

# Pre-implantation genetic testing:

Who will benefit and what is the level of evidence?

**Professor Hakan Yaralı, MD**

Anatolia IVF and Women's Health Center

&

Hacettepe University, School of Medicine, Dept. of OB/GYN



# Contemporary Goals of IVF

- Reduce number of embryos transferred
- Maintain high live birth rate per transfer



**Need for Optimal Embryo Selection**

# How we define objectivity in medical science?

Assessment	Validated	Measurable	Repeatable	Unbiased	Consensus	Conclusion
Objective	Yes	Yes	Yes	No	Yes	Real
Subjective	Partially	No	Maybe	Maybe	No /Maybe	Maybe Real



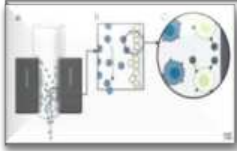
# Sperm viability assessment – none is validated today



Sperm Binding Ability Assessment



Sperm Head Birefringence Assessment



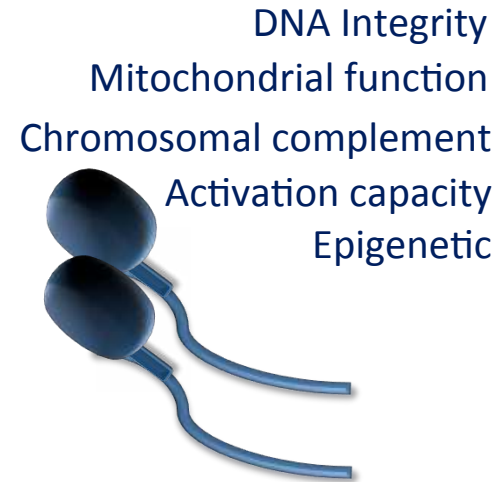
Magnetic-Activated Cell Sorting  
for Sperm Preparation



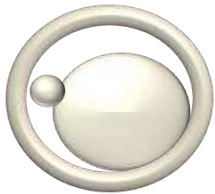
Sperm DNA Molecular Targetting



Real-Time Fine Sperm Morphology  
Assessment (IMSI)



# Oocyte morphology assessment: not validated



Cumulus-oocyte complex expansion

Zona Pellucida

Polar Body Shape & Euploidy

Vacuoles or refractile bodies

Dark cytoplasm or diffuse granulation

Central Granulation

Smooth endoplasmic reticulum clusters

Oocyte Shape

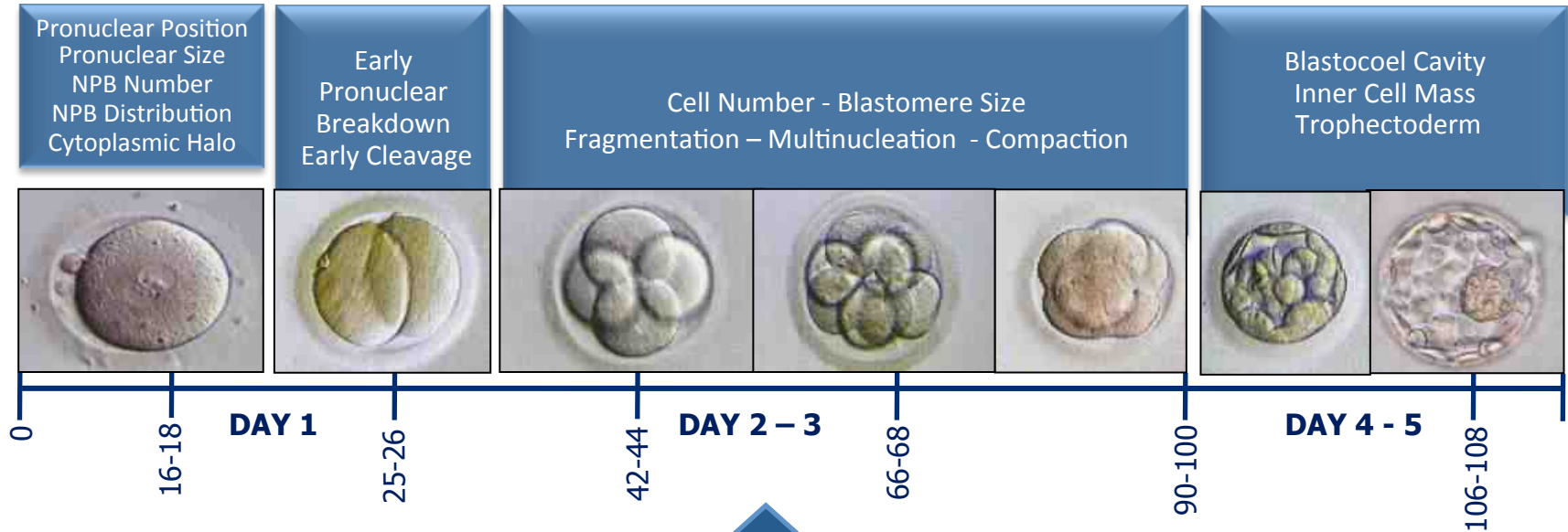
Periviteline space



1. Rienzi, et al. Human Reproduction update, 2011.

2. Alpha Eshre Consensus document, 2011.

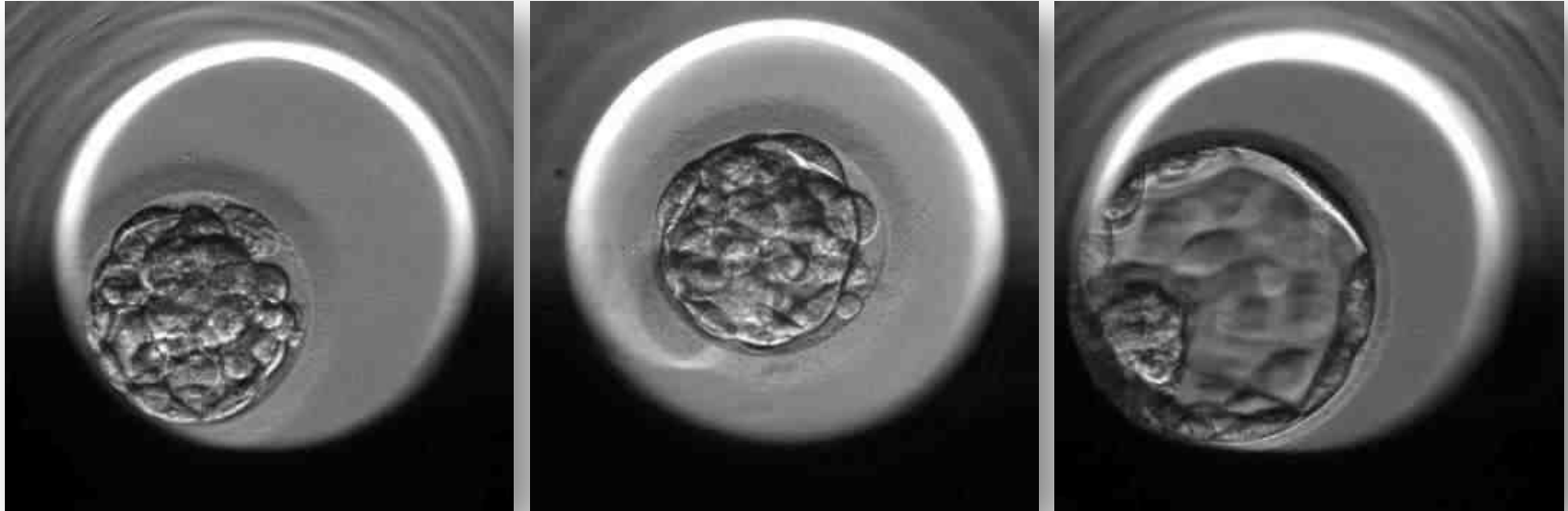
# Embryo morphological assessment: low accuracy



1. Guerif, et al. 2007.
2. Racowsky, et al. 2009.

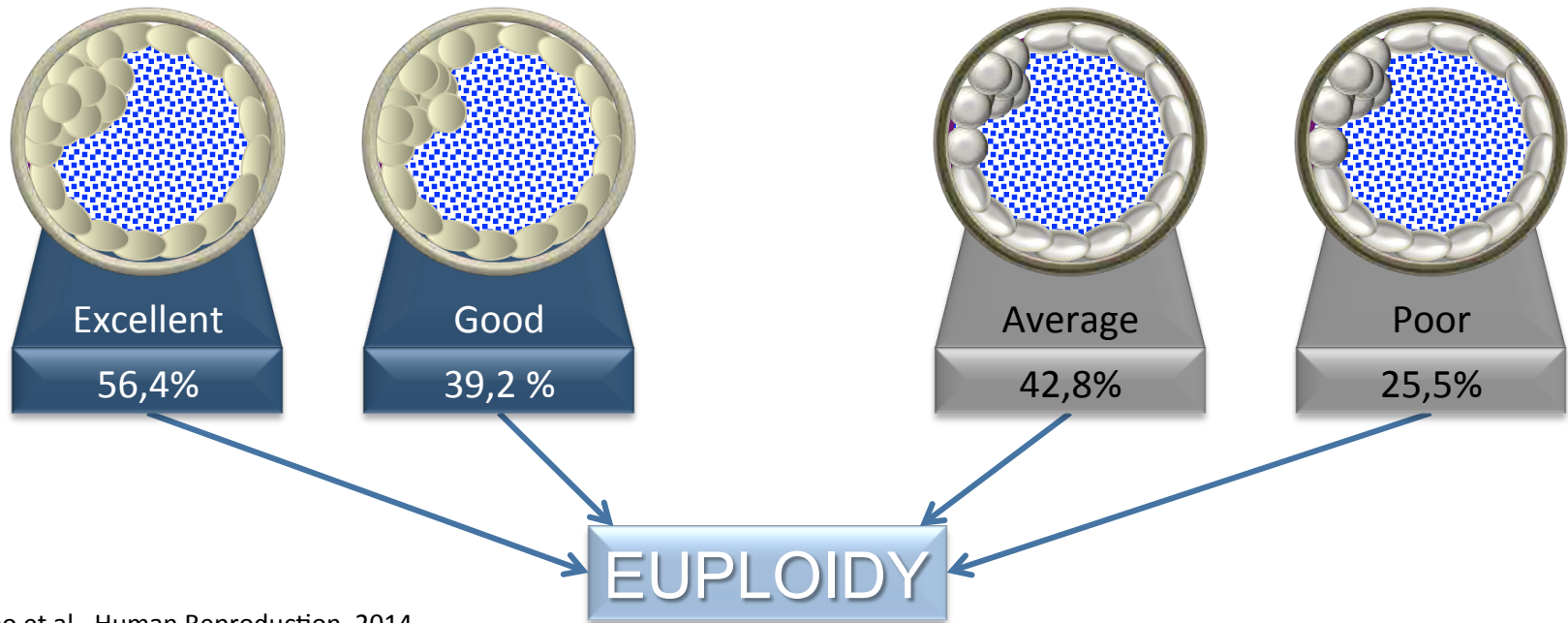
  
**AUC  $\approx$  0.7**

# Blastocyst culture to enhance selection



1. Blake, et al. 2007.
2. Papanikolaou, et al. 2008.

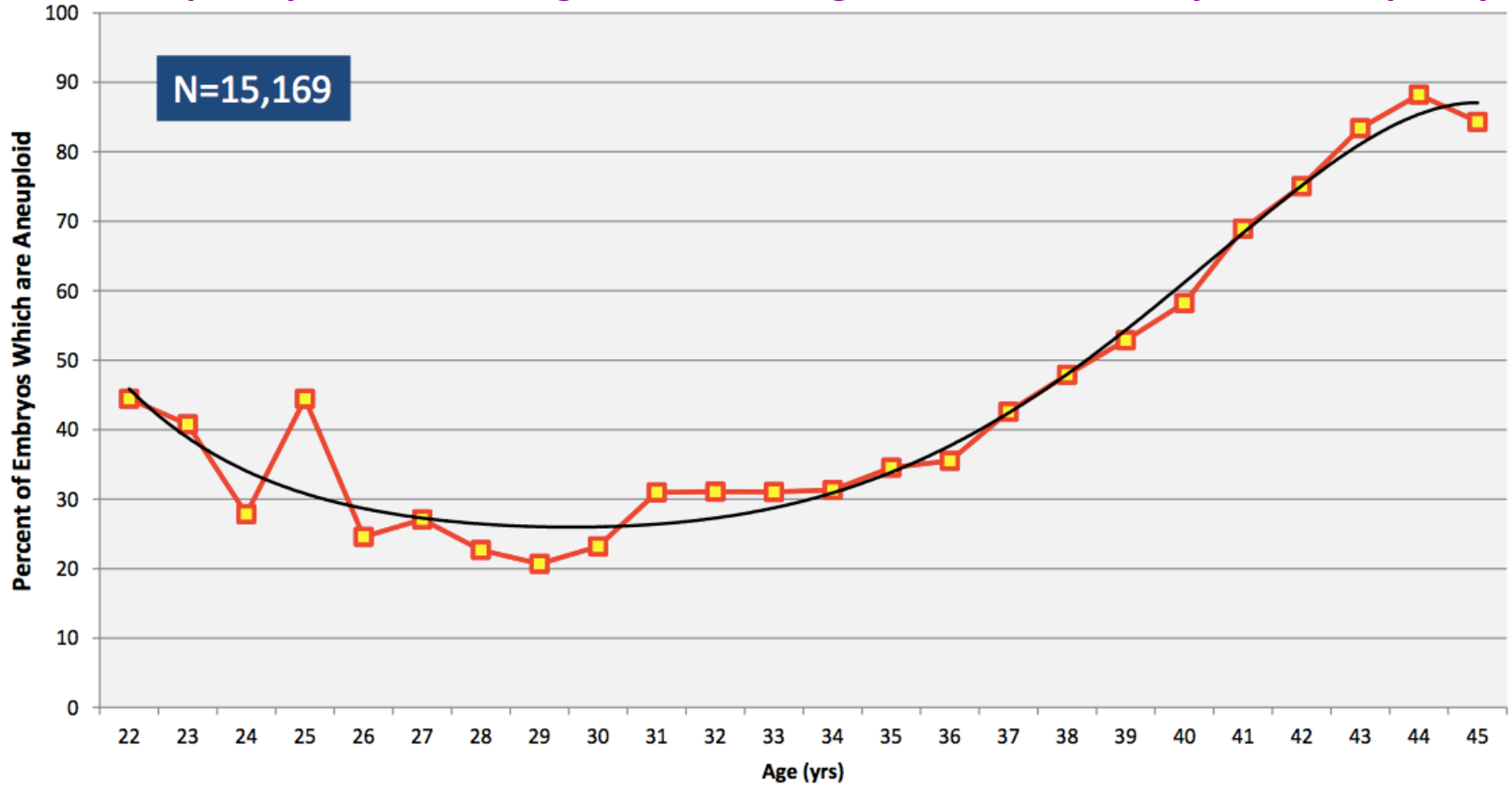
# Blastocyst morphology can not be relied on to ensure the transfer of the chromosomally normal embryo



# Why test for aneuploidy?

- Embryo selection
  - Morphology is a poor predictor of embryo viability
- Aneuploidy is the principal cause of
  - Failed implantation
  - Miscarriage
  - Down syndrome etc

# Contemporary Understanding of Maternal Age and Human Embryonic Aneuploidy



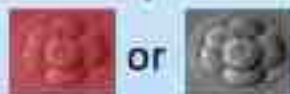
## Why test for aneuploidy?

Standard embryo evaluations do not reveal embryos with the wrong number of chromosomes

IVF treatment usually results in the production of several embryos



Ideally, one embryo is transferred to the uterus

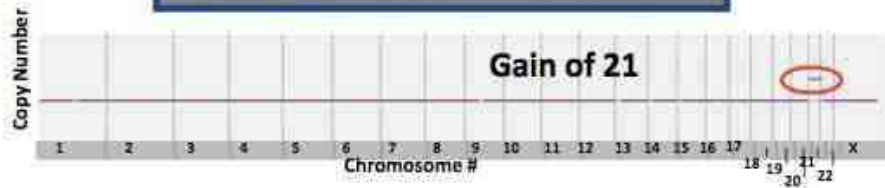


after chromosome screening

Munne et al., 1993

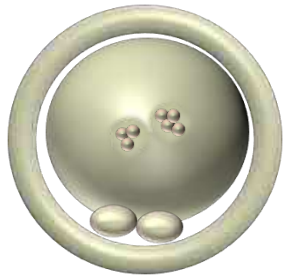
Is aneuploidy testing beneficial?

# Is transferring an aneuploid embryo acceptable?

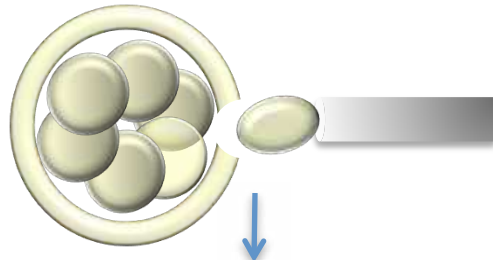
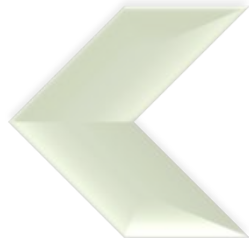


# PGS: Back or Forward to meet the challenge?

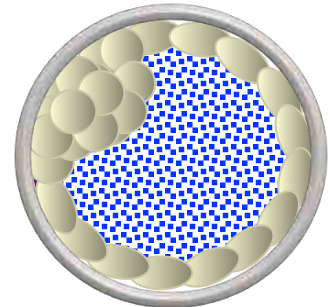
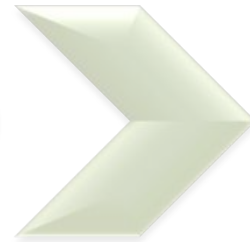
*“If the facts don’t fit the theory, change the facts.”*



- Male and mitotic errors not detected
- Amplification efficiency of both PBs low
- Higher rate of mis-diagnosis



Full chromosomal  
complement  
analysis



- Meiotic and mitotic aneuploidies
- More robust genetic diagnosis
- Reduced biopsy damage to the embryo

1. Geraedts, et al. 2011.

2. Scott, et al. 2012.

1. Schoolcraft, et al. 2010, 2011.

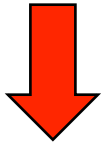
2. Fragouli, et al. 2010.

3. Capalbo, et al. 2012

Minimum requirements..

# Appropriate culture conditions should be maintained

**Temperature:  $37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$**



pH

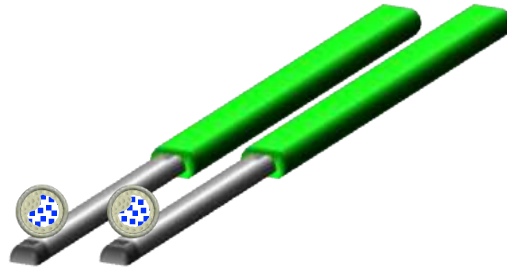
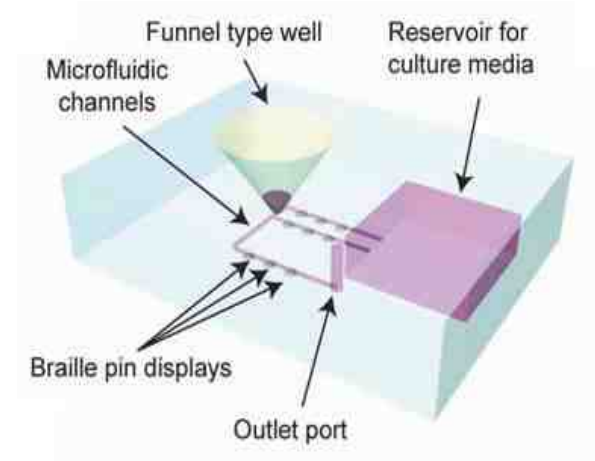


ROS production  
and REDOX state



# We must not forget solid foundation in our labs

1. Optimized and standardized culture system
2. Efficient Biopsy technology
3. Efficient vitrification program



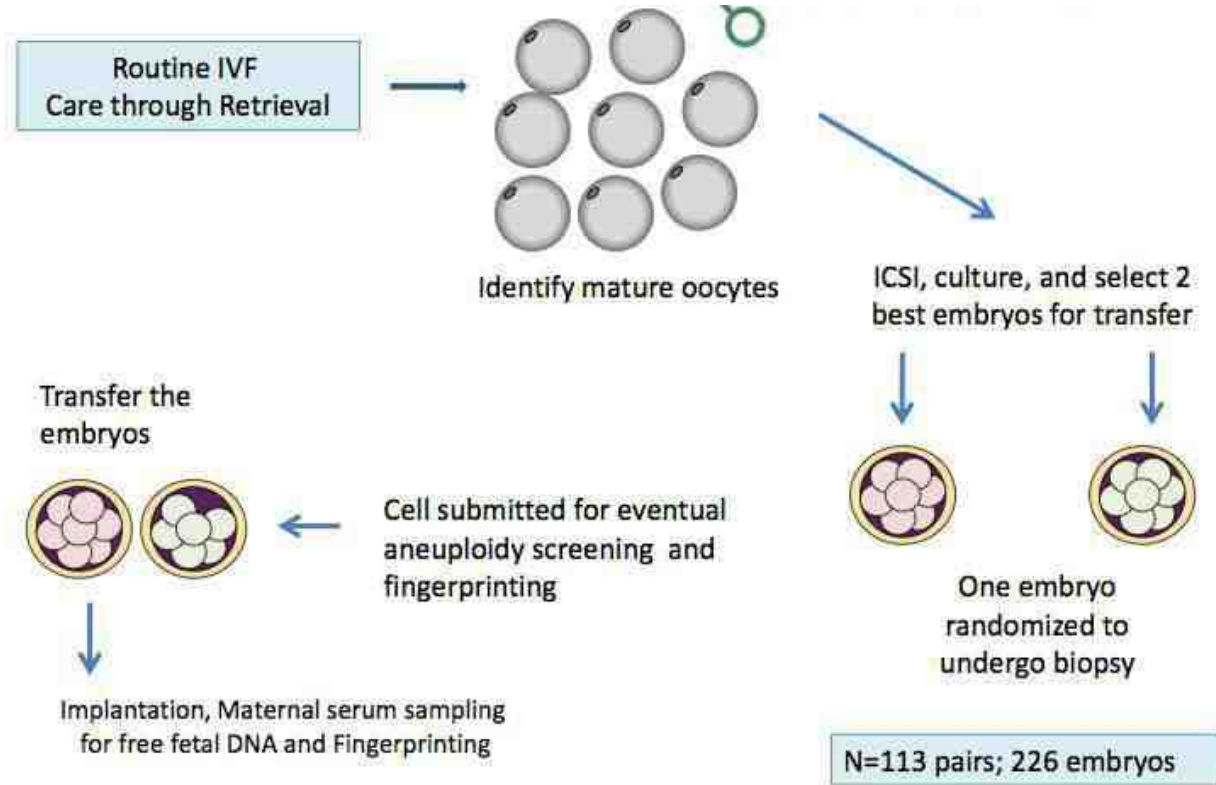
## Pros and cons of different preimplantation aneuploidy testing platforms

Method	Cost	Speed	Segmental detection	Mosaic detection
aCGH	\$\$	+++	+++	+++
qPCR	\$\$	++++	+	+
NGS	\$	+	++++	++++
SNP array	\$\$\$	++	++	++

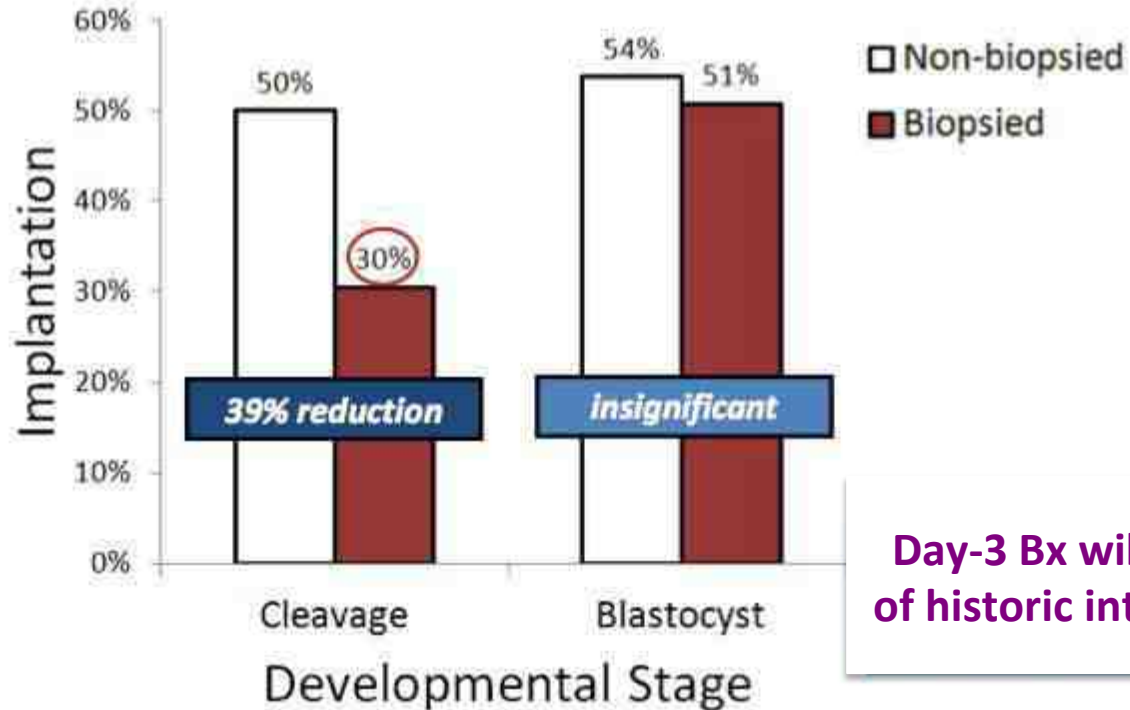
# Does aneuploidy testing work?

- High accuracy (>98%) testing of 24 chromosomes
- 5 RCTs
- All report significant advantages of aneuploidy testing
- None have presented any negative findings

# Does Embryo Biopsy Impact the Development Potential?



# Overall Implantation Rates



# Aneuploidy Testing Improves Outcome-RCT 1

- Women <35 yr-old (good prognosis patients)
- D-5 Bx; a-CGH; fresh D-6 ET
- Ongoing pregnancy rate (>20 weeks) per cycle started
  - Control: 41.7% (morphology only)
  - a-CGH: 69.1% (p=0.009)

## Aneuploidy Testing Improves Outcome-RCT 2

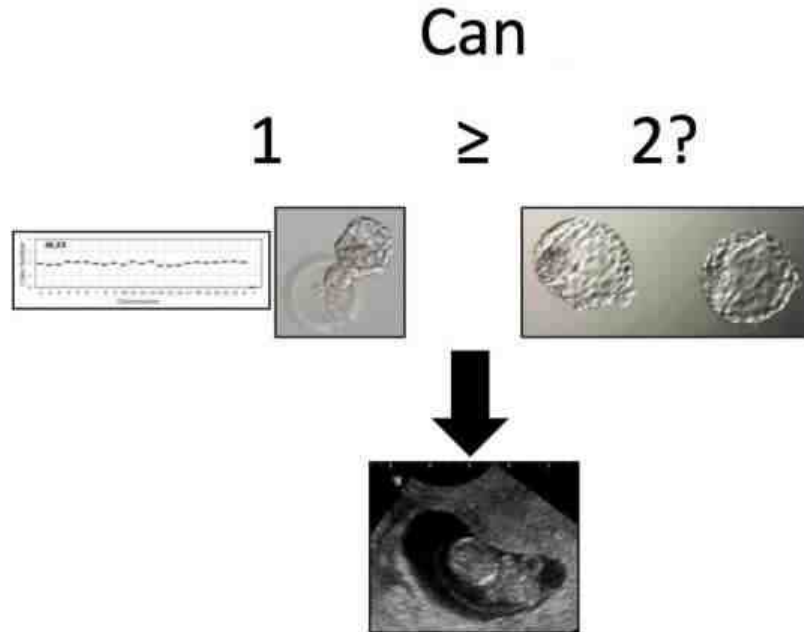
- Women >35 yr-old (mean 39 yr)
- Blastocyst Bx; SNP array; vitrification; transfer in later cycle

	Implantation rate	Miscarriage rate
Control	38.7 % <sup>a</sup>	17.4 % <sup>b</sup>
After testing	56.7 % <sup>a</sup>	0 % <sup>b</sup>

**a, b:** P<0.05

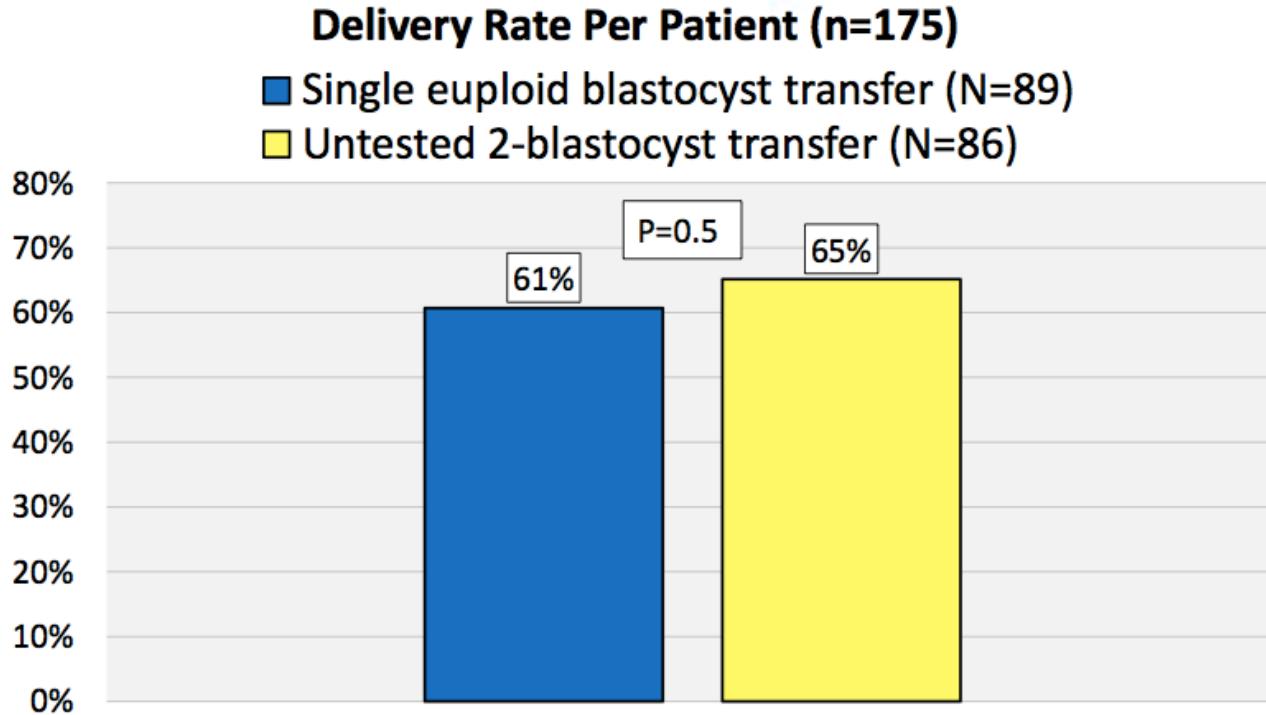
# Aneuploidy Testing Improves Outcome-RCT 3

- Women <43 yr-old (BEST Trial)
- D-5 Bx; qPCR; fresh D-6 transfer

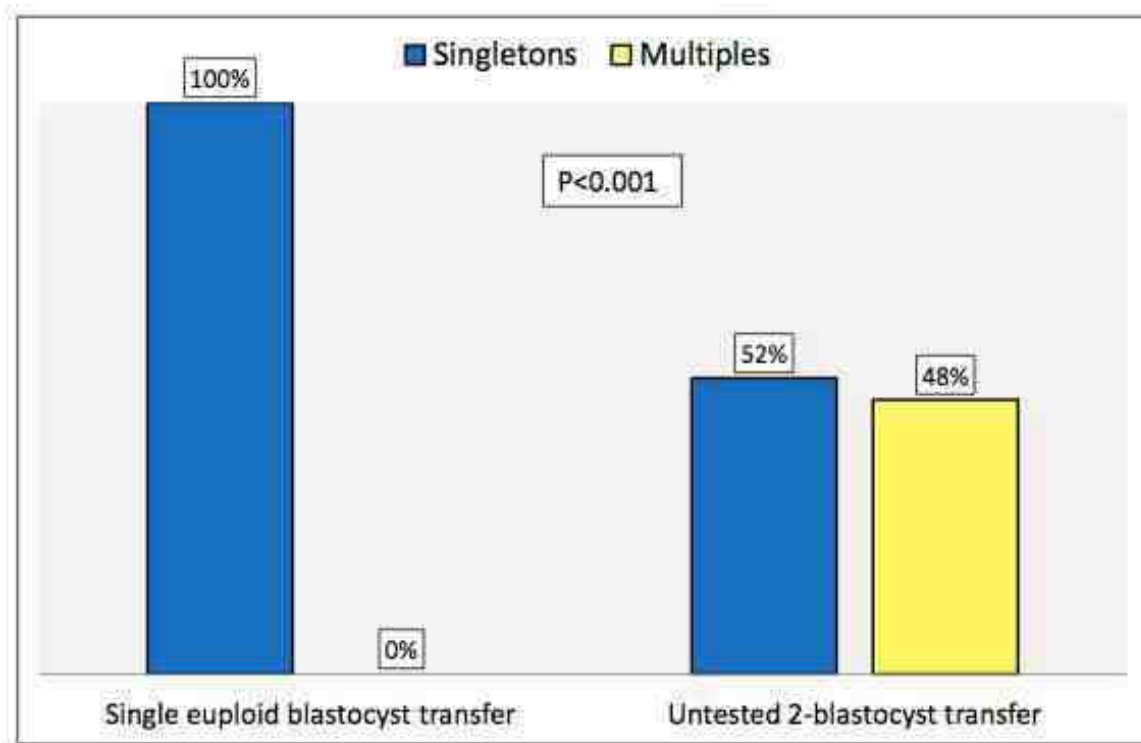


Forman et al. Fertil Steril 2013

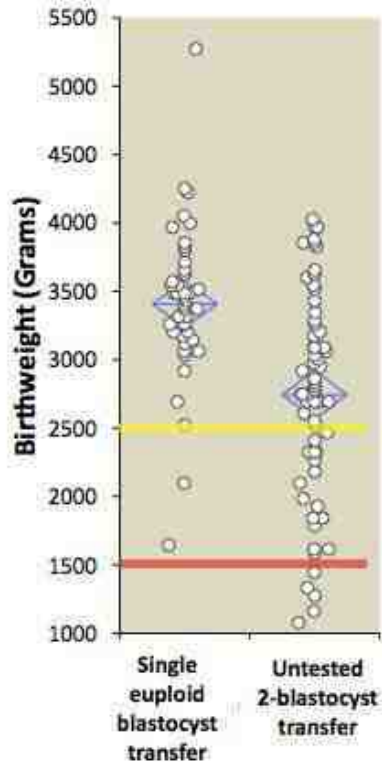
## RCT 3-Same delivery rate.. (RCT)



## RCT 3-Eliminates multiples..



# Better obstetric outcome..



- **Mean Birthweight:**

3408 562g – Single euploid

2745 743g – 2-Blastocyst  
( $P < 0.001$ )

- **Low birthweight (<2,500g):**

4.4% (2/45) – Single Euploid

31.9% (22/69) – 2-Blastocyst  
( $P < 0.001$ )

- **Very low birthweight (<1,500g):**

0% (0/45) – Single Euploid

7.2% (5/69) – 2-Blastocyst ( $P = 0.06$ )

## Aneuploidy Testing Improves Outcome-RCT 4

- Women 20-42 yr-old (mean 32 yr)
- D-5 Bx; qPCR; fresh D-6 transfer

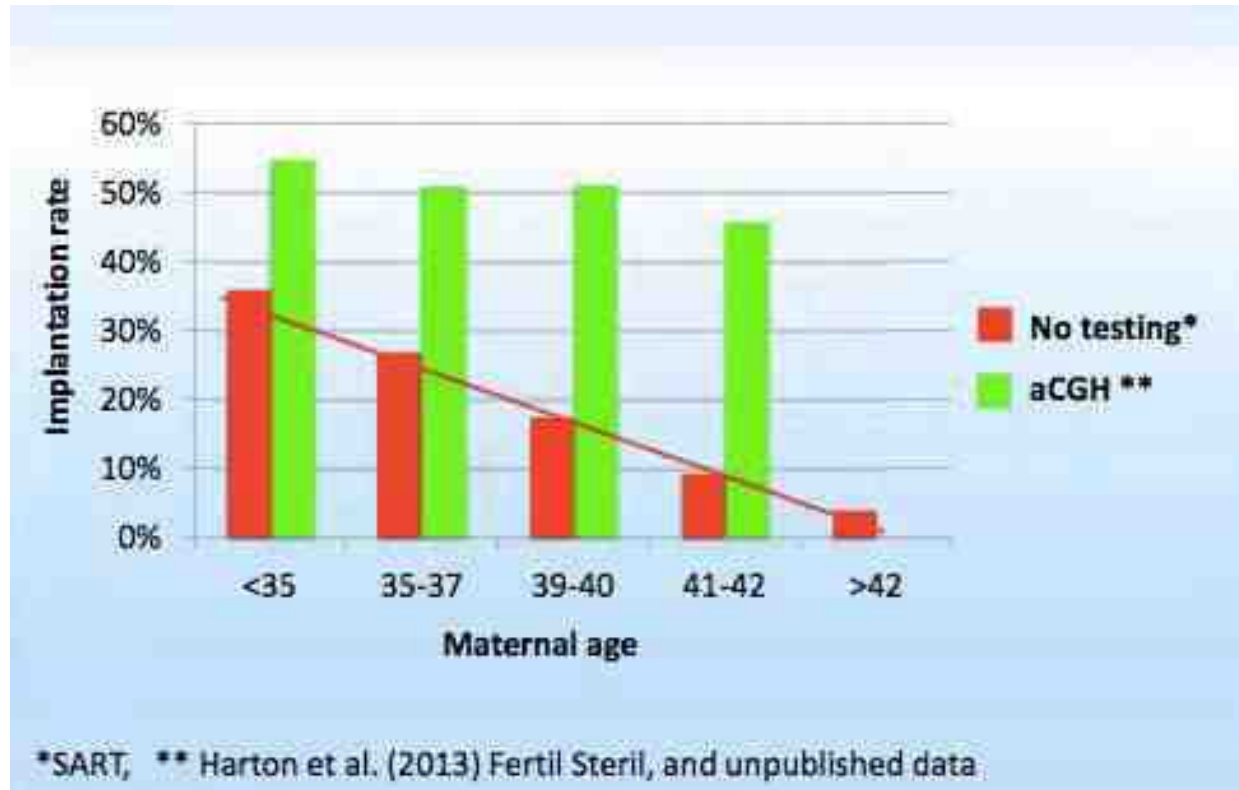
	Sustained Implantation rate	Delivery rate/cycle
Control	47.9 % <sup>a</sup>	67.5 % <sup>b</sup>
After testing	66.4 % <sup>a</sup>	84.7 % <sup>b</sup>

<sup>a, b</sup> = p<0.01

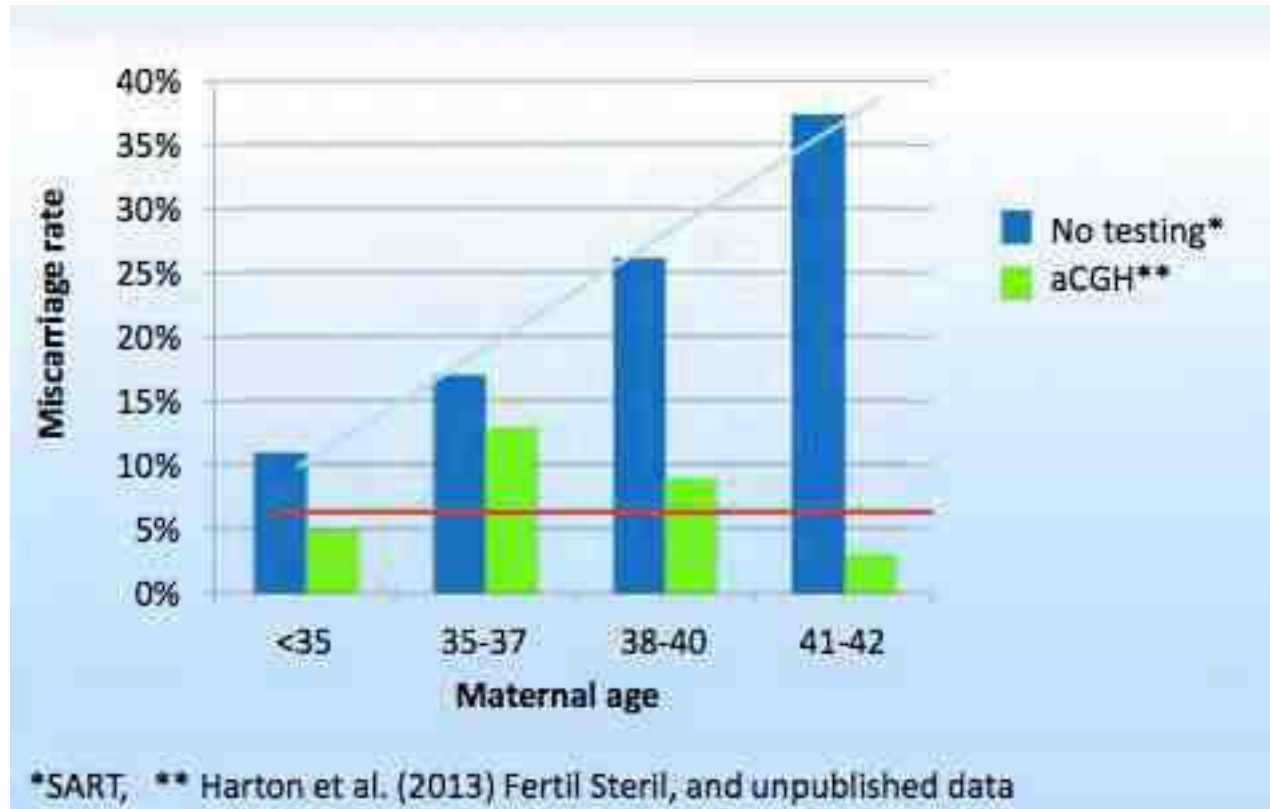
## Aneuploidy Testing Improves Outcome-Conclusions..

- One third to two third increase in implantation rate
- Remarkably similar results despite different clinics

# Aneuploidy Testing Improves Outcome

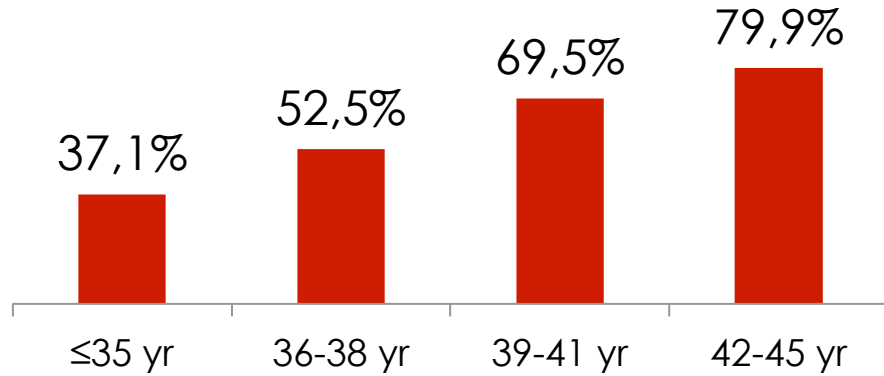


# Aneuploidy Testing Improves Outcome

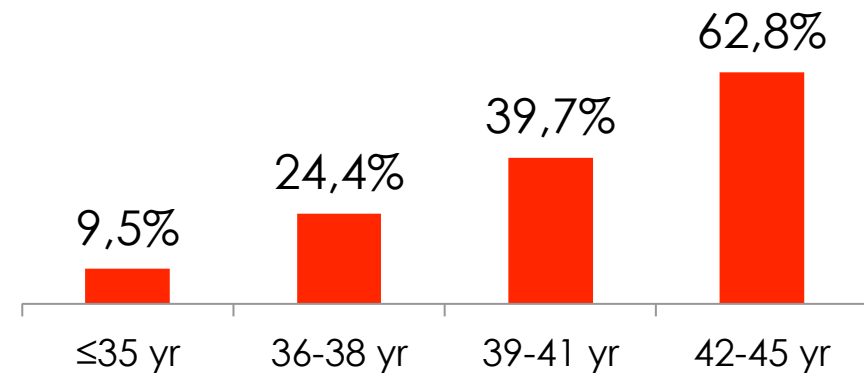


# PGS results stratified according to maternal age at OPU (GENERA; 630 cycles)

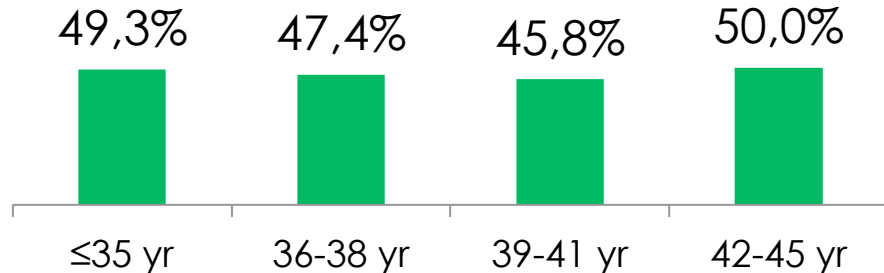
## Blastocyst aneuploidy rate



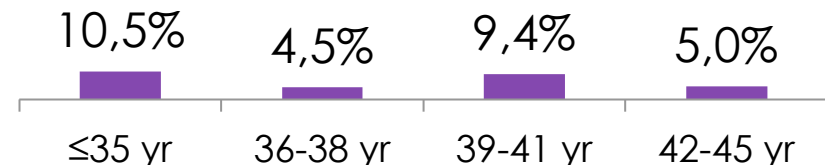
## Embryo cancellation rate



## Life birth rate / ET



## Abortion rate



# Our Results-Blastocyst Bx (a-CGH)

Start COS

Blast Bx

≥1 Euploid embryo

Pregnancy/ET



75 patients  
(88 cycles)

47 patients (63%)  
(52 cycles; 60%)

32 patients (43%)  
(31 cycles; 35%)

12/17 <sup>a</sup>

**a:** All SET; 1 biochemical, 1 miscarriage.

**Ongoing PR= 10/17= 59%**

## Clinical Experience

### *Misdiagnoses*

#### Clinical Error Rate

Per embryo	0.2%
Per transfer	0.3%
Per ongoing pregnancy	0.1%

- 4974 embryos
- 2976 gestations (62.1%)
- 10 errors
  - 1 tetraploid
  - 2 monosomies
  - 7 trisomies
- 3168 transfers
- 2354 ongoing / delivered (72.1%)
- Mean age 38.4 years
- 10 errors
  - 7 found in losses
  - 3 found in ongoing preg.

Mosaicism evaluated in 4 samples – 100% mosaic

## Should all patients be offered aneuploidy testing?

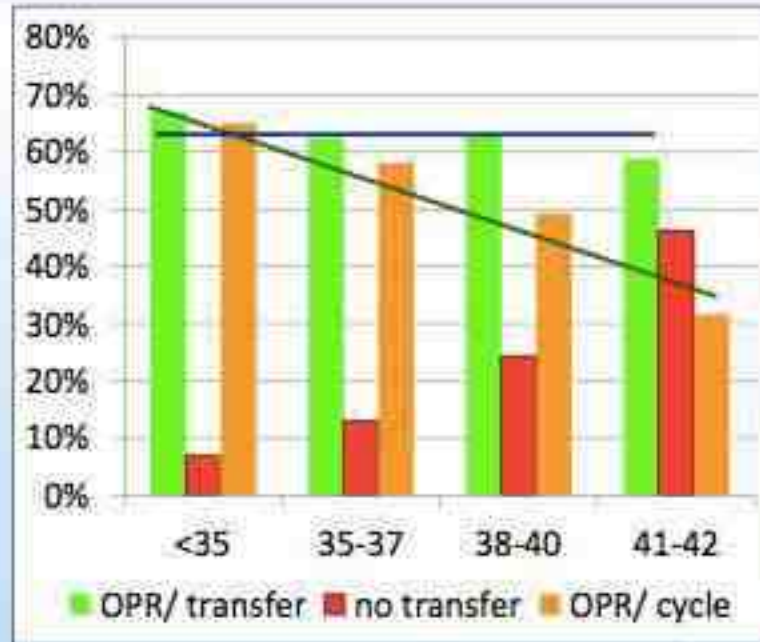
- Traditional aneuploidy testing are:
- Advanced maternal age: >34 ?; >36 ?; >38 ?
- Recurrent miscarriage- 2 or more ?; 3 or more ?
- Recurrent implantation failure; 2 or more ?; 3 or more ?
- Good evidence that good prognosis patients benefit

# Should all patients be offered aneuploidy testing?



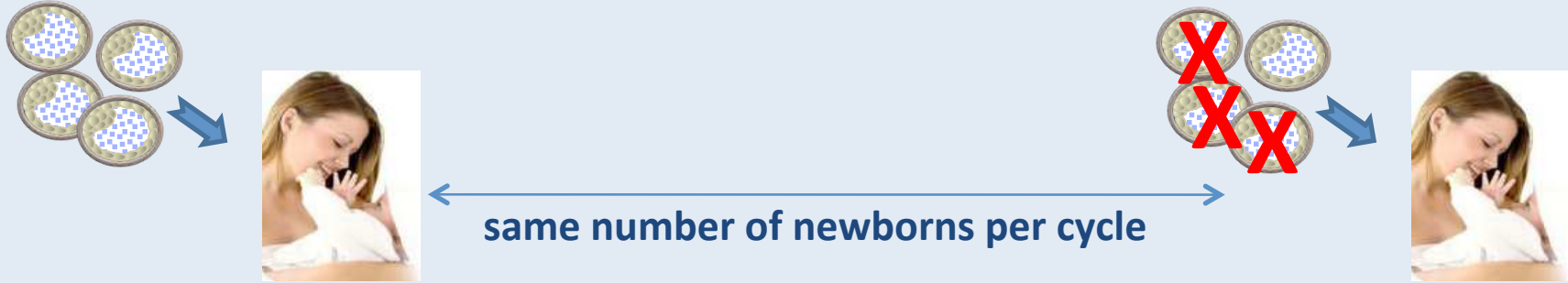
Harton et al. (2013) Fertil Steril, and unpublished data

# Should all patients be offered aneuploidy testing?



Harton et al. (2013) Fertil Steril, and unpublished data

**EFFICACY** = *in IVF refers to the number of baby born per stimulation cycle*



**EFFICIENCY** = *it describes the extent to which time, effort or cost is well used for intended task or purpose*

Lower abortion rate



Lower abnormal pregnancies



Less time to pregnancy



Single embryo transfer and less multiple pregnancy



More Cost-effective



# Aneuploidy Testing-Conclusions

- Avoids multiples (very efficient eSET strategy)
- Avoids aneuploid transfers and pregnancies
  - Miscarriage; Down syndrome etc
- Avoids cryopreservation of aneuploid embryos
- Faster time to pregnancy

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At. Caddesi No: 1, 06450  
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Phone: +90 312 454 0000  
Fax: +90 312 454 0001  
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