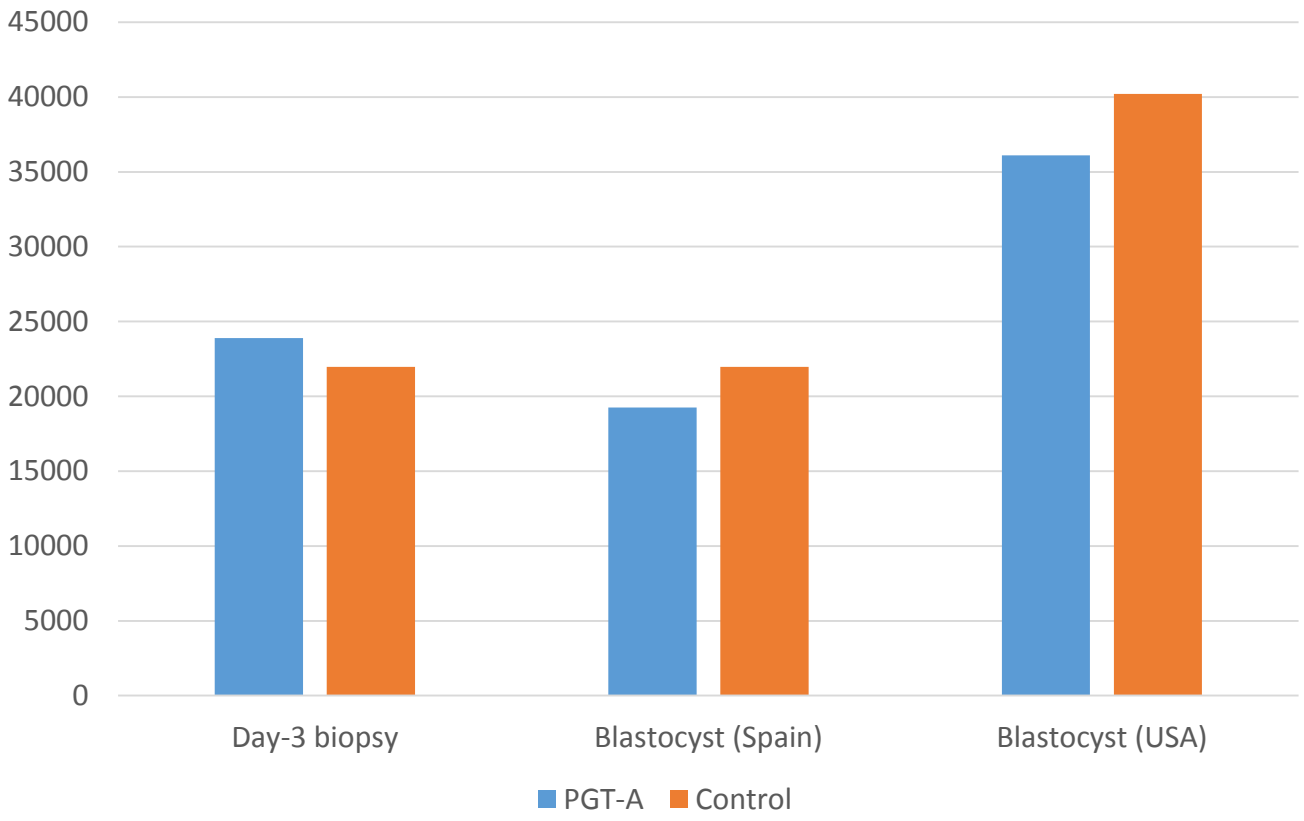
| | PGT-A | Non PGT-A |
|---|---|---|
| **No. of cycles** | 100 | 105 |
| **IVF lab cost** | 5490x**100** (549,000) | 5490x**105** (576,450) |
| **Drug cost** | 1200x**100** (120,000) | 1200x**105** (126,000) |
| **Vitrification cost** | 1100x**13** (14,300) | 1100x**55** (60,500) |
| **Cost of additional transfers** | 1950x**1** (1950) | 1950x**35** (68,250) |
| **Cost of PGD-A + day-3 embryo biopsy** | 3890x**100** (389,000) | --- |
| **Cost of D&C+POC** | 1023x**1** (1023) | 1023x**21** (21,483) |
| **Cost of medical treatment of ectopic** | --- | 2040x**2** (4080) |
| **Mean cost/baby day-3** | 1075,273/**45 babies (23,895)** | 856,763/**39 babies (21,968)** |
| **Estimated cost (€)/baby blastocyst** | **19,250** | **21,968** |
| **Estimated cost ($)/baby USA** | **36,098** | **40,211** |

*ClinicalTrials.gov NCT01571076 Igenomix-IVI*

*Rubio et al. F&S, 2017*

# RCT- Advanced Maternal Age

## Cost-effectiveness estimation per baby at home




*ClinicalTrials.gov NCT01571076 Igenomix-IVI*

*Rubio et al. F&S, 2017*

# Conclusions

PGT-A In AMA

## 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

# RCT- Severe Male Factor

## Clinical outcome after the first attempt: fresh transfer

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

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

*ClinicalTrials.gov NCT01571076 Igenomix-IVI*
*Rubio et al. F&S Submitted*

# RCT- Severe Male Factor (Interim analysis)

## Cumulative clinical outcome after cryotransfers

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

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

*ClinicalTrials.gov NCT01571076 Igenomix-IVI*
*Rubio et al. F&S Submitted*

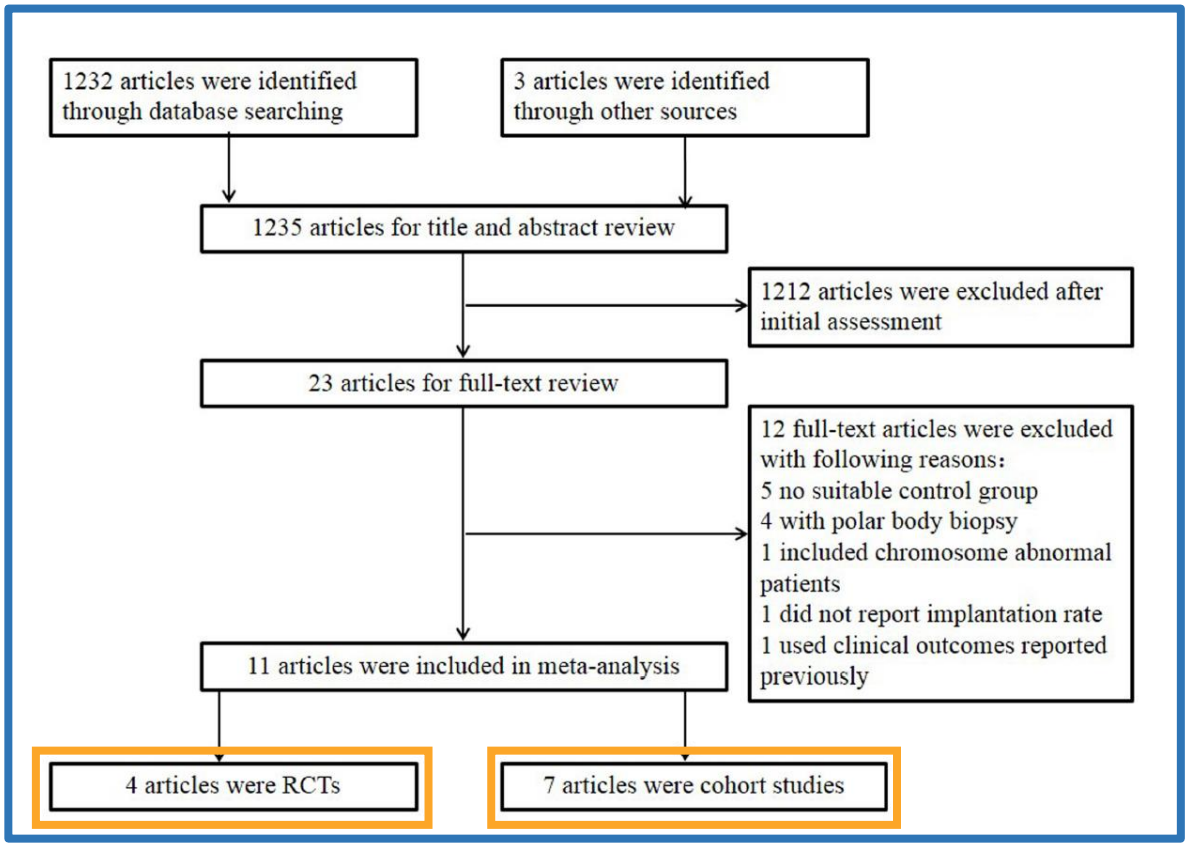
# Meta-Analysis on PGt-A for 24 chromosomes

PLOS ONE

RESEARCH ARTICLE

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

Minghao Chen[1☯], Shiyou Wei[2☯], Junyan Hu[3☯], Song Quan[1*]




*Chen et al., PLoS One, 2015*

# Meta-Analysis on PGT-A for 24 chromosomes

PLOS ONE

RESEARCH ARTICLE

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

Minghao Chen[1☯], Shiyou Wei[2☯], Junyan Hu[3☯], Song Quan[1*]

**No benefit of PGT-A on CLB**

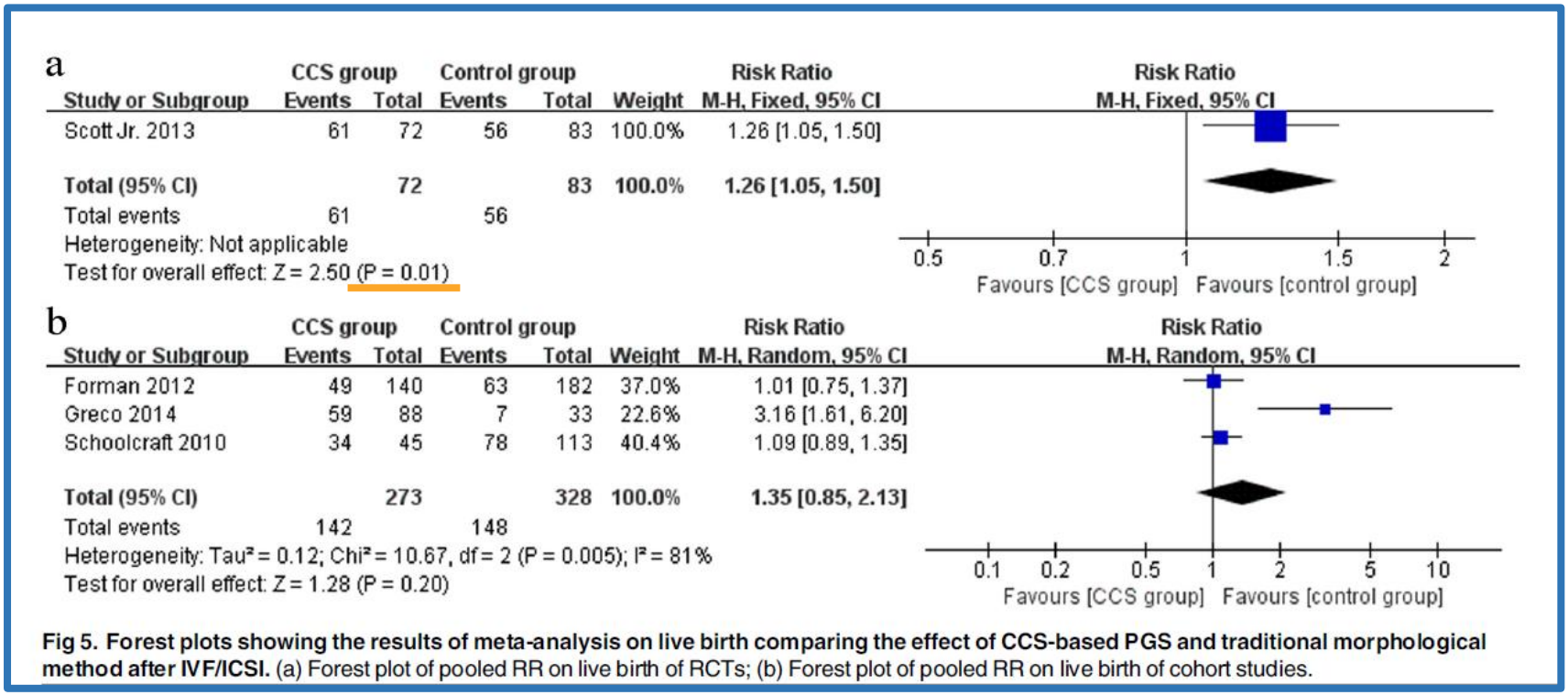



**Fig 5. Forest plots showing the results of meta-analysis on live birth comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI.** (a) Forest plot of pooled RR on live birth of RCTs; (b) Forest plot of pooled RR on live birth of cohort studies.

*Chen et al., PLoS One, 2015*

# Meta-Analysis on PGT-A for 24 chromosomes

PLOS ONE

RESEARCH ARTICLE

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

Minghao Chen[1☯], Shiyou Wei[2☯], Junyan Hu[3☯], Song Quan[1*]

**Decreased miscarriage with PGT-A**

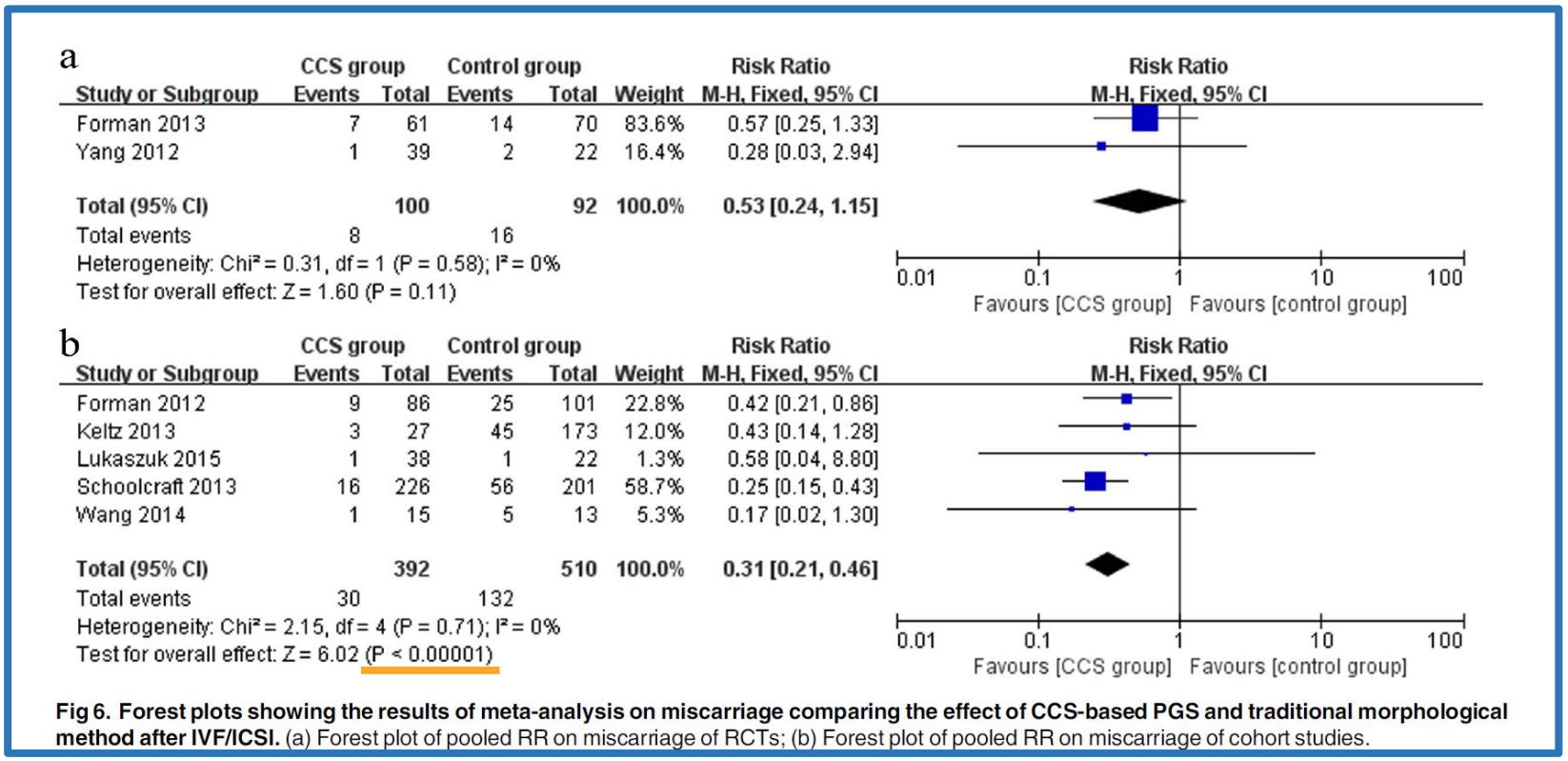



**Fig 6. Forest plots showing the results of meta-analysis on miscarriage comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI.** (a) Forest plot of pooled RR on miscarriage of RCTs; (b) Forest plot of pooled RR on miscarriage of cohort studies.

*Chen et al., PLoS One, 2015*

# Meta-Analysis on PGT-A for 24 chromosomes

PLOS ONE

RESEARCH ARTICLE

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

Minghao Chen[1☯], Shiyou Wei[2☯], Junyan Hu[3☯], Song Quan[1*]

**Decreased multiple pregnancy with PGT-A**

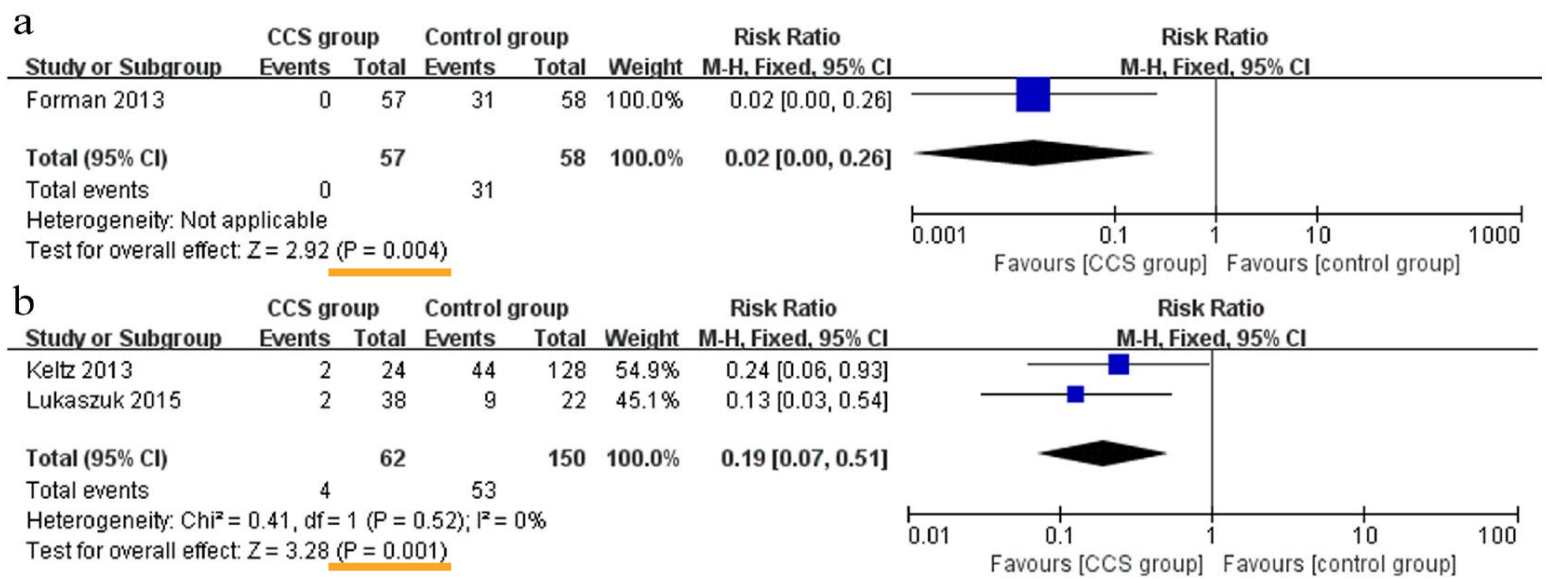



**Fig 7. Forest plots showing the results of meta-analysis on multiple pregnancy comparing the effect of CCS-based PGS and traditional morphological method after IVF/ICSI.** (a) Forest plot of pooled RR on multiple pregnancy of RCTs; (b) Forest plot of pooled RR on multiple pregnancy of cohort studies.

*Chen et al., PLoS One, 2015*

# Meta-Analysis on PGT-A for 24 chromosomes

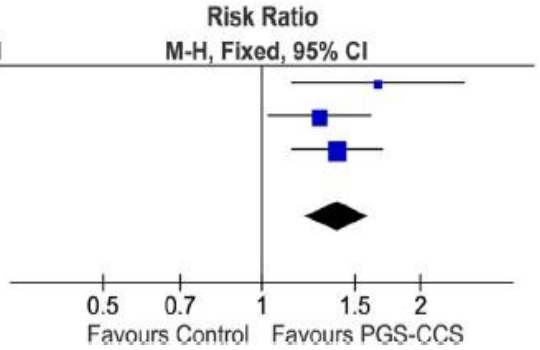
RCTs

**Improved sustained implantation with PGT-A**

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

| Study or Subgroup | PGS-CCS Events | PGS-CCS Total | Control Events | Control Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Yang et al. 2012 | 38 | 55 | 20 | 48 | 14.5% | 1.66 [1.14, 2.42] |
| Forman et al. 2013 | 54 | 87 | 83 | 172 | 37.8% | 1.29 [1.03, 1.61] |
| Scott et al. 2013 | 89 | 134 | 78 | 163 | 47.7% | 1.39 [1.14, 1.70] |
| **Total (95% CI)** | | **276** | | **383** | **100.0%** | **1.39 [1.21, 1.60]** |
| Total events | 181 | | 181 | | | |

Heterogeneity: Chi² = 1.29, df = 2 (P = 0.53); I² = 0%
Test for overall effect: Z = 4.61 (P < 0.00001)

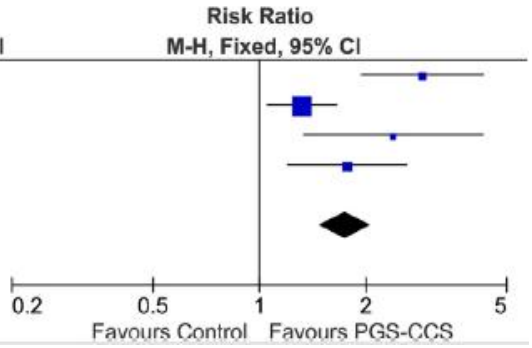



Observational

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

| Study or Subgroup | PGS-CCS Events | PGS-CCS Total | Control Events | Control Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Sher et al. 2009 | 34 | 94 | 39 | 311 | 14.9% | 2.88 [1.94, 4.29] |
| Forman et al. 2012 | 77 | 140 | 76 | 182 | 54.6% | 1.32 [1.05, 1.65] |
| Lee et al. 2015 | 25 | 55 | 12 | 63 | 9.2% | 2.39 [1.33, 4.29] |
| Feichtinger et al. 2015 | 29 | 110 | 60 | 403 | 21.2% | 1.77 [1.20, 2.62] |
| **Total (95% CI)** | | **399** | | **959** | **100.0%** | **1.75 [1.48, 2.07]** |
| Total events | 165 | | 187 | | | |

Heterogeneity: Chi² = 13.10, df = 3 (P = 0.004); I² = 77%
Test for overall effect: Z = 6.48 (P < 0.00001)



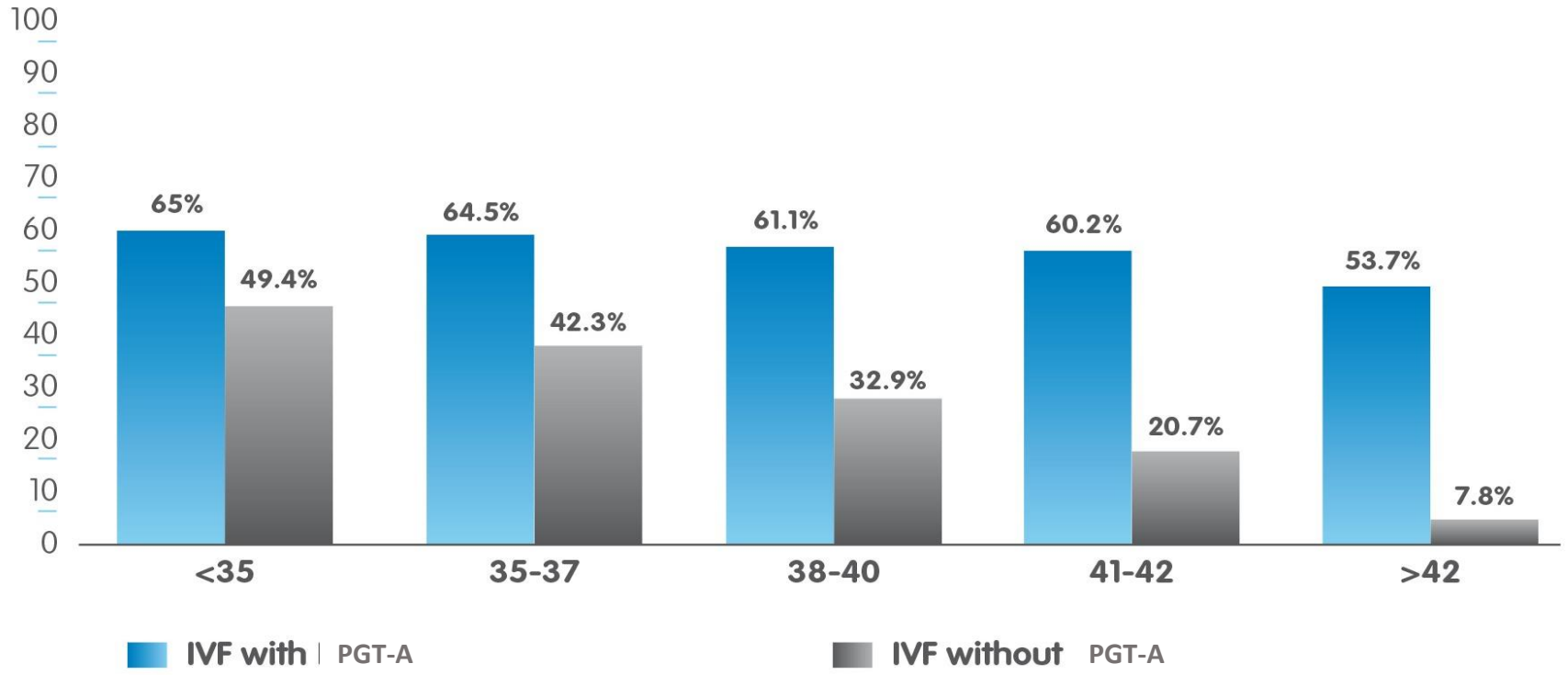

*Dahdouh et al, F&S, 2015*

# PGT-A: the NGS era

## Blastocyst biopsies and NGS cycles performed in 2017

**>100.000 trophoectoderm biopsies analysed worldwide**

### 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

**Table 2** Characteristics of aneuploid embryos transferred that led to implantation

| Patient | n Embryos transferred | Embryos transferred | Outcome |
|---|---|---|---|
| 1 | 1 | 43, XY, -13, -15, -18 | Normal birth, 46, XY |
| 2 | 1 | 45, XY, -21 | Normal birth, 46, XY |
| 3 | 2[a] | 45, XY, -21<br>46, XX | Normal birth, 46, XY |
| 4 | 2[b] | Partial 47, XX,17p11.2-pter<br>45, XY, -22 | Normal ongoing 46, XX |
| 5 | 2[c] | 47, XY, +22<br>Partial 45, XY,-1plar-p36, 12 | Normal ongoing 46, XY |
| 6 | 1[d] | 45, XY, -21 | Chemical pregnancy |

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

Fertility and Sterility® Vol. 97, No. 4, April 2012

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

Richard T. Scott Jr., M.D.,[a,b] Kathleen Ferry, B.S.,[a] Jing Su, M.S.,[a] Xin Tao, M.S.,[a] Katherine Scott, M.S.,[a] and Nathan R. Treff, Ph.D.[a,b]

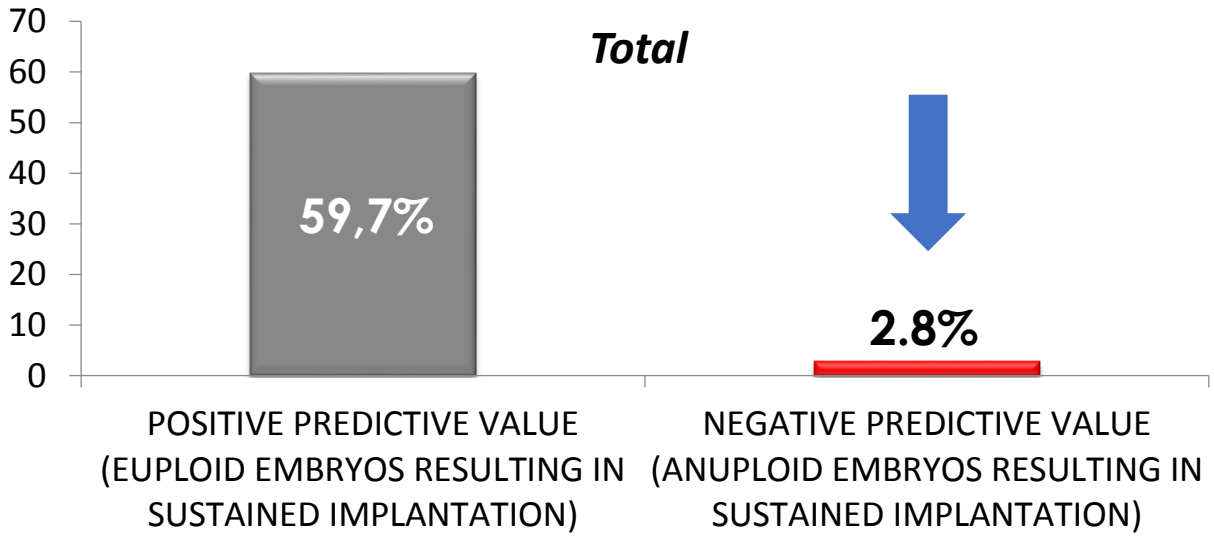
**SNP array**: of the 99 embryos assigned aneuploid, 4 (4%) sustained implantation

**O-31** Monday, October 19, 2015 11:15 AM

**A PROSPECTIVE, BLINDED, NON-SELECTION STUDY TO DETERMINE THE PREDICTIVE VALUE OF PLOIDY RESULTS USING A NOVEL METHOD OF TARGETED AMPLIFICATION BASED NEXT GENERATION SEQUENCING (NGS) FOR COMPREHENSIVE CHROMOSOME SCREENING (CCS).** M. D. Werner, J. M. Franasiak, K. H. Hong, C. R. Juneau, X. Tao, J. Landis, K. M. Upham, N. R. Treff, R. T. Scott. RMA, NJ, NJ.

**ASRM Abstracts** Vol. 104, No. 3, Supplement, September 2015

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



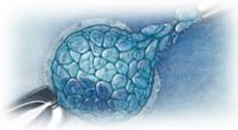

*Scott et al., F&S 2012*

# 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

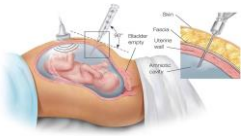
Gk, *dia* + *gnosis*, **knowledge**

## PGT-A



- ✓ **Invasiveness**: none or extremenly low
- ✓ **Prevalence** (Chromosomal risk) **20-90%**
- ✓ **No result rate:** ~1%
- ✓ **Mosaicism:** present 6%
- ✓ **Accuracy:** ~**98-99%**

## PRENATAL DIAGNOSIS



- ✓ **invasiveness 0.2-1%** Abortion risk
- ✓ **Chromosomal risk** (prevalence) **0.1-4%**
- ✓ **No result rate:** ~1%
- ✓ **Mosaicism:** present 1-2% CVS
- ✓ **Accuracy:** ~**98-99%**

*Alfirevic et al., 2009*

# 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%** concordan ce (N=22)

*Xu et al., PNAS 2016* → **85.7%** concordance (N=42)

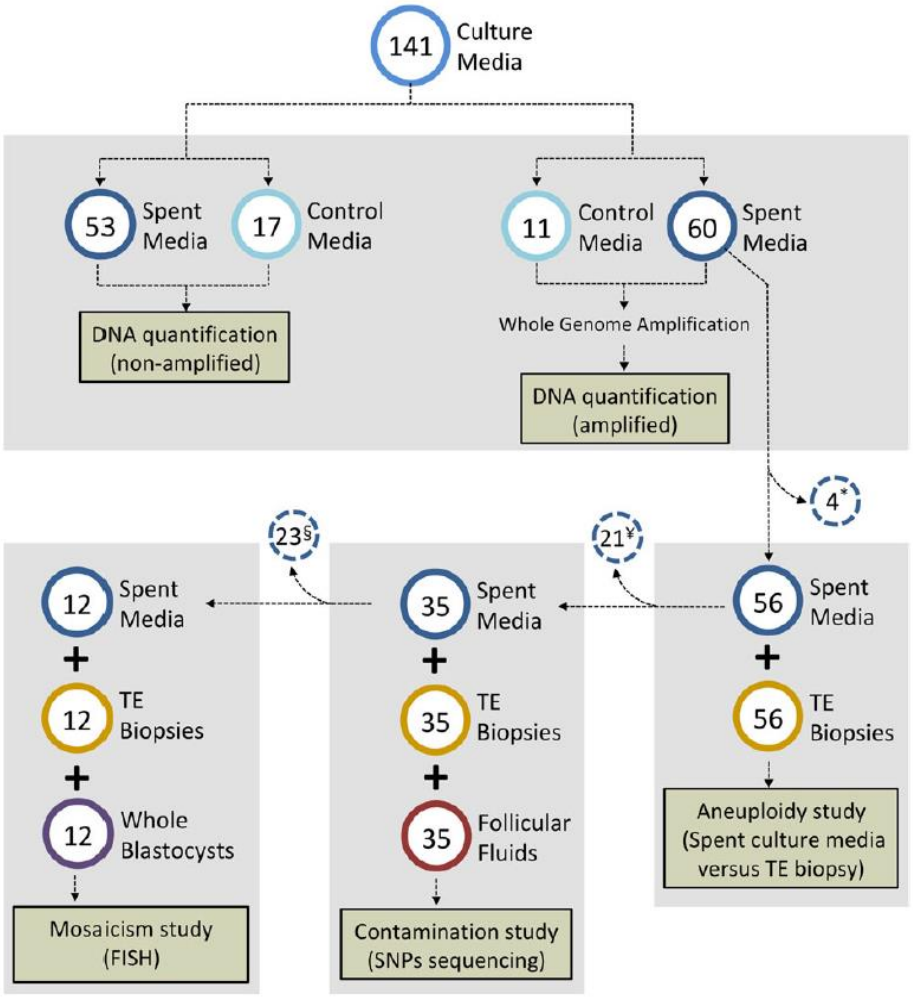
Low number of concordance studies

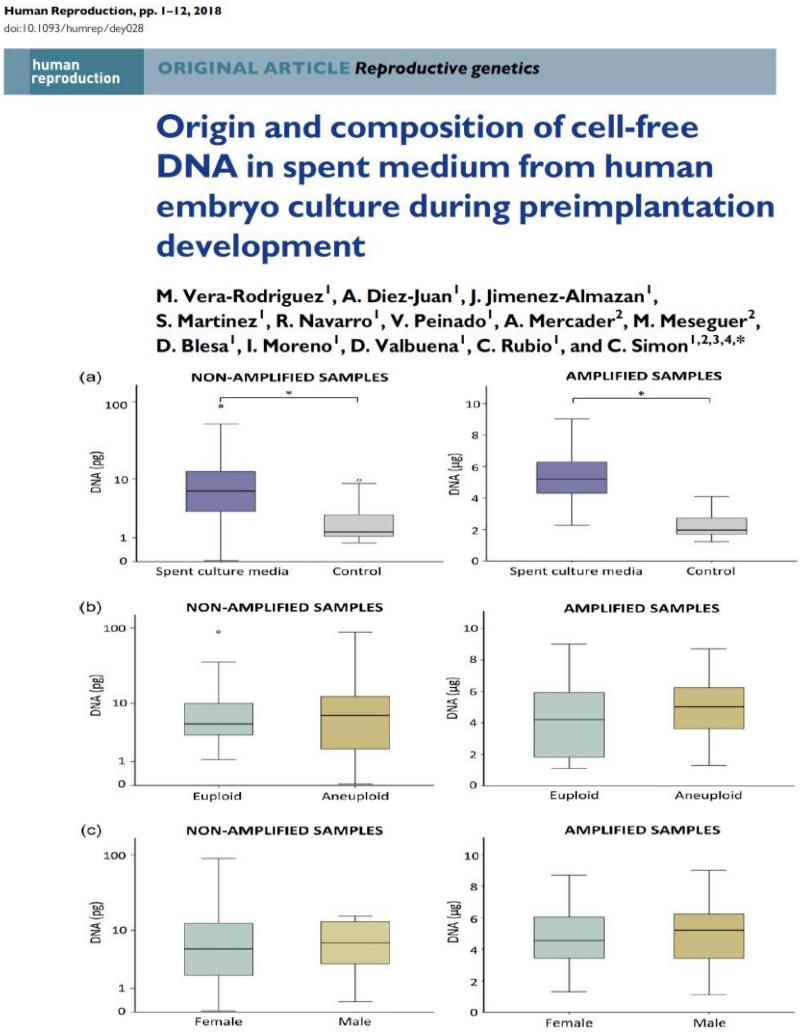
High variability between results

- Embryo mosaicism
- Maternal DNA contamination

*Hammond et al., FS 2017*

# niPGT-A: our previous results





Human Reproduction, pp. 1–12, 2018
doi:10.1093/humrep/dey028
human reproduction ORIGINAL ARTICLE Reproductive genetics

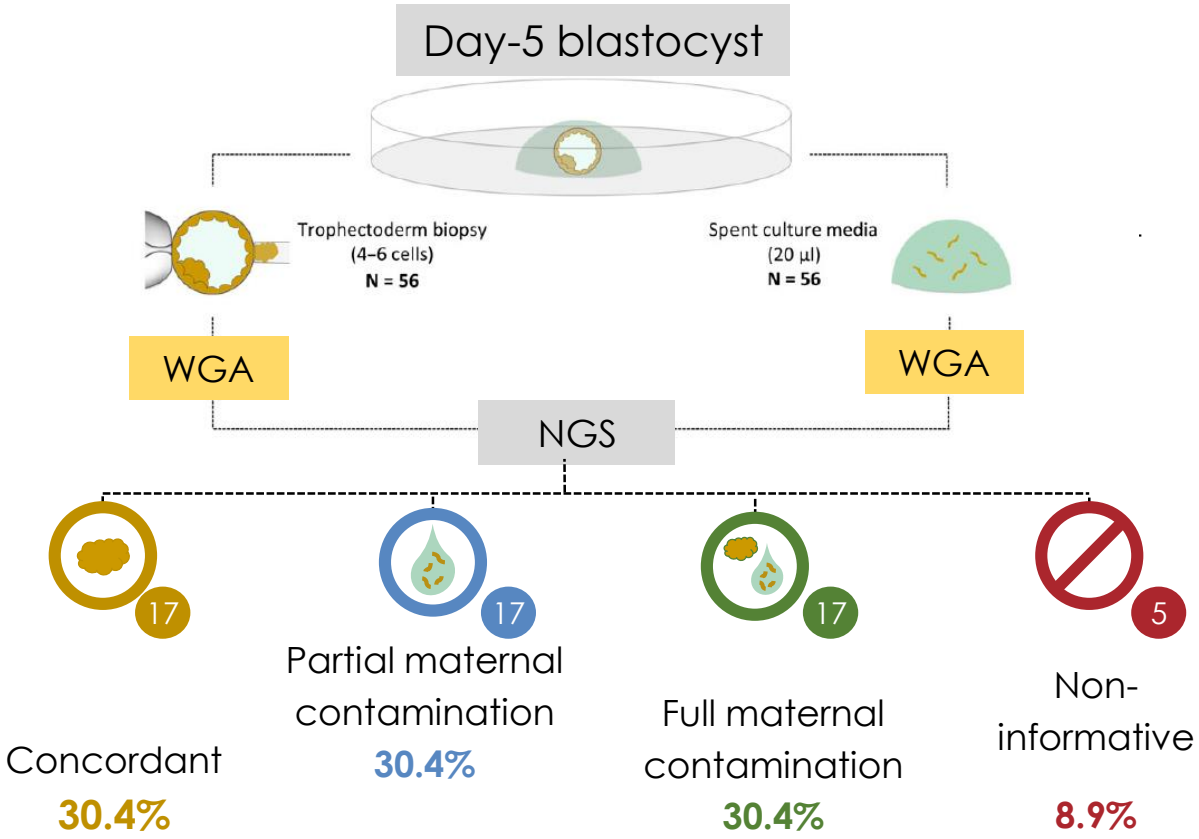
**Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development**

M. Vera-Rodriguez[1], A. Diez-Juan[1], J. Jimenez-Almazan[1], S. Martinez[1], R. Navarro[1], V. Peinado[1], A. Mercader[2], M. Meseguer[2], D. Blesa[1], I. Moreno[1], D. Valbuena[1], C. Rubio[1], and C. Simon[1,2,3,4,*]



*Vera-Rodriguez et al., Hum Reprod 2017*

# niPGT-A: our previous results



# niPGT-A: optimization of the protocol

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

**Conventional Incubator**

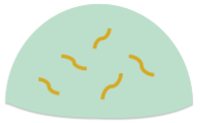
**NO Hatching on D3**

**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

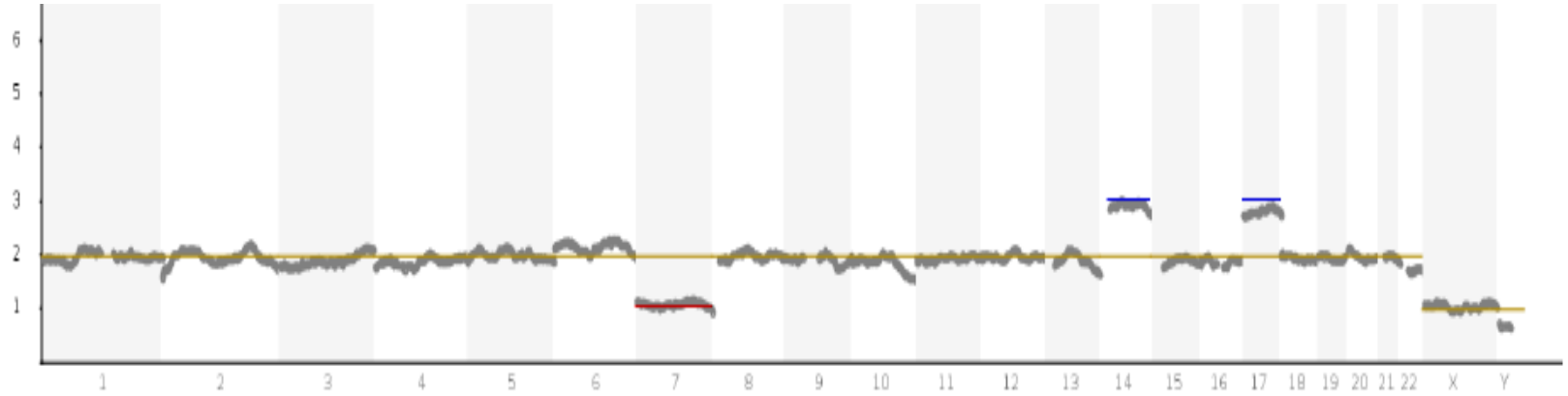
Non-informative

*Rubio et al., ESHRE 2018*

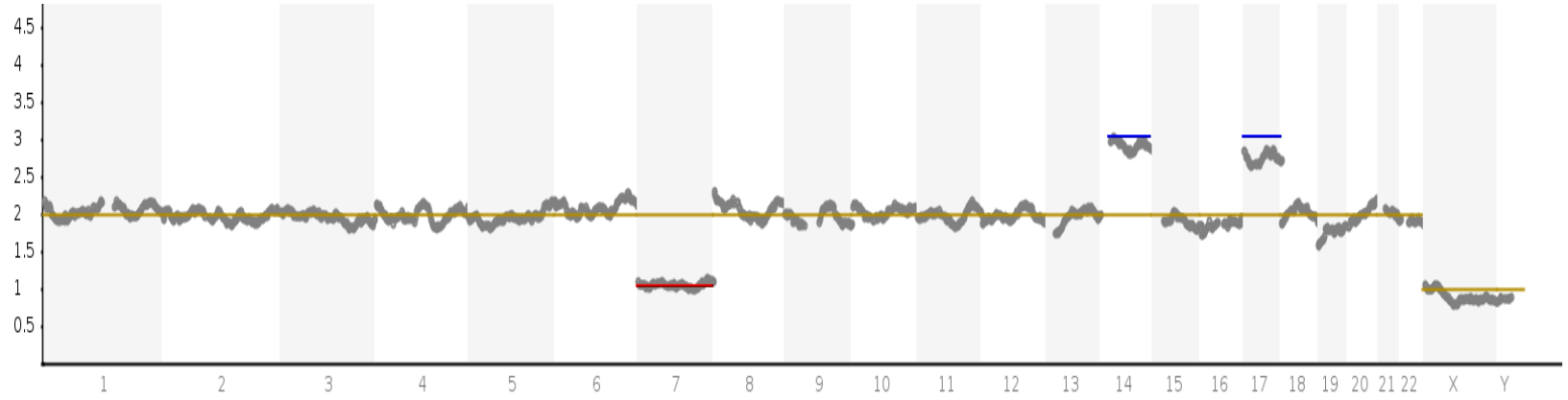
# niPGT-A: Igenomix/Genera Pilot Study

NGS profiles of trophectoderm biopsies and spent culture media

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



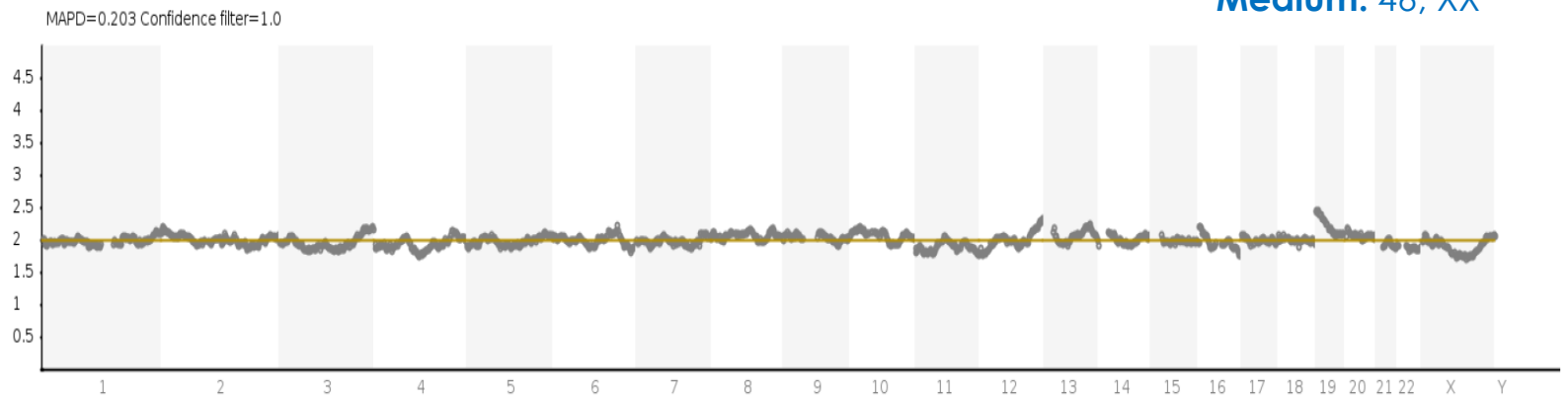

**Trophectoderm biopsy:** 47, XY, -7+14+17



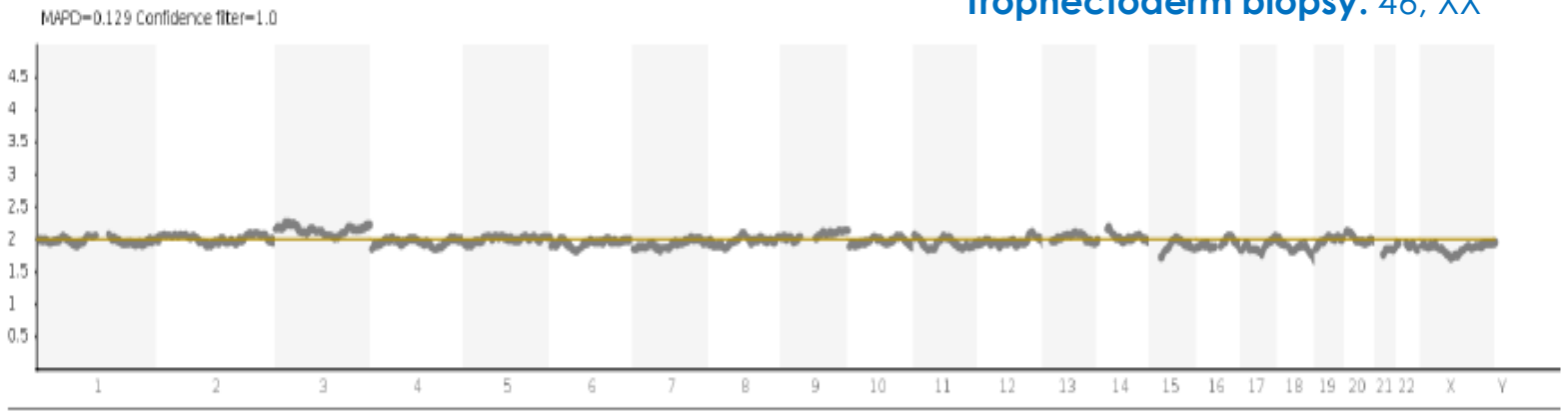
# niPGT-A: Igenomix/Genera Pilot Study

NGS profiles of trophectoderm biopsies and spent culture media

**Medium:** 46, XX




**Trophectoderm biopsy:** 46, XX



# niPGT-A: optimization of the protocol

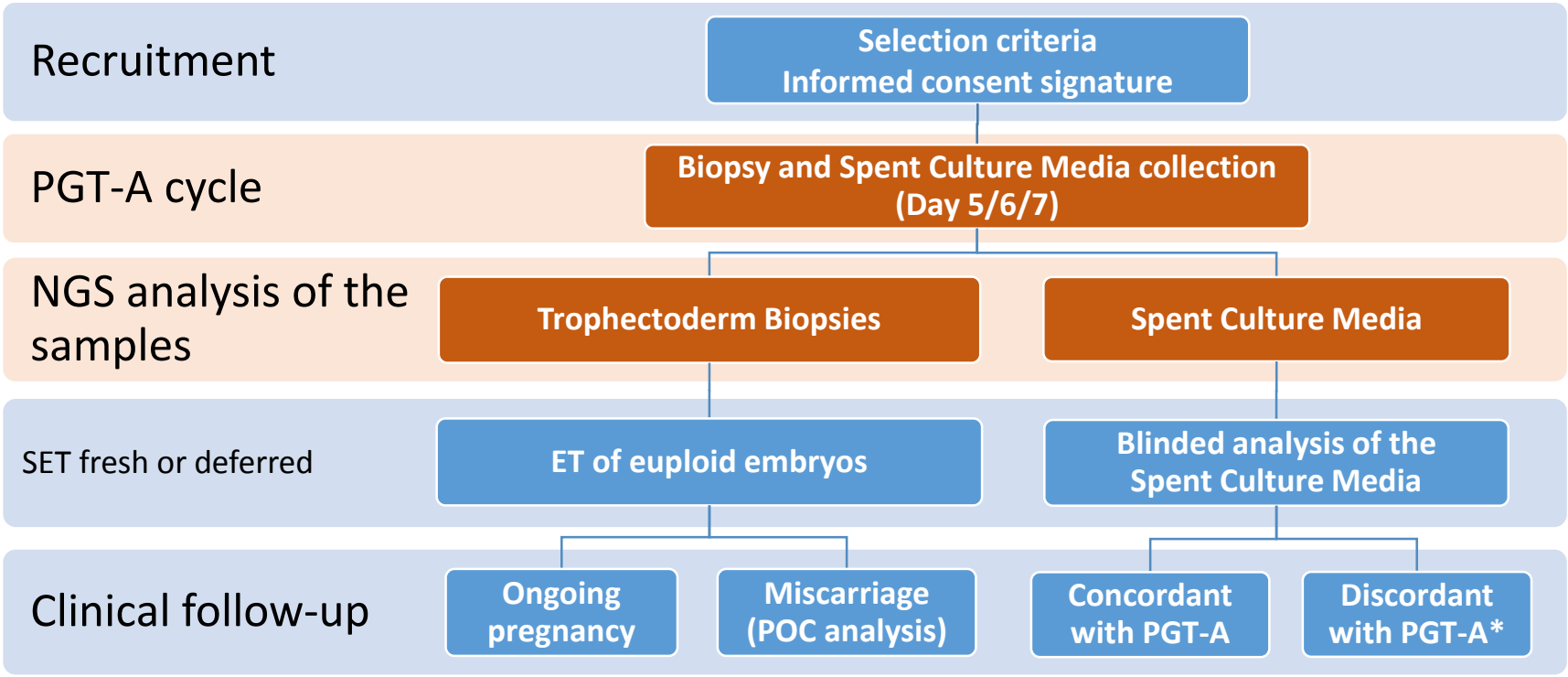
## Summary Pilot Study ni PGT-A (Igenomix-Genera)

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

**ESHRE 2018 SELECTED ORAL PRESENTATION**

# niPGT-A: Multicenter study

## Study flowchart




*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 -

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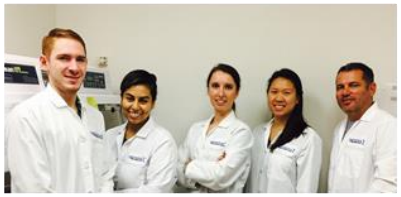

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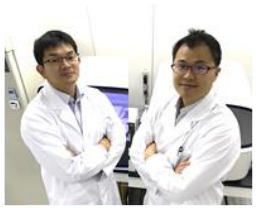

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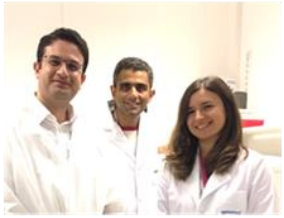

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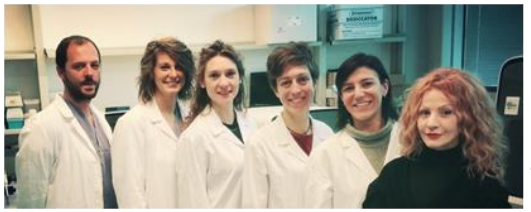

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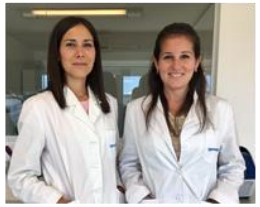

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JAKUBOWSKA MILENA
KAYALI, REFIK
MAE HOOVER, LARISSA
PHILLIPS, KIMBER
SNEIDER, ALYSSA
STANKEWICZ, TIFFANY
YEH, CHRISTINE

Technicians

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BAUTISTA, ABELARD
BOJI NESCU, ANCA
DUENAS, FRANCISCO
CUI, KATHY
GRIFFIN, MARISA
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LOANIDIS, ALEXANDROS
NGUYEN, VI
PENA, DAYTERNA
PHAM, QUON
SANTI, ANNAI
SEN, GURKAN
SHAIBI, DEREK
TUYEN, KENNY

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ESTEVES MORAES, CAMILA
RIBOLDI, MARCIA
UEHARA DE SOUZA, MARIANE

Technicians

DE GODOI IGLESIAS LIMA, GABRIELLE
SANTANA, LEILA

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GARCIA PASCUAL, CARMEN MARÍA

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CRISTINA COYOTECATL

## DUBAI

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CHOPRA, RUPALI

Technicians

ALNIMS, HAYA
ROY CHOWDGHURY, SHEWETA
SHARMA, SHEWETA
WARRIER EDAKUNNY, SHRUTI

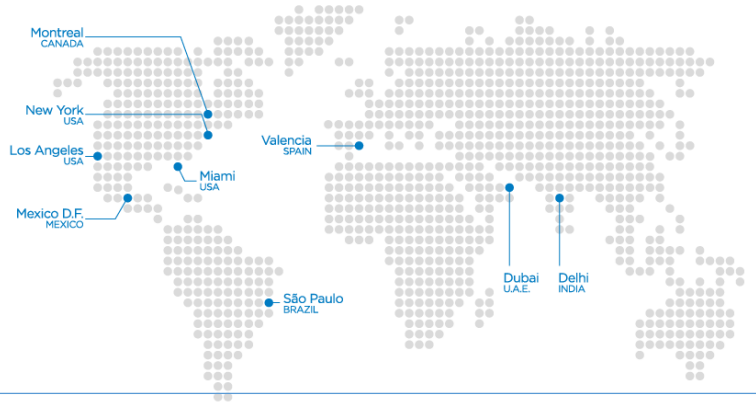
## INDIA

Scientists

KHAJURIA, RAJNI
SINGH BUTTAR, BRINDERJIT

Technicians

UPADHYAY, DIVYESH




Collaborators:

Unión Europea
Fondo Europeo de Desarrollo Regional
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MARIE CURIE ACTIONS

INCLIVA
Instituto de Investigación Sanitaria

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Instituto de Salud Carlos III

VNIVERSITAT DE VALÈNCIA

Stanford University

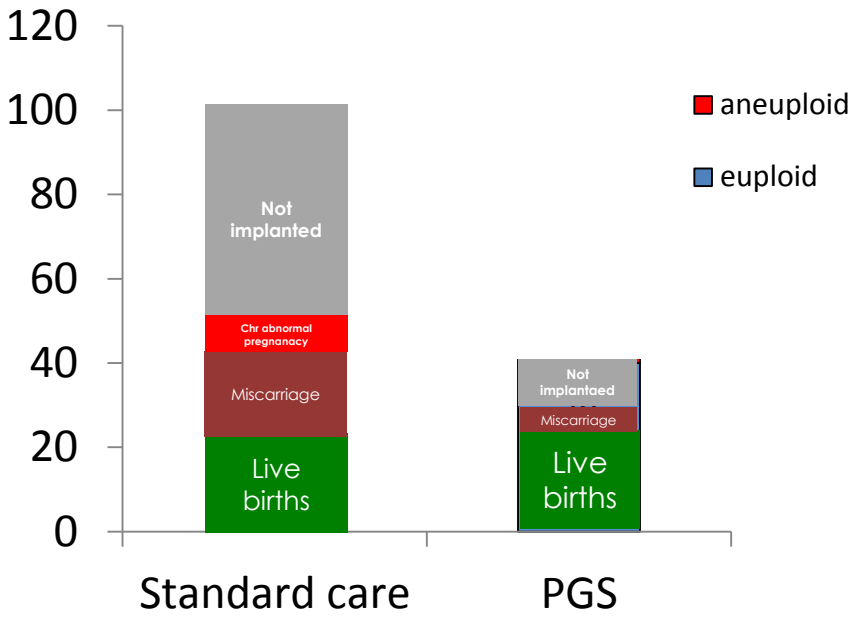
IVI

BCM Baylor College of Medicine

# 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.

Minear et al., Annu. Rev. Genomics Hum. Genet. 2015
(adapted Allyse et al., *Prenat. Diagn.* 2013)

# Desperation is expensive: one patients bill

Prontogest £760.00
Intralipids £300.00
Full Blood Count (FBC) £40.00
Progesterone (Prog) £30.00
HCG & Prog £70.00
NK Assay £310.00
HCG & Prog £70.00
HCG & Prog £70.00
HCG & Prog £70.00
HCG & Prog & FBC £110.00
HCG & Prog £70.00
HCG & Prog £70.00
Prog £30.00
Prog & FBC £70.00
Prog £30.00
NK Assay £310.00
Prog £30.00
Prog £30.00
5+6 Scan £110.00
6+4 Scan £110.00
7+1 Scan £110.00
8+0 Scan £0.00
9+0 Scan £110.00
10+0 Scan £110.00
12+4 Scan (FMC) £230.00

Blood Tests (HIV & Hep) £200.00
Hormone Profile £90.00
Rubella £45.00
Full Immune Blood Test £805.00
E2 £30.00
Progesterone (Prog) £30.00
E2 & LH £60.00
E2 & LH £60.00
E2, LH, FSH & Prog £120.00
E2 & LH £60.00
E2, LH & FSH £90.00
E2 (x2), LH, FSH & Prog £150.00
E2 (x2), LH, FSH & Prog £150.00
E2 (x2), LH, FSH & Prog £150.00
E2, LH, FSH & Prog £120.00
E2 (x2), LH, FSH & Prog £150.00
E2 (x2), LH, FSH & Prog £150.00
E2 (x2), LH, FSH & Prog £150.00
Prog (x2), FBC, HCG £140.00
NK Assay £310.00
IVIG £1400.00

TOTAL £13,271

*Slide from Nick Macklon*