PGS 2.0

Will it work this time?

Thorir Hardarson

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Content

- Background – PGS 1.0
- PGS 2.0 – what is new?
- Will it work?

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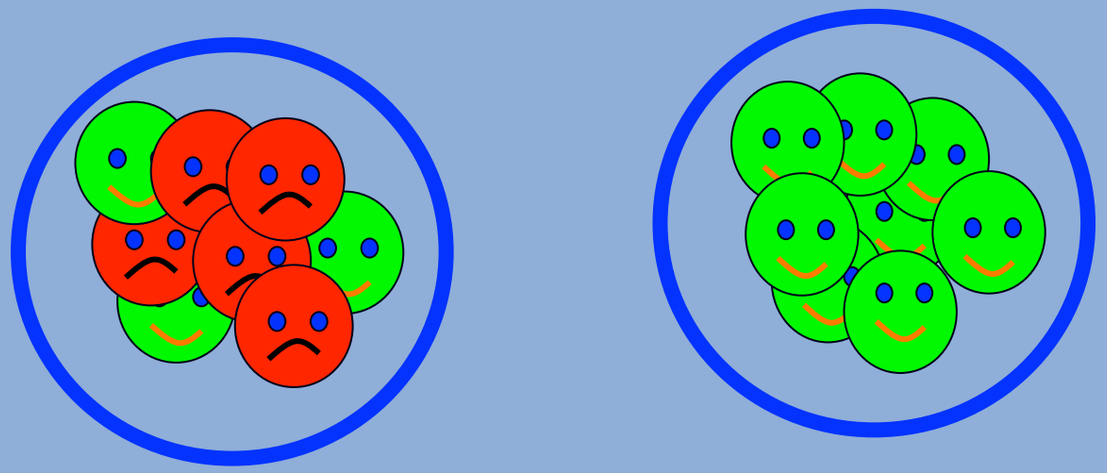
PGS

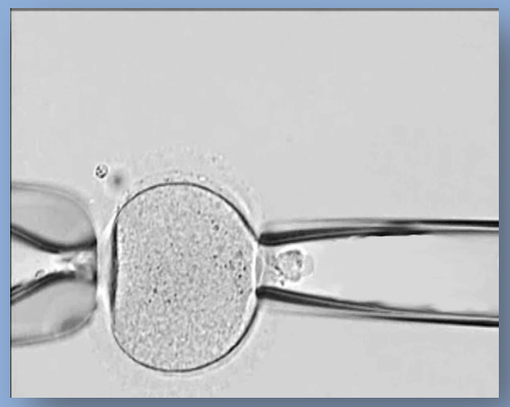
(Preimplantation Genetic Screening)

Transfering a chromosomally normal embryo increases the chance of a life birth



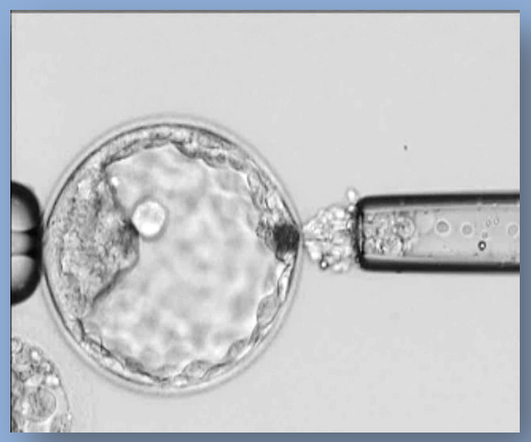
Invasive - Biopsy

Polar Body

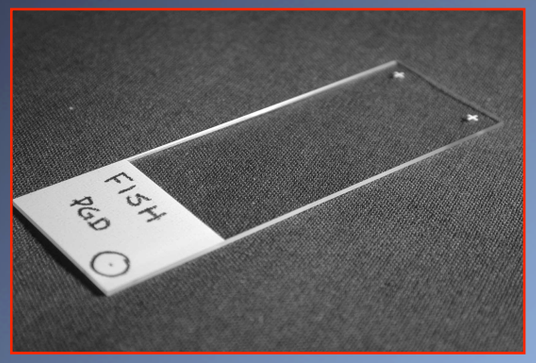


Blastomere

Blastocyst



FISH

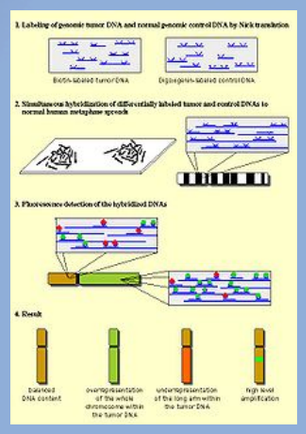


Number of 5-9 chromosomes, Known translocations, deletions

PCR

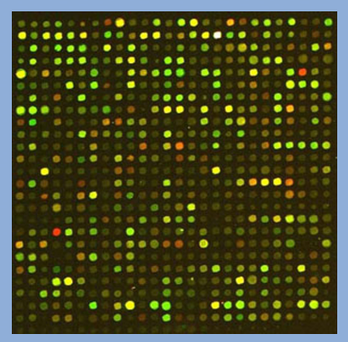
Specific desease genes/ absence

CGH



All chromosomes, translocations, deletions

Microarrays



All chromosomes, translocations, deletions,, specific genes and expression

Patient groups

Advanced Maternal Age (AMA)

IVF failure (2-3 failures)

Altered karyotype

Repeated misscarrages

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Prospective randomized controlled studies

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 5, 2007 VOL. 357 NO. 1

Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial

T. Hardarson[1,3], C. Hanson[2], K. Lundin[2], T. Hillensjö[1], L. Nilsson[2], J. Stevic[2], E. Reismer[1], K. Borg[1], M. Wikland[1] and C. Bergh[2]

[1]*Fertility Centre Scandinavia, Carlanderska Hospital, Box 5418, 402 29 Göteborg, Sweden;* [2]*Department of Reproductive Medicine, Sahlgrenska University Hospital, 413 45 Göteborg, Sweden*

[3]Correspondence address. E-mail: thorir.hardarson@fcivf.com

BACKGROUND: Advanced maternal age (AMA) is an important parameter that negatively influences the clinical pregnancy rate in IVF, in particular owing to the increased embryo aneuploidy rate. It has thus been suggested that only transferring euploid embryos in this patient group would improve the pregnancy rate. The purpose of this study was to test whether employing preimplantation genetic screening (PGS) in AMA patients would increase the clinical pregnancy rate. **METHODS:** We conducted a two-center, randomized controlled trial (RCT) to analyze the outcome of embryo transfers in AMA patients (≥38 years of age) after PGS using FISH analysis for chromosomes X, Y, 13, 16, 18, 21 and 22. The PGS group was compared with a control group. The primary outcome measure was clinical pregnancy rate after 6–7 weeks of gestation per randomized patient. **RESULTS:** The study was terminated early as an interim analysis showed a very low conditional power of superiority for the primary outcome. Of the 320 patients calculated to be included in the study, 56 and 53 patients were randomized into the PGS and control groups, respectively. The clinical pregnancy rate in the PGS group was 8.9% (95% CI, 2.9–19.6%) compared with 24.5% (95% CI, 13.8–38.3%) in the control group, giving a difference of 15.6% (95% CI, 1.8–29.4%, $P = 0.039$). **CONCLUSIONS:** Although the study was terminated early, this RCT study provides evidence against the use of PGS for AMA patients when performing IVF. Trial registration number: ISRCTN38014610.

Keywords: AMA; PGS; embryo biopsy; RCT; IVF

Human Reproduction Vol.19, No.12 pp. 2849–2858,
Advance Access publication October 7, 2004

Comparison of blasto preimplantation gene in couples with adva randomized controlle

Catherine Staessen[1,3], Peter Plat Tournaye[1], Michel Camus[1], Pau

[1]Centre for Reproductive Medicine and [2]Centr (Vrije Universiteit Brussel), Laarbeeklaan 101

[3]To whom correspondence should be addresse

BACKGROUND: It is generally accepte implantation and a higher abortion ra group to assess the possible benefit of p outcome after assisted reproductive tec trial (RCT) was carried out comparing rescence *in situ* hybridization (FISH) fo years) with a control group without PG allocated to the trial, an oocyte pick- control cycles). RESULTS: Positive ser controls: 35.8% (19.6%) [%/per embry fewer embryos were transferred in the rate (with fetal heart beat) was 17.1% $P = 0.09$). We observed a normal diploi arguments in favour of PGD-AS for i there are no restrictions in the number

Key words: age/aneuploidy screening/FSH/pre

Why did PGS 1.0 not work?

- The biopsy
- Too few chromosomes analysed
- Wrong patient groups

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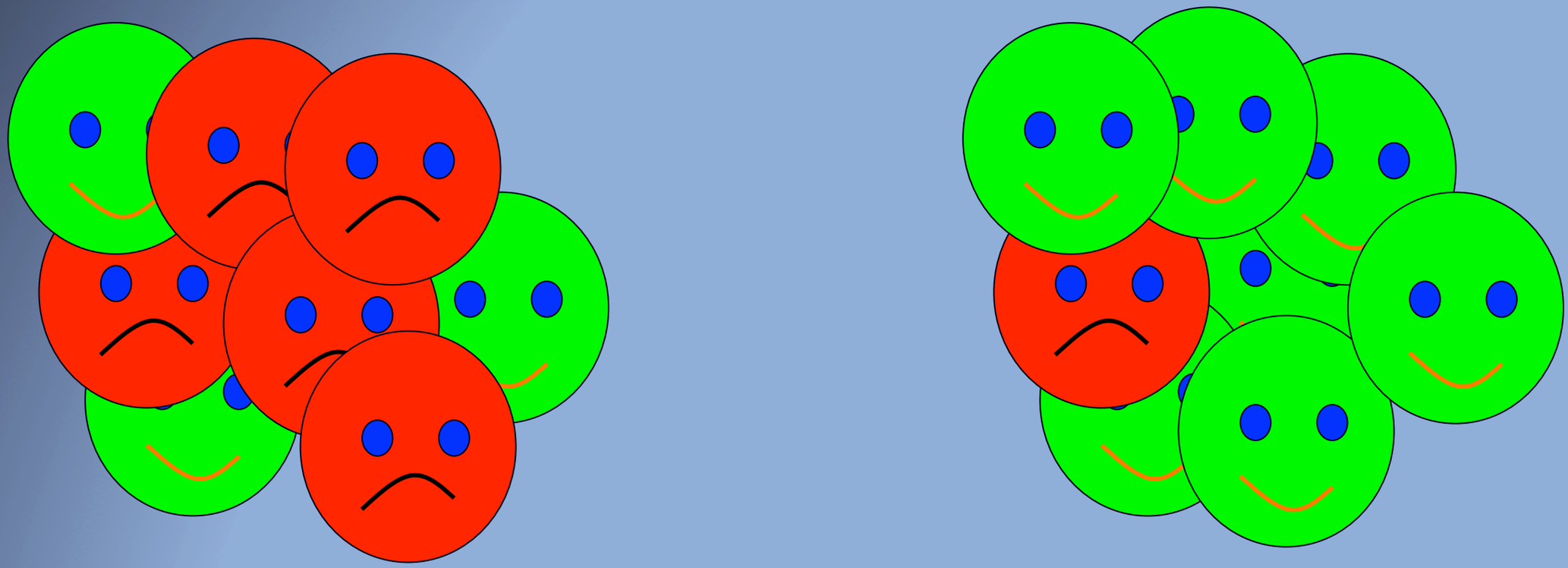
Mosacism



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PGS 2.0

- Blastocyst biopsy
  - **<u>Less mosacism</u>**

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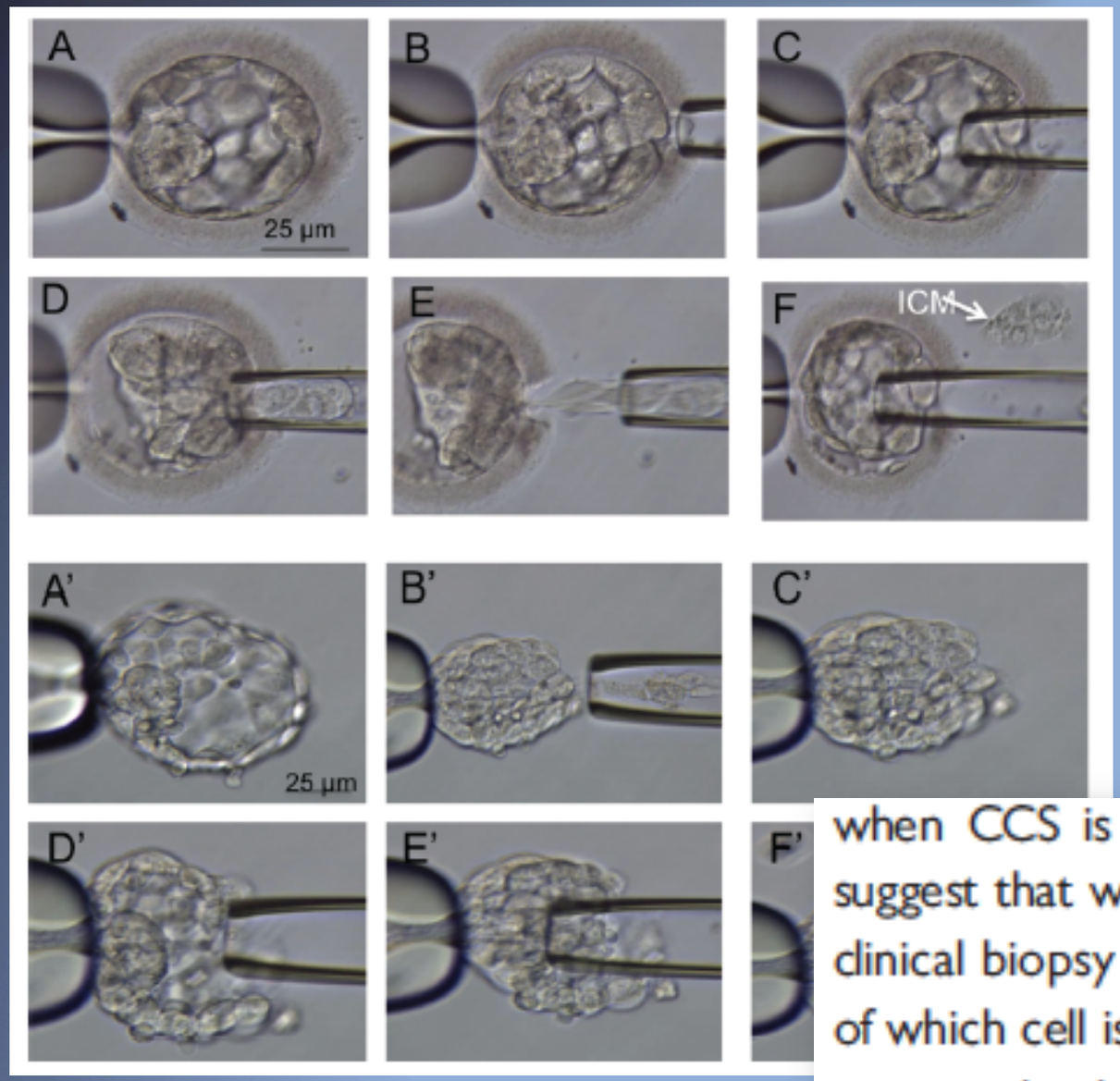
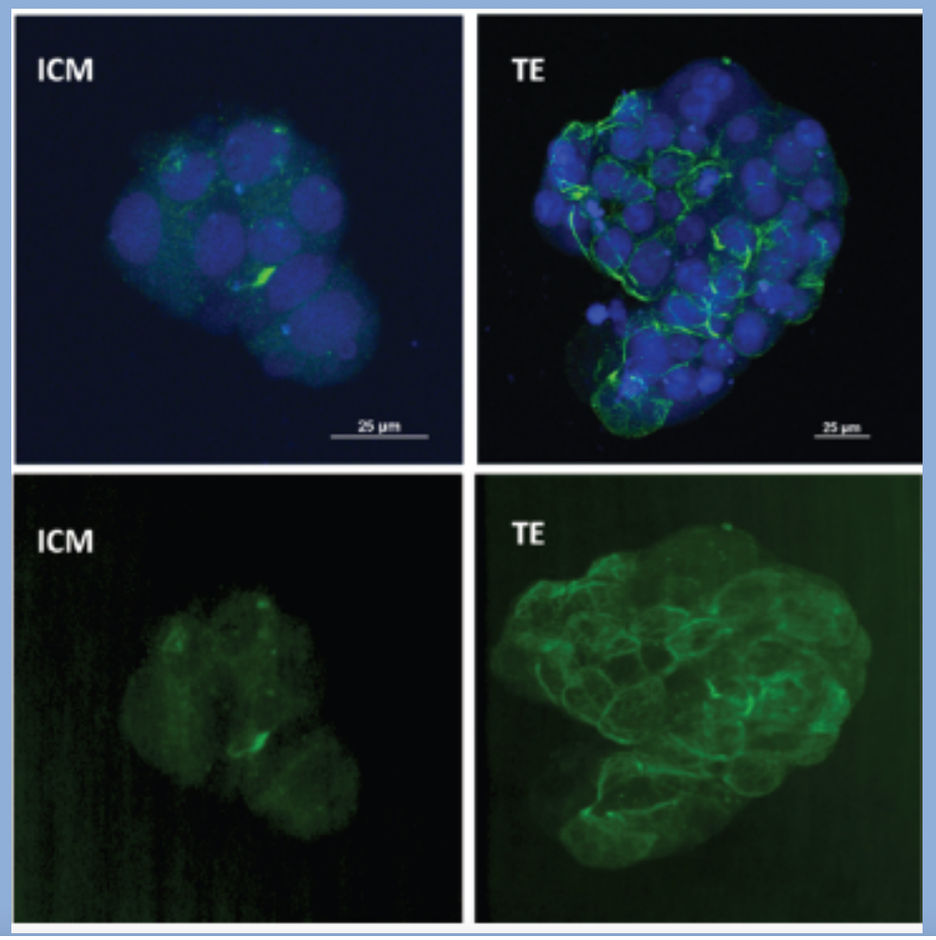
FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage

Antonio Capalbo[1,*], Graham Wright[2], Thomas Elliott[2], Filippo Maria Ubaldi[1], Laura Rienzi[1], and Zsolt Peter Nagy[2]





when CCS is performed at the blastocyst stage. All these findings suggest that when good morphology blastocysts are considered, a TE clinical biopsy can correctly classify the embryo karyotype regardless of which cell is biopsied. Furthermore, data indicate that mosaicism is not a major issue for blastocyst stage PGS programs considering the low prevalence of mosaic diploid/aneuploid embryos and the high detection rate of clinically relevant mosaicism. The biological features of

PGS 2.0

- Blastocyst biopsy
  - **<u>Less mosacism</u>**
  - **Better survival**

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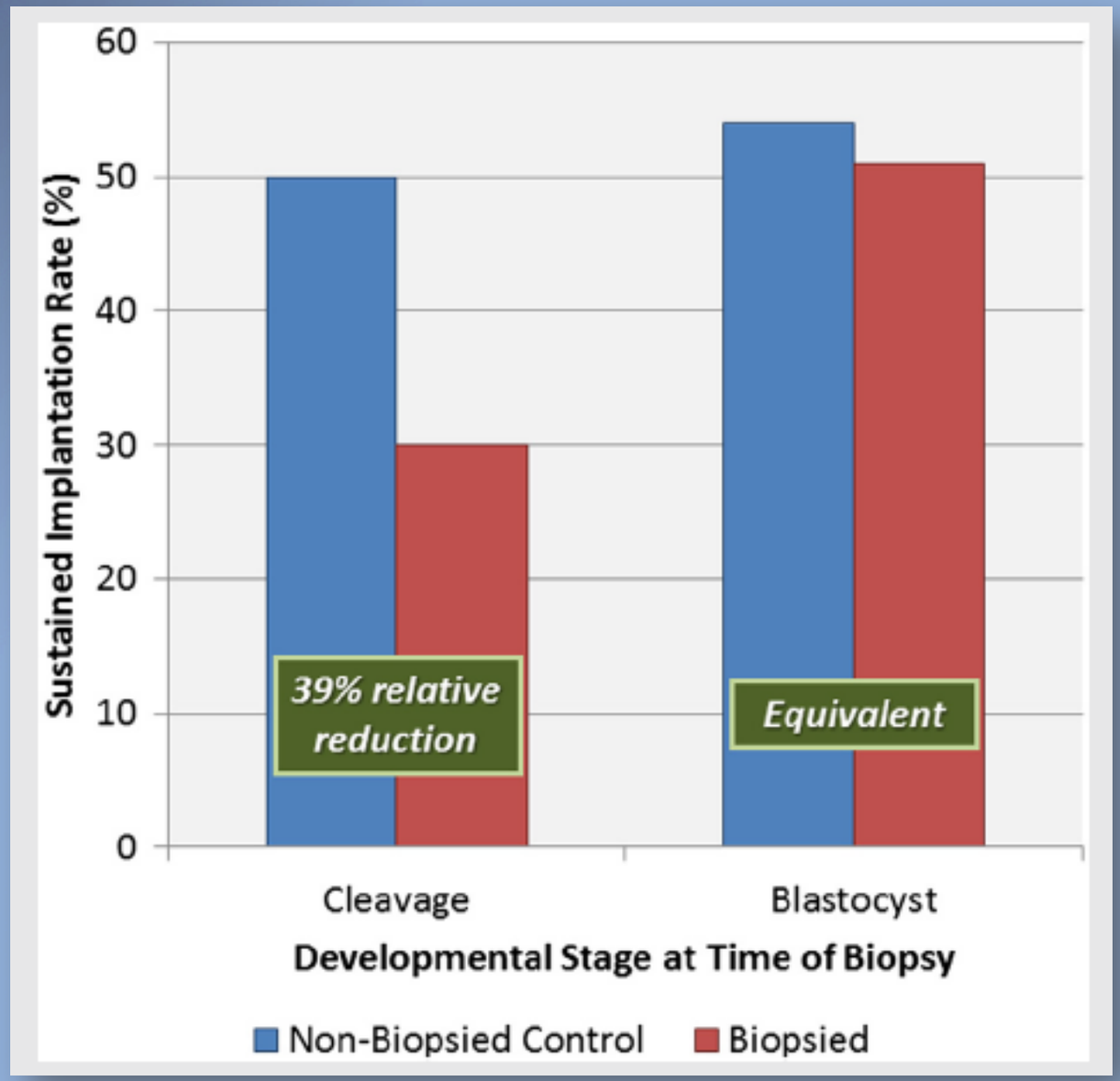
Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial



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PGS 2.0

- Blastocyst biopsy
  - **Less mosacism**
  - **Better survival**
- Array CGH + +
- All chromosomes analyzed + +
- Delayed transfer (vitrification - natural cycle)
- New patient groups

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PGS 2.0 - results

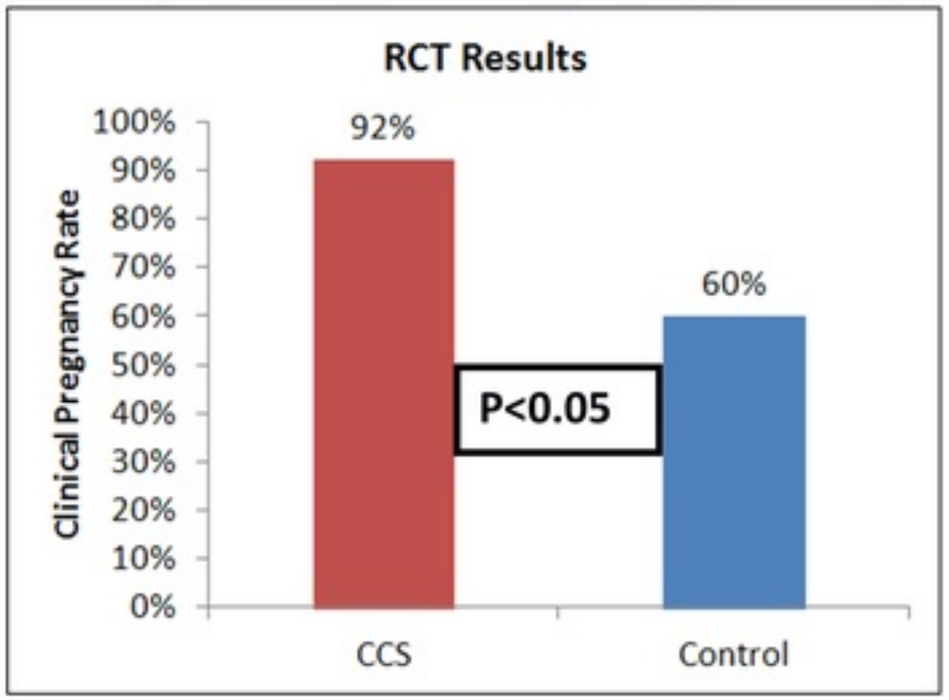
	Preg rate PGS / transfer	preg rate Contr / transfer	Diff	
Scott R 2010	92% (n=13)	60% (n=15)	32%	Retrospect, SNParray
Forman EJ 2012	55% (n=140)	42% (n=182)	13%	Retrospect, SET, qPCR
Yang Z 2012	69% (n=55)	42% (n=48)	27%	Prospect rand, SET, Blue Gnome array
Mir P 2013	60% (n=320)	N D	N A	Prospect, 1,5 emb/ transfer, Blue Gnome array
Keltz M 2013	61.5%* (n=39)	32.5%* (n=394)	29%	Retrospect, BlueGnome array
Greco E 2014	68.3% (n=43)	21.2% (n=33)	43.9 %	

* Ongoing preg rate/started cycle

ASRM 2012

Good Prognosis Patients, TE D5 Biopsy and Fresh D6 Transfer – Randomized Control Trial

Maternal age: CCS = 34, Control = 32 years; <1 prior failed cycle



Scott et al., ASRM 2010

First-time IVF patients with a good prognosis (age <35, no miscarriage)

	aCGH (n=55)	Morphology alone (n=48)	P value
Grade 5/6	31	28	
Grade 4	21	19	0.677
Grade 3	3	1	
Clinical Pregnancy	70.9%	45.8%	0.017
Ongoing Pregnancy	69.1%	41.7%	0.009
MAB	2.6%	9.1%	0.597

Yang et al., Molecular Cytogenetics 2012

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Making Dreams Conceivable

ASRM 2012

Comprehensive chromosome screening (CCS) with vitrification, results in improved clinical outcome in women >35 years: a randomized control trial

William B Schoolcraft, Eric Surrey, Debra Minjarez,
Robert Gustofson, Richard T Scott Jr* & Mandy G Katz-Jaffe

Colorado Center for Reproductive Medicine
**Reproductive Medicine Associates of New Jersey*

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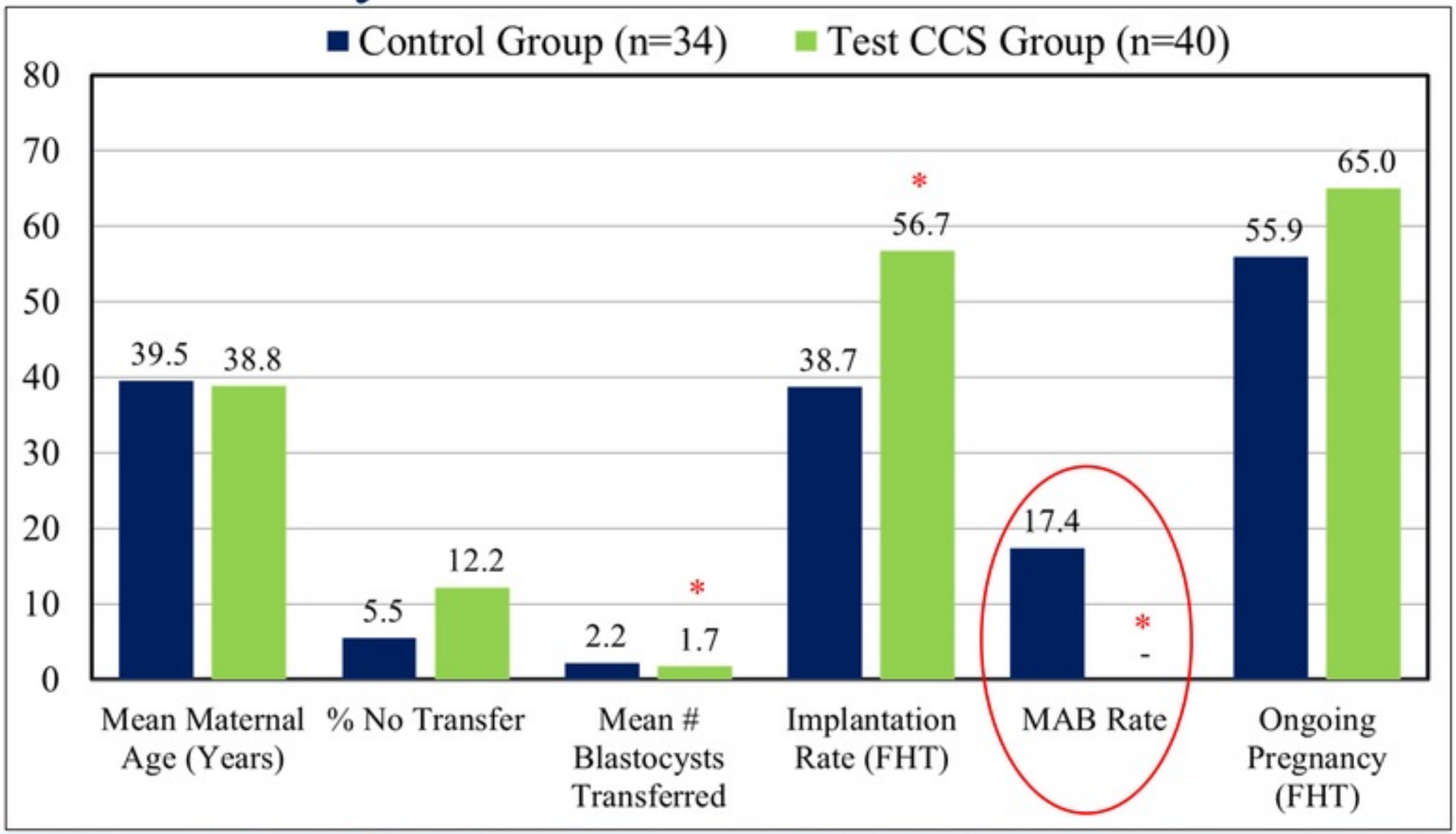
Cycle and Transfer Outcome



Fishers Exact Test; *Significance = P<0.05

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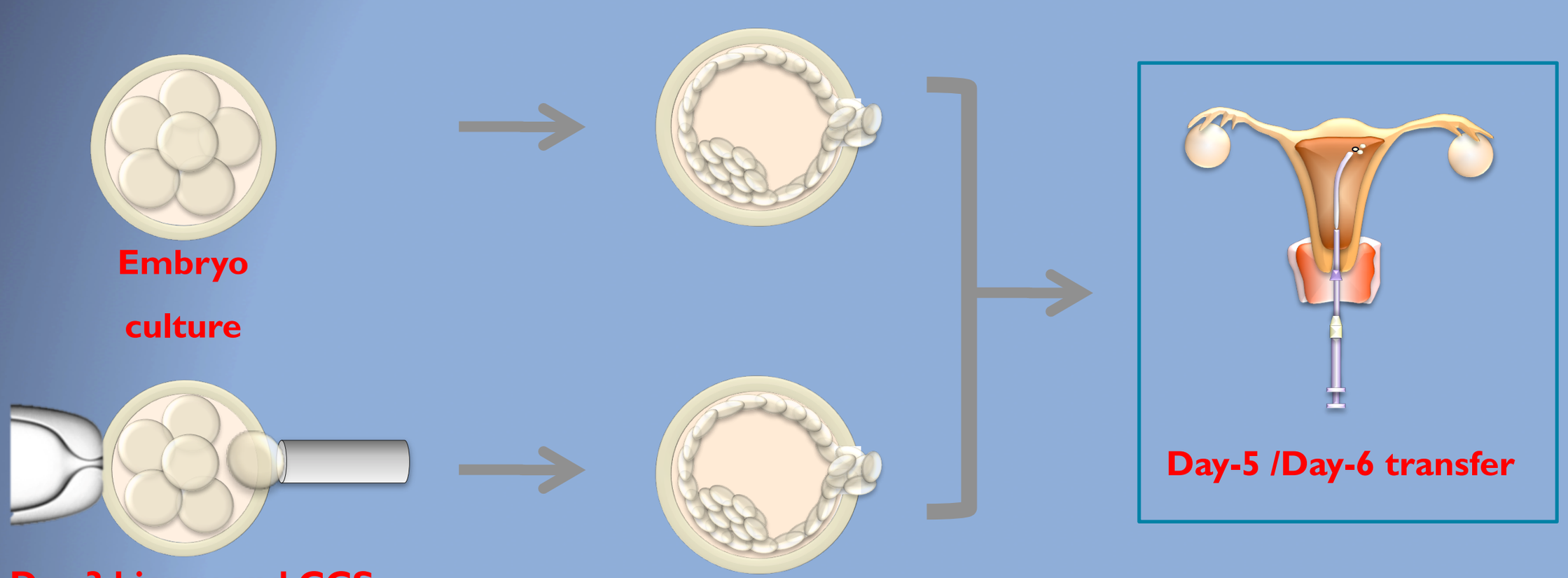
RCTs Design

- **Sample size:** 120 patients per arm for 15 points difference in the endpoints of ongoing pregnancy rates per cycle and delivery rates (α 5%, β 20%).
- **Patient allocation:** through computer-generated randomization into two groups: conventional blastocyst transfer or day-3 biopsy with transfer of euploid blastocysts.



ClinicalTrials.gov NCT01571076

Day-3 RCT in AMA (May 2012- April 2014)

Inclusion criteria:

- ✓ Women Age: 38-41 years
- ✓ Normal Karyotypes
- ✓ First or second ICSI cycle
- ✓ ≥5 MII from 1 or 2 cycles
- ✓ Sperm: ≥5 million sperm/mL

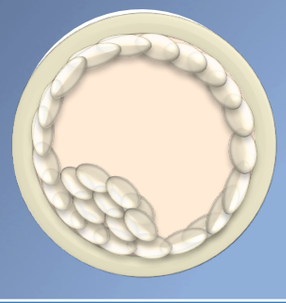
Exclusion criteria:

- ✓ Previous abnormal pregnancy
- ✓ Previous PGS/PGD cycles
- ✓ ≥ 2 previous miscarriages
- ✓ ≥ 2 previous IVF failures
- ✓ Uterine abnormalities

Interim, Rubio et al., ESHRE 2014

ClinicalTrials.gov NCT01571076

Day-3 RCT in AMA (May 2012- April 2014)



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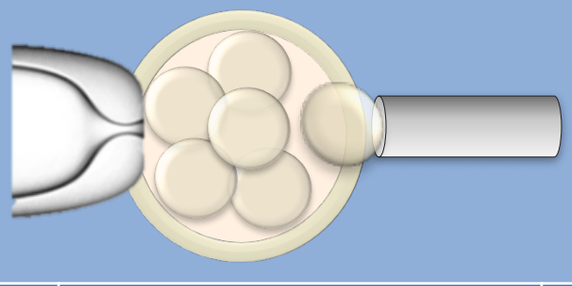


	Blastocyst	CCS	*P-value*
No. of cycles performed	86	75	---
Mean age (SD)	39.0 (2.8)	39.5 (3.0)	*NS*
Percentage of transfers	96.5	70.7	*p<0.0001*
Mean embryos/transfer	1.8 (0.6)	1.3 (0.7)	*p<0.0001*
No. of pregnancies	39	33	---
No. of miscarriages (%)	17 (43.6)	1 (3.3)	*p<0.0001*
Ongoing PR/transfer*	26.5	60.4	*p=0.0001*
Ongoing PR/cycle*	**25.6**	**42.7**	*p=0.0294*
Ongoing IR	18.4	58.6	*p=0.0001*

**12 weeks ongoing pregnancies*

** p<0.05 Two-side Fishers´ test*

Interim, Rubio et al., ESHRE 2014

RESULTS (May 2012- July 2014)

Study Group

Inclusion criteria:

- ✓ Sperm count ≤2 million /mL
- ✓ Women Age <38 years
- ✓ Normal Karyotypes
- ✓ First or second ICSI cycle
- ✓ ≥5 MII from one or two cycles

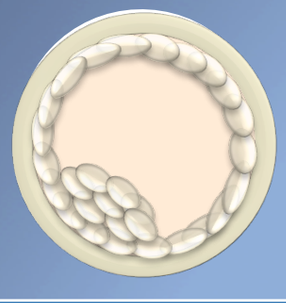
Exclusion criteria:

- ✓ Previous abnormal pregnancy
- ✓ Previous CCS/PGD cycles
- ✓ ≥ 2 previous miscarriages
- ✓ ≥ 2 previous IVF failures
- ✓ Uterine abnormalities

Interim, Rubio et al., ASRM 2014

ClinicalTrials.gov NCT01571076

RESULTS (May 2012- July 2014)



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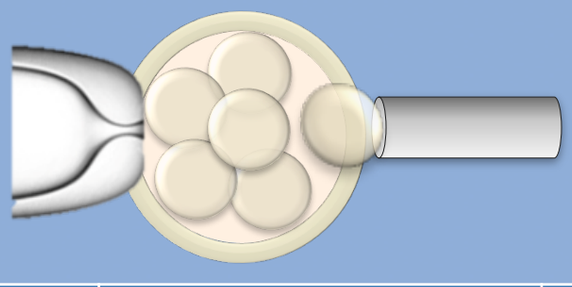

	Blastocyst	CCS	*P-value*
No. of cycles performed	41	44	---
Mean age (SD)	32.6 (3.4)	33.0 (2.8)	*NS*
Percentage of transfers	97.6	88.6	*NS*
Mean embryos/transfer (SD)	**1.9 (0.6)**	**1.5 (0.7)**	*P=0.0079*
No. of pregnancies	23	29	---
No. of miscarriages (%)	**6 (26.1)**	**1 (3.4)**	*P=0.0237*
Ongoing PR/transfer*	42.5	71.8	*P=0.0078*
Ongoing PR/cycle*	**41.5**	**63.6**	*P=0.0334*
Ongoing IR	22.7	55.2	*P=0.0001*

12 weeks ongoing pregnancies* * *p<0.05 T-Student and one-side Fishers´ test* ***Interim, Rubio et al., ASRM 2014*

RCT in Göteborg

- Woman, max 39 years, min 3 IVF with ET of fresh embryos without a clinical pregnancy
- Where we expect at least 8-10 oocytes
- Normal to high responders (AMH min 1.5 ng/ml AFC min 12)
- Ejaculated spermatozoa
- Randomized on day 1
- Blastocyst culture, <u>all</u> vitrified (even the control group)

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Power-analysis

Power-analys has shown that to detect a difference in pregnancy rates (week 18) between PGS and control groups of at least 15% (25% to 40%) we need to randomize 112 patients in total (alfa 0.05, beta 0.20).

To compensate for drop-outs (ca 10%) a **total of 130 patients will need to be recruited**.

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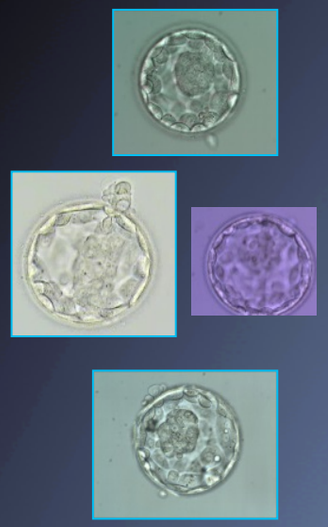
Study plan – PGS



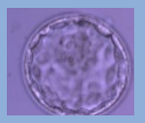
Day 5-6

Biopsy of TC cells

”Tubing”

Vitrification of blastocysts directly after biopsy

Rewarming of ***”normal”*** blastocyst



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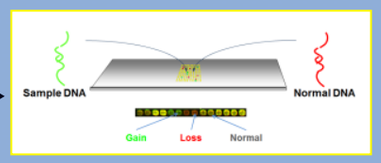
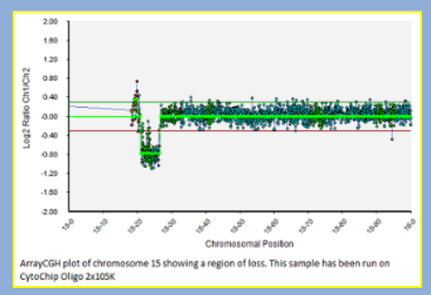


BlueGnome



Microarray analysis aCGH

Results

Will it work?

- The theory speaks for it
- It could still fail though…….
  - May need PGS 3.0 or 3.4.8
  - May be too expensive for ”main younger patients”
- New techniques:
  - Next Generation Sequencing
  - SNA / Karyomapping
  - More…..

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Future

- Sequencing based single platform for translocations, aneuploidy screening and single gene disorders (done on same platform and multiple indications simulateneously)?
- Ethical problems off knowing ”too much”
- Limitation on creating ”perfect mutation free baby” is number of embryos available at present
- PGD just part of a wider genetic infusion into IVF as part to a mission to help couples have a healthy singleton (e.g. carrier screening for all couples and fertility panel of actionable known genetic variants which affect fertility and can be used to make changes to therapy.

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Conclusion

- PGS will become an integral part of IVF
- PGS/PGD will become increasingly important as we learn more of how to intrepid the information we get.

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Those that can afford it, will get pregnancy through IVF

"Everything else is irresponsible"

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