Preimplantation Genetic Diagnosis

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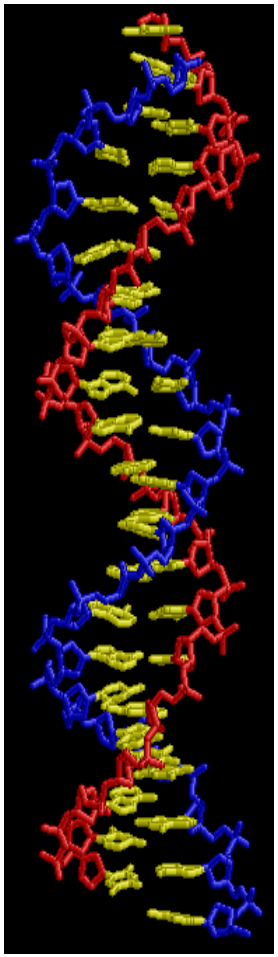
Indications PGD



Single Gene Defects

Gender Selection
X-Linked Diseases

PGD

HLA Typing

Structural Chromosomal Aberrations
Deletions
Translocations

PGS=CCS

Aneuploidy
Advanced Maternal Age
Recurrent Pregnancy Loss
Multiple Failed IVF cycles

Screening vs. Diagnosis

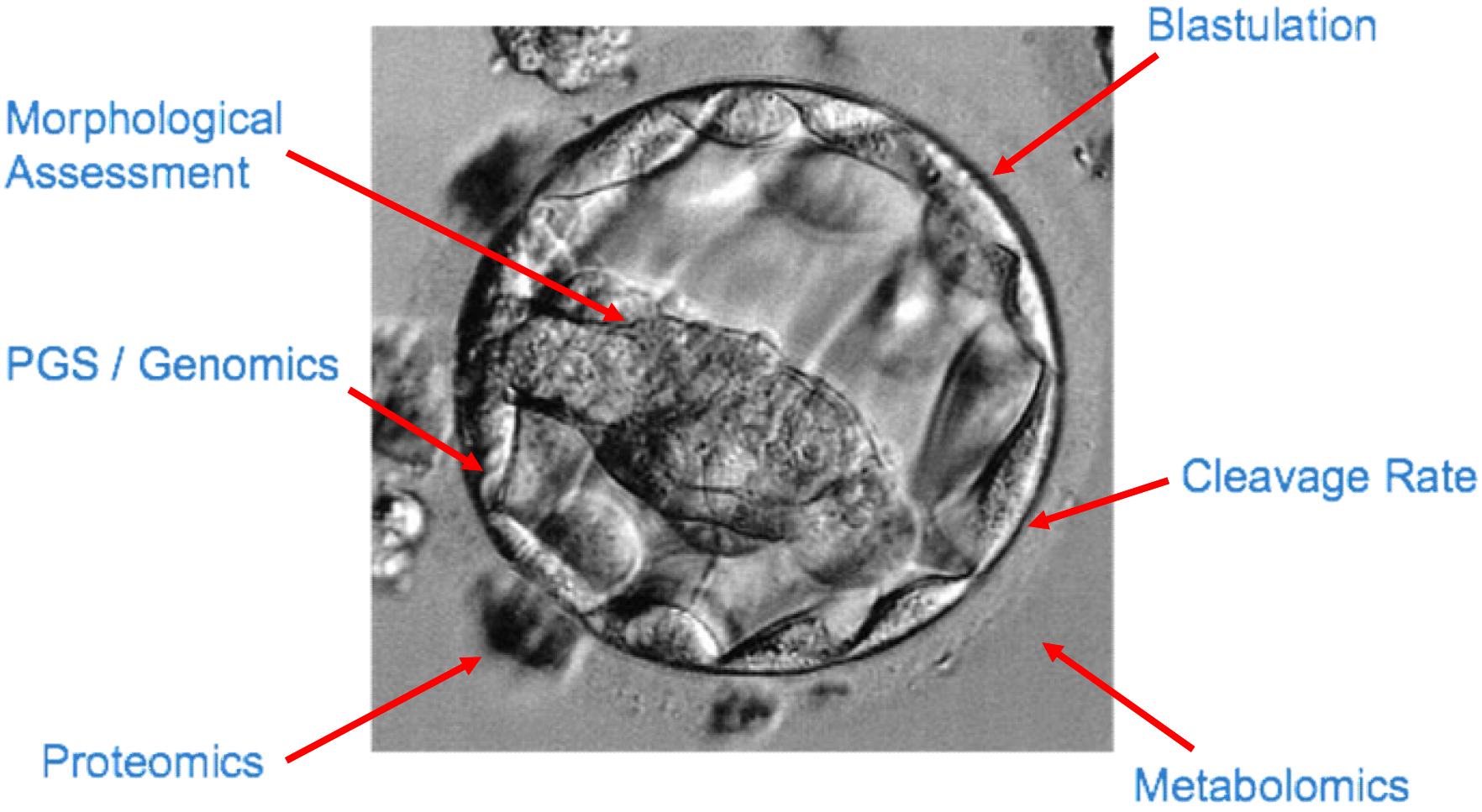
TABLE 1

Screening versus diagnostic testing of chromosome copy number in preimplantation embryos.

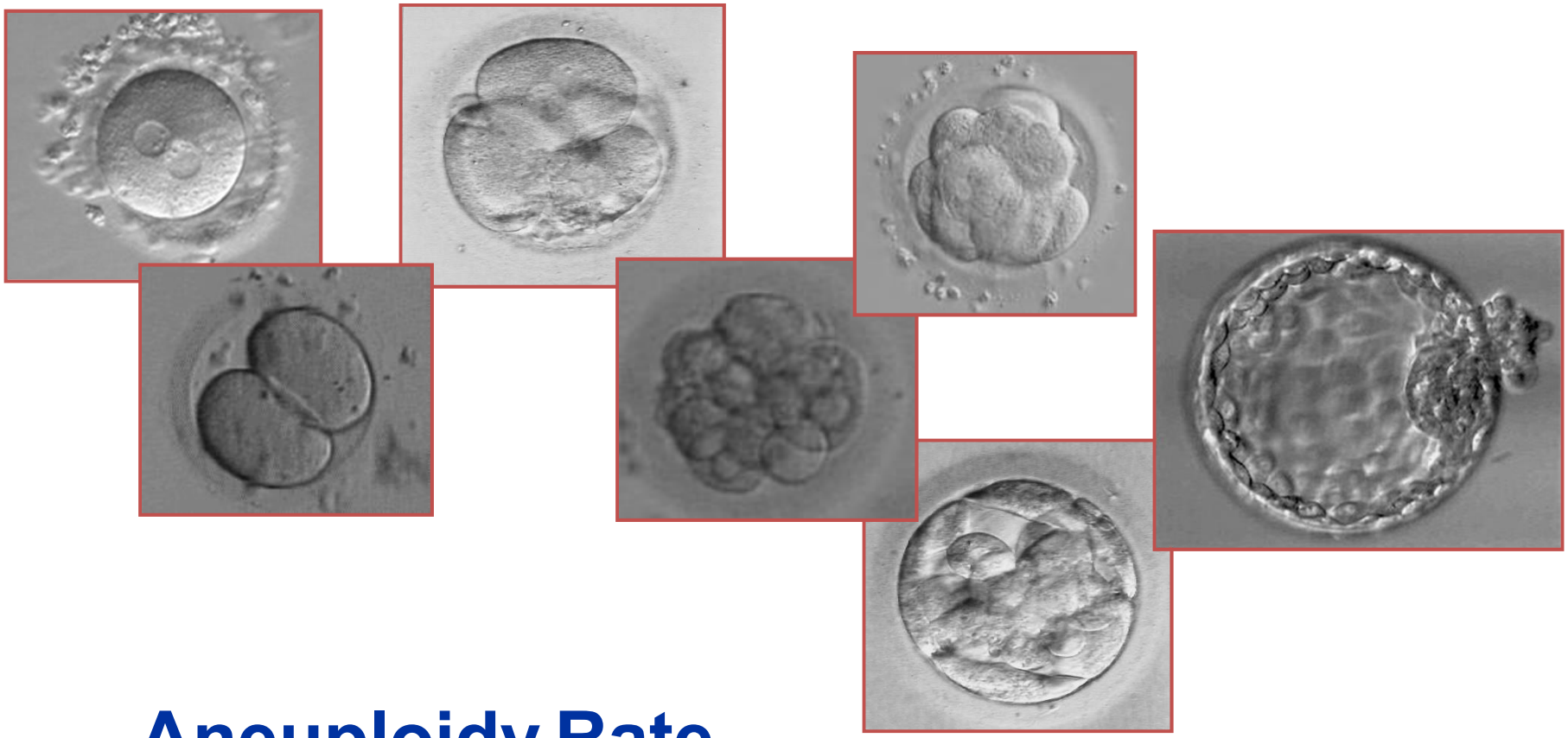
Screening	Diagnosis
All patients	Specific indications
Minimally invasive	Invasive
All embryos	Good-quality embryos only
Rapid with fresh transfer	Rapid with fresh transfer, or not time limited with vitrification
High efficiency	Moderate efficiency
Direct or indirect	Direct
Accurate	Highly accurate
Low false negatives acceptable	Tolerate false positives
	No false negatives
Clinically effective	Validation of diagnostic accuracy
Randomized control trials	
Low cost	Medium to high cost

Handyside. 24-chromosome copy number analysis. Fertil Steril 2013.

Embryo Assessment



Embryo Development



Aneuploidy Rate

80% → 40%

Munne et al, 2013; Yang et al, 2012; Peterson, et al. *GSN* 2012

The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening

Jason M. Franasiak, M.D.,[a] Eric J. Forman, M.D.,[a,b] Kathleen H. Hong, M.D.,[a,b] Marie D. Werner, M.D.,[a,b] Kathleen M. Upham, B.S.,[b] Nathan R. Treff, Ph.D.,[a,b] and Richard T. Scott Jr., M.D.[a,b]

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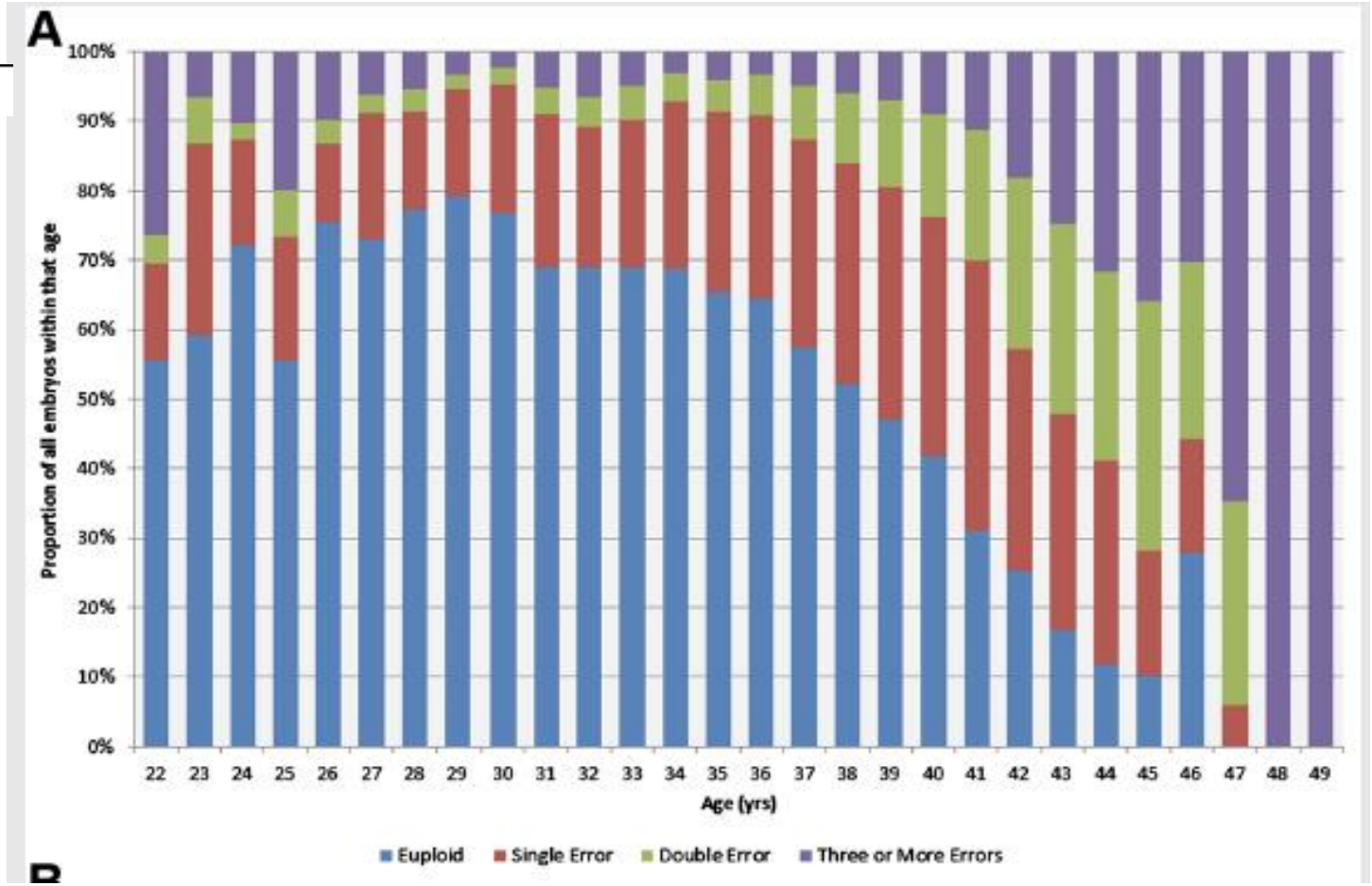
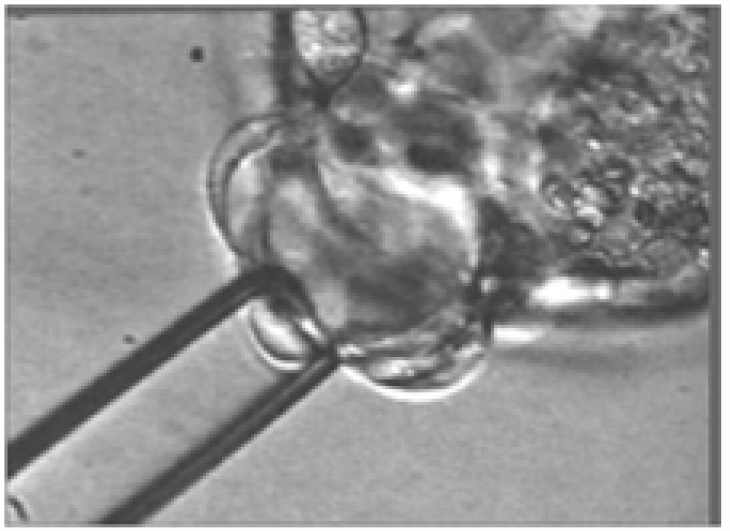


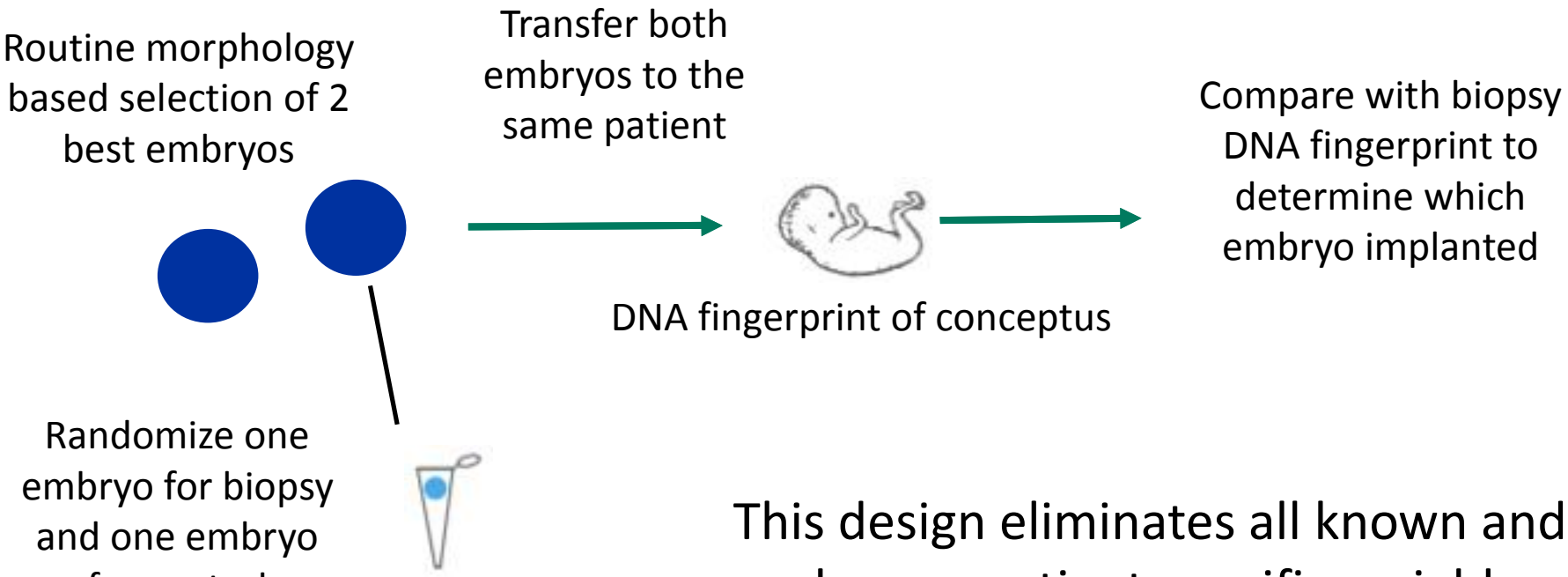
Trophectoderm Biopsy

- Move away from D3 biopsy
- More Cells
- Biopsy only “viable” embryos
- More accurate testing



- Blastocyst biopsy
- D5/6
- Accurate determination of chromosomal component
- Multiple cells ripped/torn/cut from embryo
- May require embryo freezing/vitrification

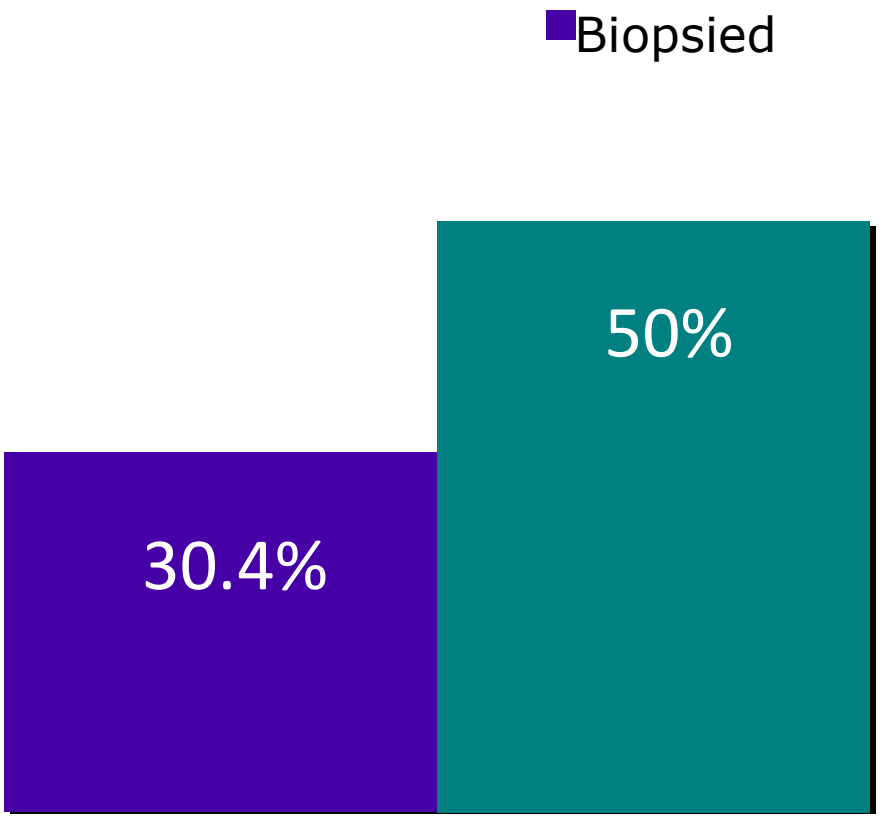
A Novel Study Design to Determine Impact of Biopsy



This design eliminates all known and unknown patient specific variables from the analysis of impact of biopsy.

Treff et al, 2010; Fertil Steril 94;477-84

Blastomere Biopsy

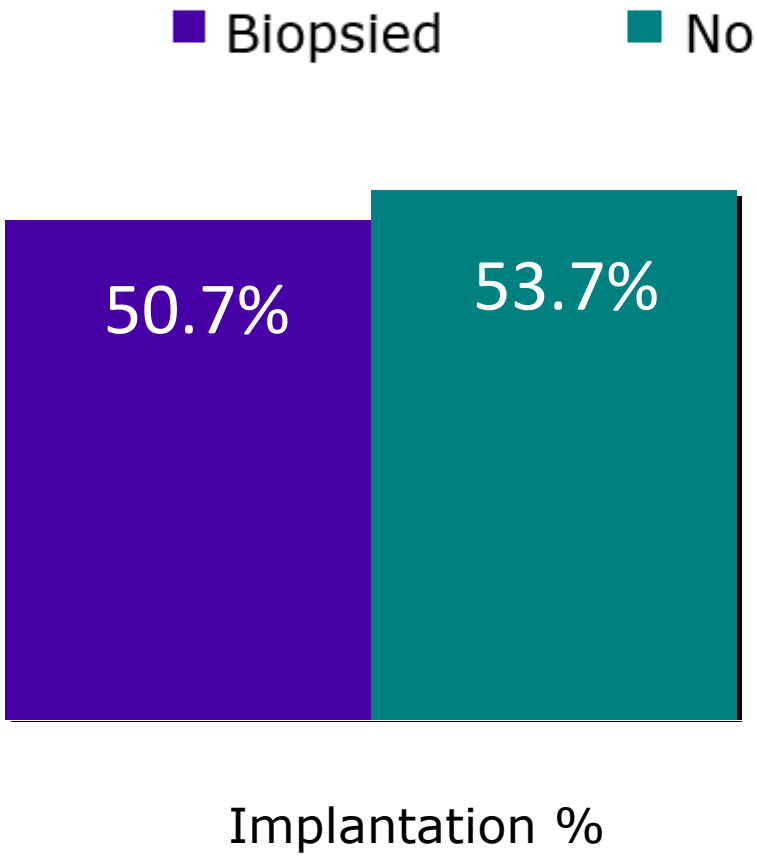


Implantation %

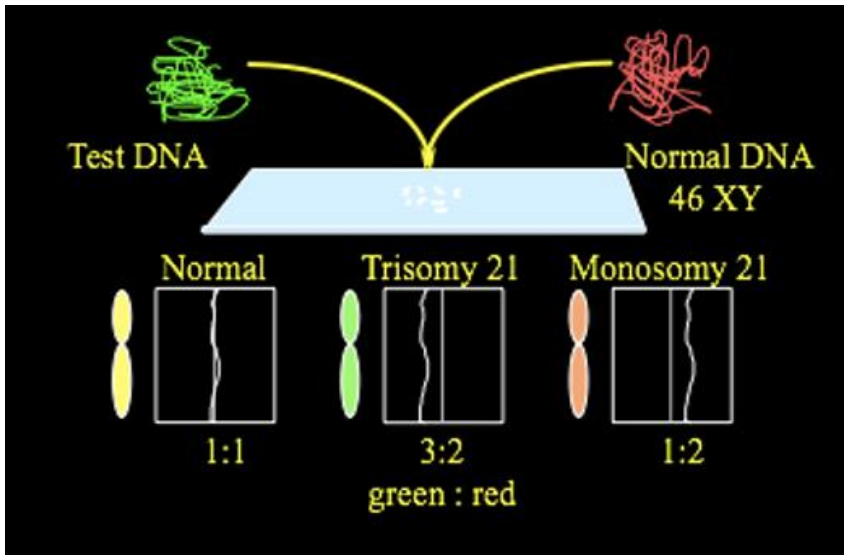
Reduction 39%

Mean maternal age 32 years

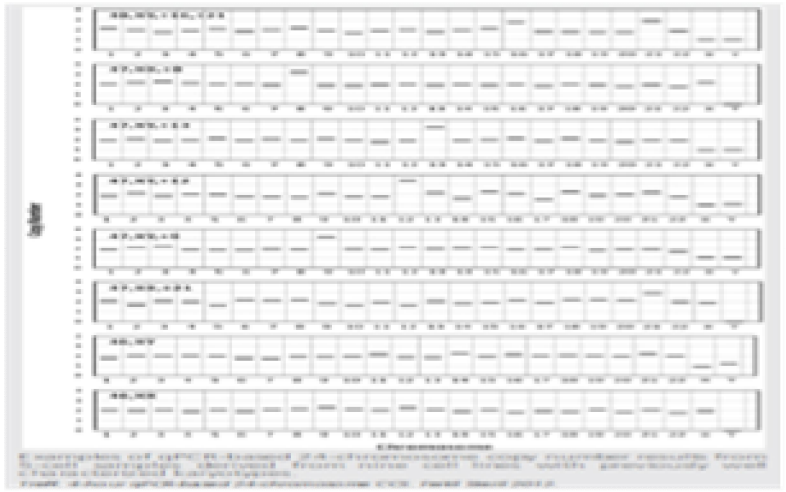
Trophectoderm Biopsy



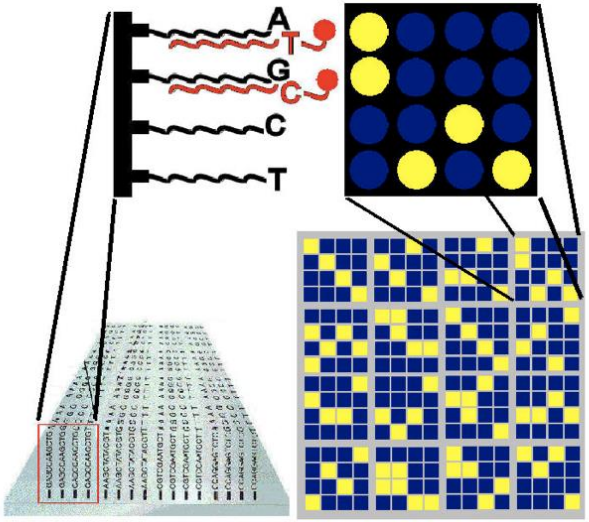
Non Significant



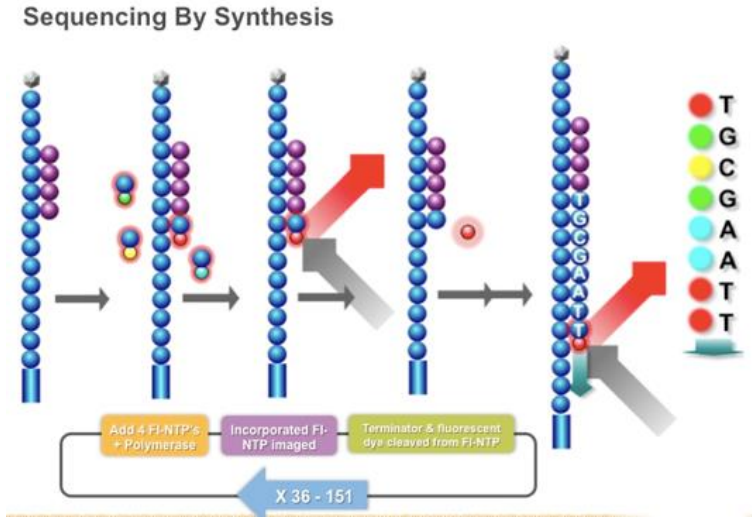
Comparative genome hybridization (CGH)



Quantitative PCR

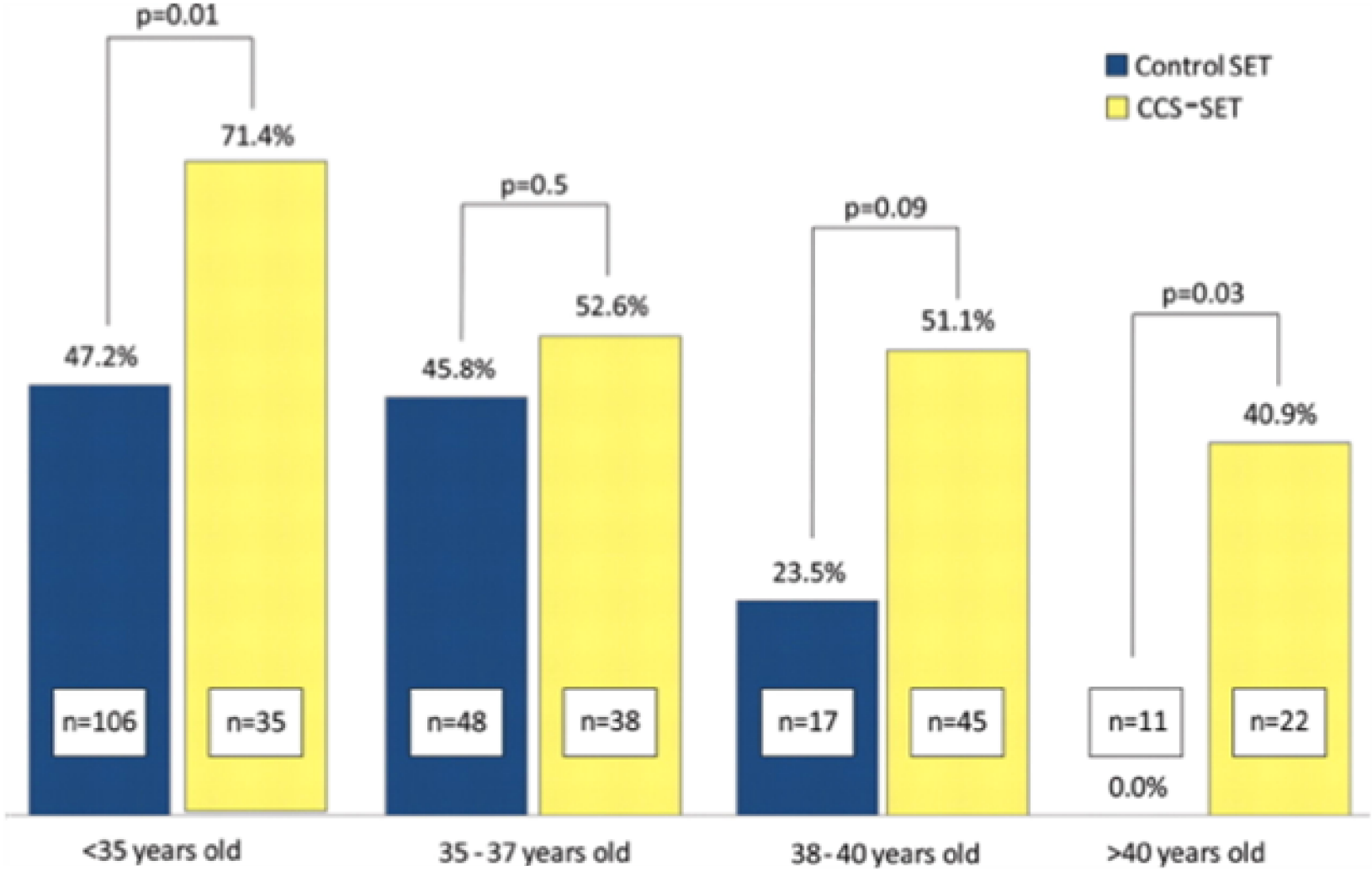


Single Nucleotide Polymorphism



Next Generation Sequencing

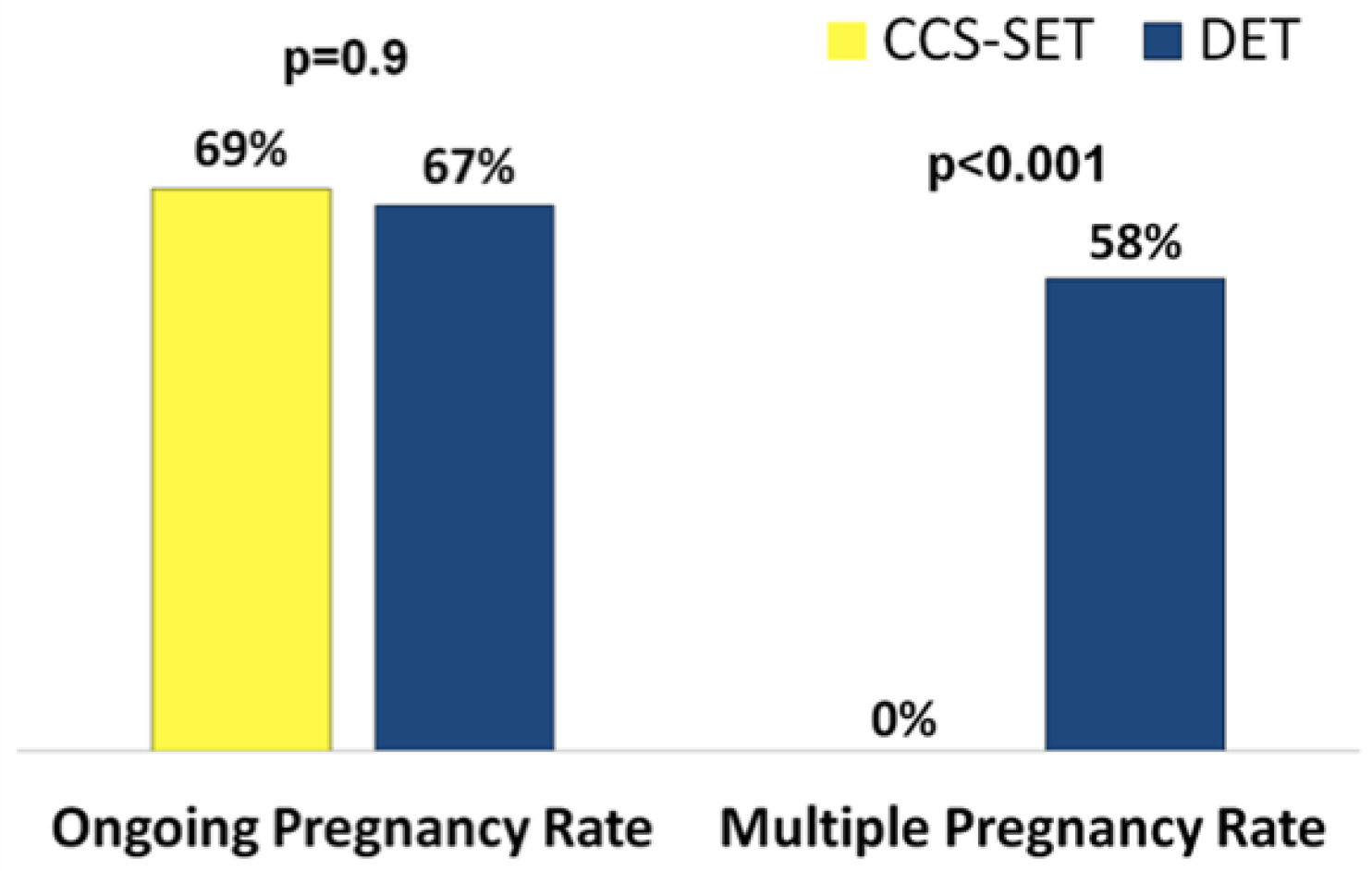
Ongoing Pregnancy SET vs. CCS SET



Forman E et al. Hum. Reprod. 2012;27:1217-1222

human reproduction

CCS-SET vs. DET RCT



Forman E et al. Hum. Reprod. 2012;27:1217-1222

PGD with TBx

- Class I data demonstrates increased implantation and delivery rates and reduced multiple gestation rates by empowering more effective SET.

- Sustained IR of 60% or higher even in women in their early forties.

Scott RT, 2013; Forman EJ, 2013

Diminished effect of maternal age on implantation after preimplantatior genetic diagnosis with array comparative genomic hybridization

Gary L. Harton, B.S.,[a] Santiago Munné, Ph.D.,[b] Mark Surrey, M.D.,[c] Jamie Grifo, M.D., Ph.D.,[d] Brian Kaplan, M.D.,[e] David H. McCulloh, Ph.D., H.C.L.D.,[d] Darren K. Griffin, Ph.D.,[f] and Dagan Wells, Ph.D.,[g,h] for the PGD Practitioners Group[i]

[a] Bluegnome, La Jolla, California; [b] Reprogenetics, Livingston, New Jersey; [c] Southern California Reproductive Center, Beverly Hills, California; [d] NYU Fertility Center, New York, New York; [e] Highland Park IVF Center, Fertility Centers of Illinois, Highland Park, Illinois; [f] School of Biosciences, University of Kent, Canterbury, United Kingdom; and [g] Reprogenetics UK and [h] Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, United Kingdom; and [i] Centers in the PGD Practitioners Group are listed at the end of the article

Equivalent ongoing pregnancy rates

TABLE 3

Comparison of ongoing pregnancy rate per embryo biopsy cycle and per transfer between day 3 biopsy or blastocyst biopsy.

Day 3 biopsy			Day 5/6 biopsy		
Age group (y)	OP/BX cycle[a,b]	OP/transfer[c,d]	Age group (y)	OP/BX cycle[a,b]	OP/transfer[c,d]
<35	43.4% (49/113)	48.5% (49/101)	<35	57.4% (85/148)	64.4% (85/132)
35–37	40.8% (31/76)	50.8% (31/61)	35–37	47.4% (46/97)	59.0% (46/78)
38–40	34.4% (44/128)	48.9% (44/90)	38–40	39.1% (45/115)	53.6% (45/84)
41–42	20.0% (16/80)	38.1% (16/42)	41–42	28.6% (18/63)	54.5% (18/33)
>42	9.3% (5/54)	5/20	>42	10.3% (4/39)	4/16

Note: OP = Ongoing pregnancy as determined by the presence of a fetal sac at ultrasound investigation.

[a] The existence of an association between age and ongoing pregnancy per embryo biopsy cycle was tested using Contingency Chi Squared (2 X 2 X 5) analysis (χ^2). χ^2 was 64.3 with 9 degrees of freedom ($P < .01$). The significance of this χ^2 value indicates that there was a significant association of ongoing pregnancy per cycle start with age.

[b] Associations between ongoing pregnancy per biopsy cycle and day 3 biopsy versus day 5/6 biopsy were tested using Chi Squared Analysis (2 X 5). χ^2 was 14.6 with 5 degrees of freedom ($.01 < P < .02$) when day 3 observations were tested using day 5/6 expectations. The significance of the χ^2 values indicates that the incidence of pregnancy per start was associated with biopsy day.

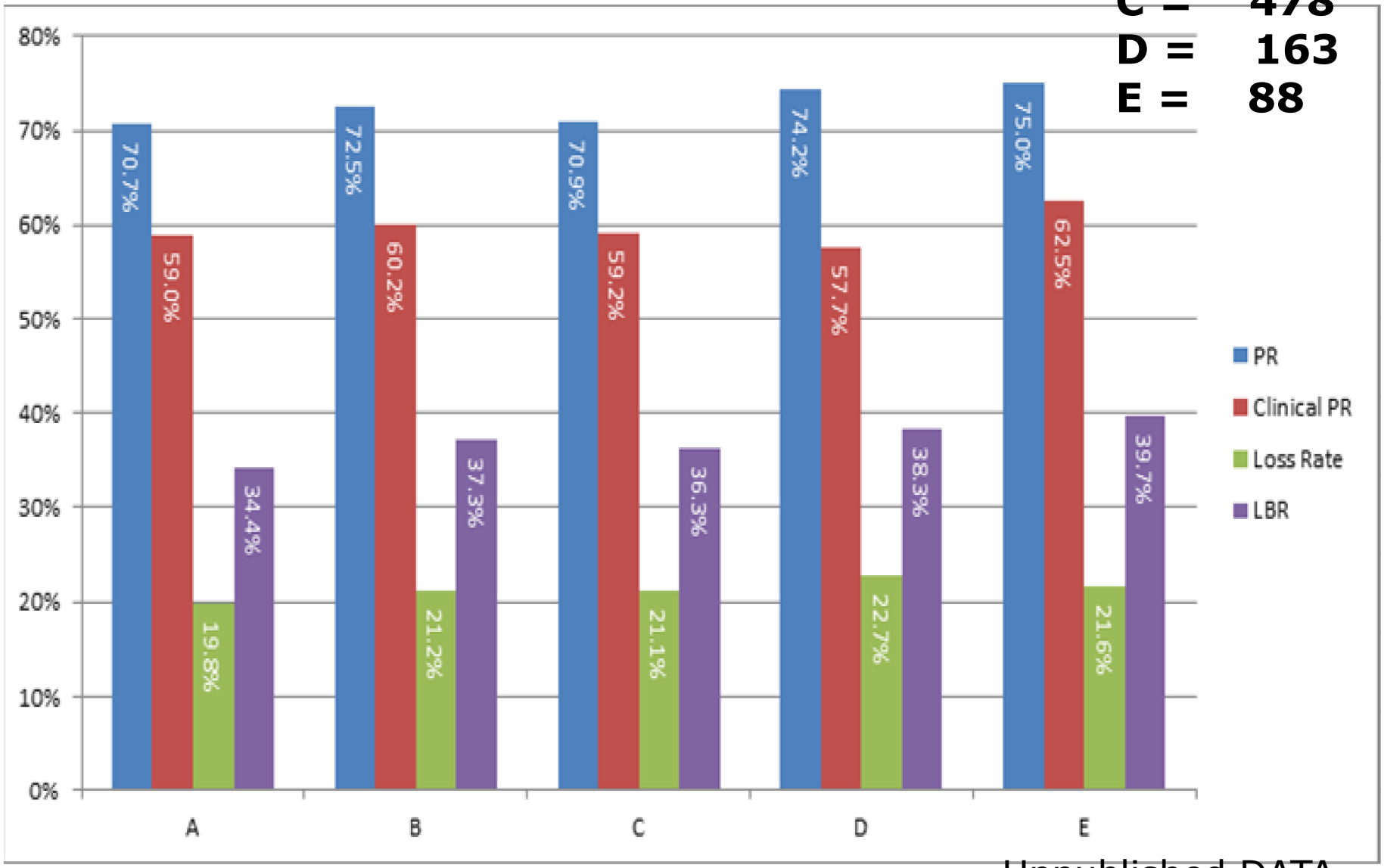
[c] The existence of an association between age and ongoing pregnancy per transfer was tested using Contingency Chi Squared (2 X 2 X 5) analysis (χ^2). χ^2 was 15.9 with 9 degrees of freedom ($.05 < P < .10$). The lack of significance of this χ^2 value indicates that there was no significant association between the incidence of ongoing pregnancy per transfer and age groups.

[d] Associations between incidence of pregnancy per transfer and day 3 biopsy versus day 5/6 biopsy were tested using Chi Squared Analysis (2 X 5). χ^2 was 18.2 with 5 degrees of freedom ($.0025 < P < .005$) when day 3 observations were tested using day 5/6 expectations. The significance of the χ^2 values indicates that the incidence of ongoing pregnancy per transfer was associated with biopsy day.

Harton. Euploid embryos mitigate maternal age effect. Fertil Steril 2013.

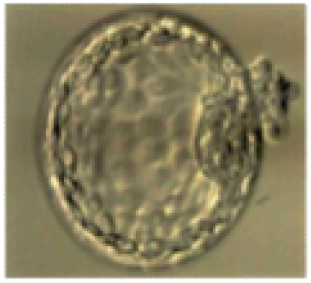
PGD Results RMA of NY

A = 646
B = 477
C = 478
D = 163
E = 88

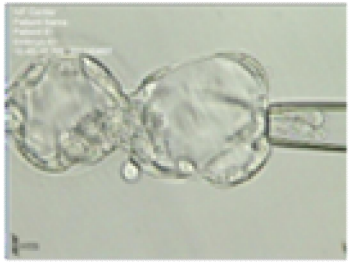


Unpublished DATA

PGD Protocol at RMA NY

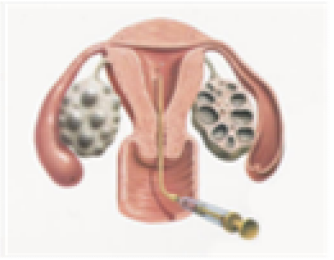


Blastocyst Stage



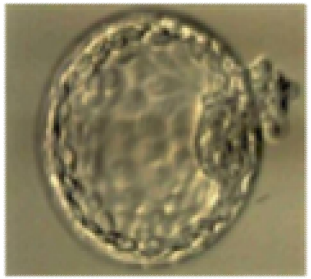
Embryo Biopsy

Quantitative PCR-CCS

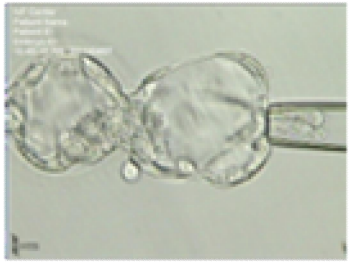


Embryo Transfer

DAY 5 → DAY 6

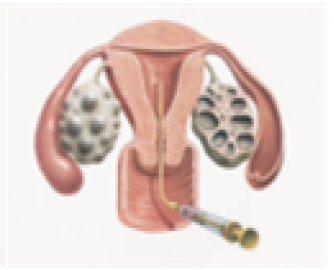


Blastocyst Stage



Embryo Biopsy

Quantitative PCR aCGH NGS

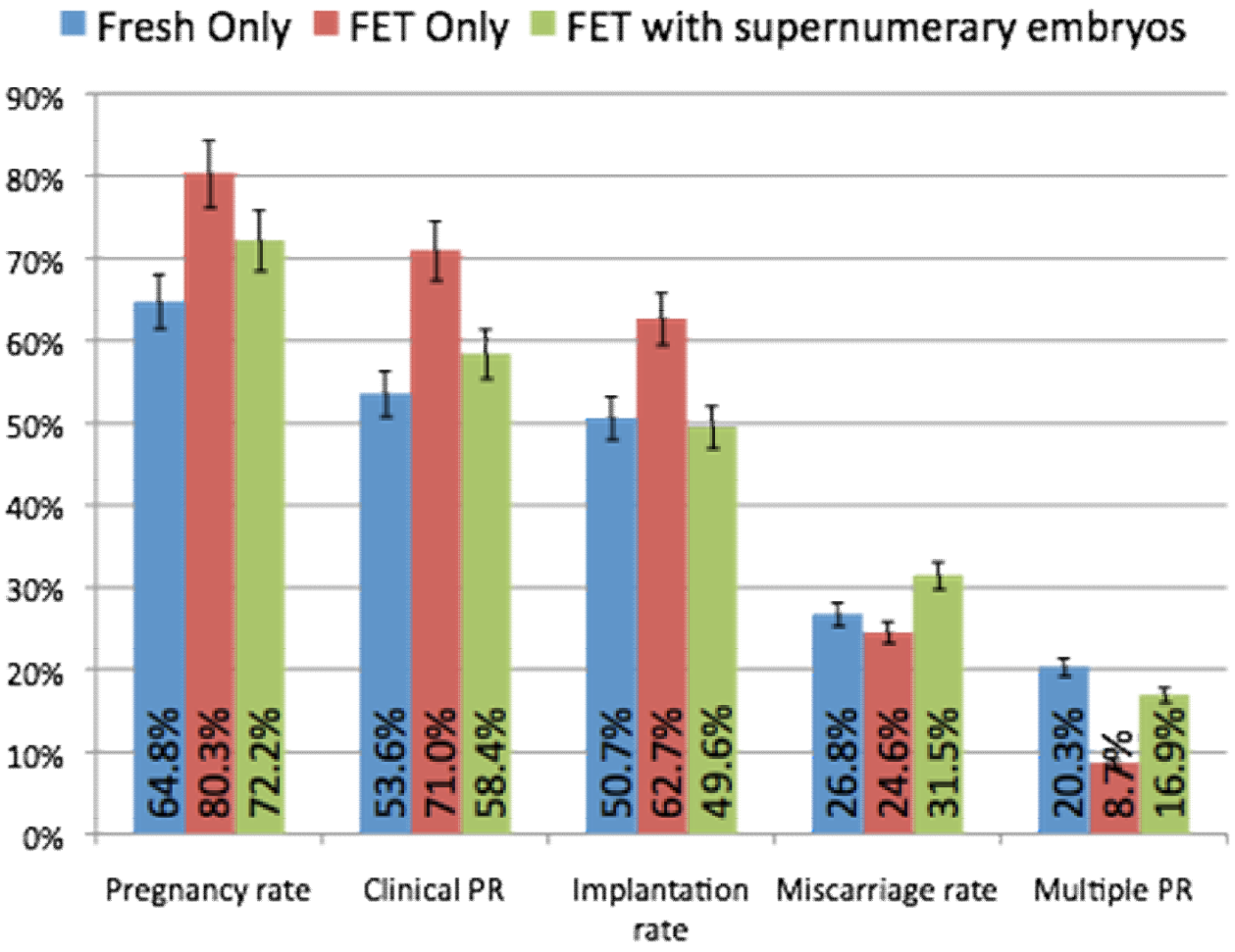


Embryo Transfer

Days 5-6 → **VITRIFY ALL EMBRYOS** → **Synthetic FET**

PGD Results RMA of NY

- IVF PGD Jan2011 - Dec 2014
- "FRESH only" (n=293) - results within 24 hrs
- "FET only " (n=290) - all embryos vitrified (no fresh ET)
- "FET after fresh ET" (n=101) - first fresh ET then FET from same cohort of embryos

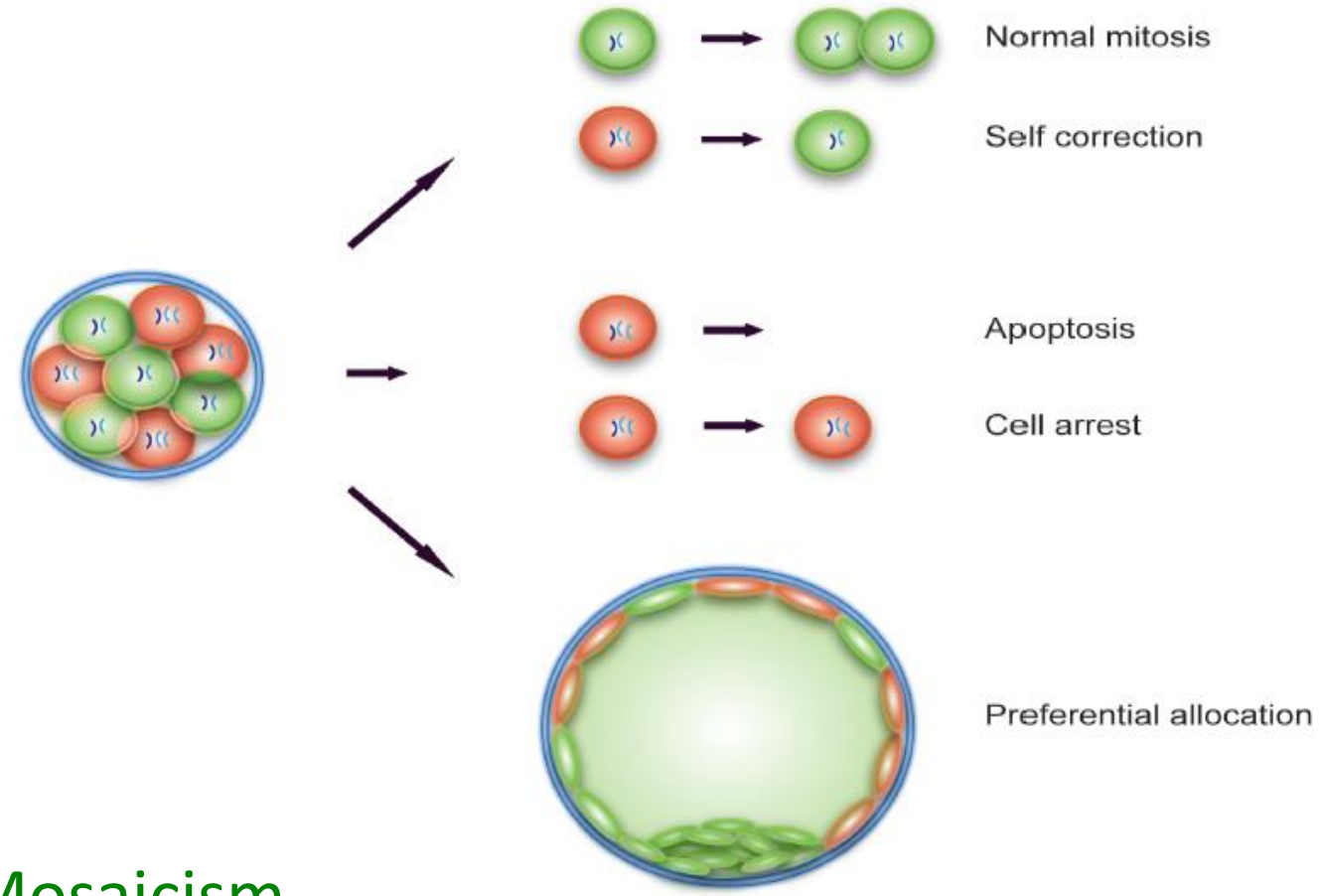


Rodriguez-Purata, M. Luna ,B. Sandler, 2015 submitted. RMA of NY

Pitfalls with PGD TBx

- Remains disappointing that a large percentage of morphologically normal euploid blastocysts fail to implant
- Loss rate is not 0%
- Blastocysts must achieve a good enough morphological quality to undergo biopsy, hence early blastocysts or regular quality blastocysts will not undergo biopsy and will be discarded

Mosaicism



Mosaicism

Day 3 - 40-60%

Day 5 - 20%

How are these cells allocated within the trophectoderm?

Are they located randomly in clusters?

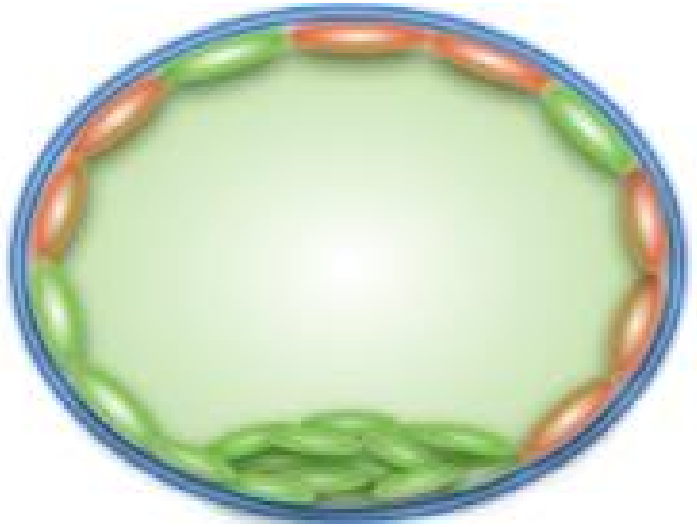
What is the probability of biopsying these cells?

When did the formation of these mosaic cells occur?

Mosaicism

"Pure Aneuploid"
False Abnormal
Lack of opportunity for implanting

Sampling Errors



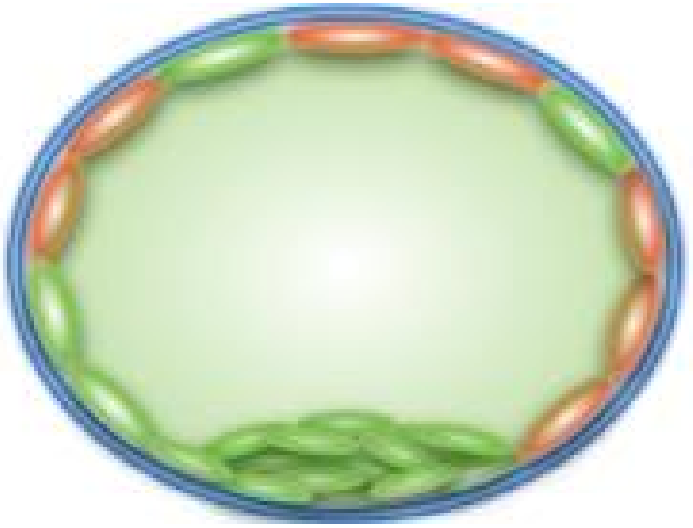
Euploid Embryo
False Normal
Failed Implantation

Mosaicism

Reciprocal Errors

Trisomy 13

Monosomy 13



Cells placed in a reaction tube and lysed → Frees DNA from all cells creating a mixture

Analyzed as a single sample

The amount of DNA from Chr 13 would be equal

Mosaicism undetected

Mosaicism

Current reporting data

PGD result: 20% mosaic

To Transfer ?

ABNORMAL RESULT: Reciprocal errors -- 40% monosomic and 60% trisomic cells

Difference = 20%

Indistinguishable from a sample that is 80% disomic and 20% trisomic

Mosaicism

A definite diagnosis as mosaic is not possible from a single trophectoderm in which all cells are lyzed and the DNA analyzed in aggregates.

"At risk for mosaicism"

If a mosaic blast has reduced IR, then deselecting those embryos would remove some of the less competent ones from the pool of transferable embryos

Reproductive Potential ?

Given the reduced accuracy of this result, deselecting reproductively competent embryos may result in a reduced pregnancy rate per VOR

Scott R, April 2016

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

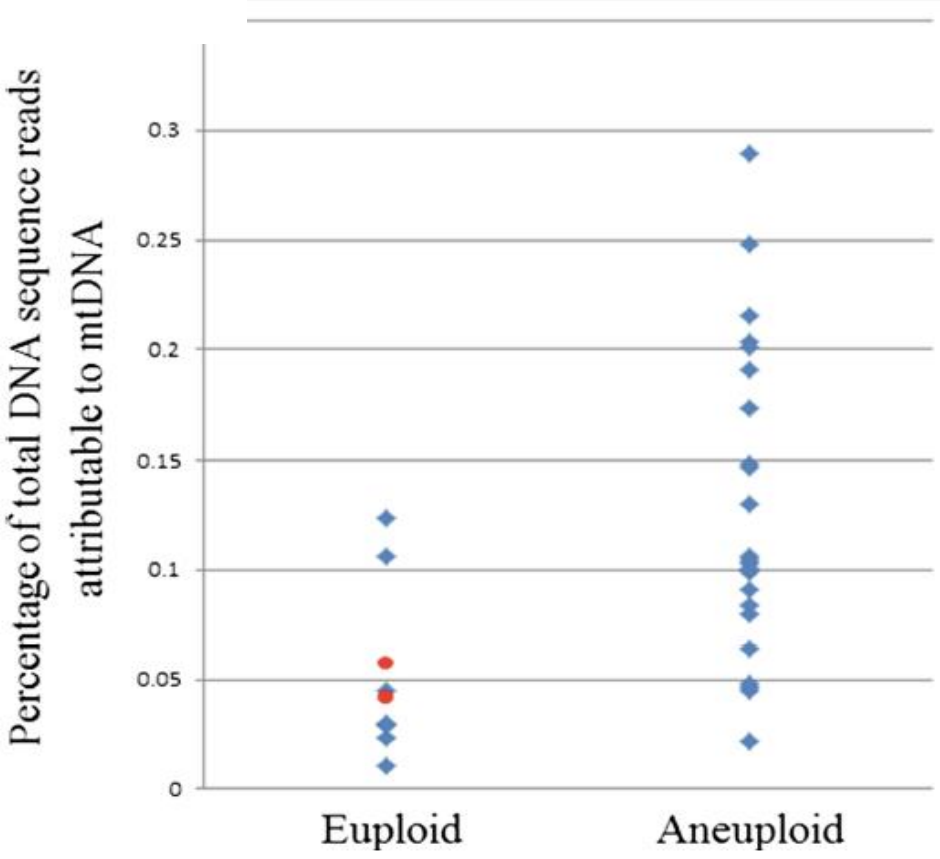
Patient No.	Chromosomal Constitution	Mosaicism†	Karyotype‡	Clinical Outcome
		percent		
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy§
11	arr 9p21.2q34.3(26,609,645-140,499,771)x3	45	NA	Biochemical pregnancy§
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy

MtDNA NGS

ORIGINAL ARTICLE

Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation

Dagan Wells,[1] Kulvinder Kaur,[2] Jamie Grifo,[3] Michael Glassner,[4] Jenny C Taylor,[2] Elpida Fragouli,[5] Santiago Munne[6]



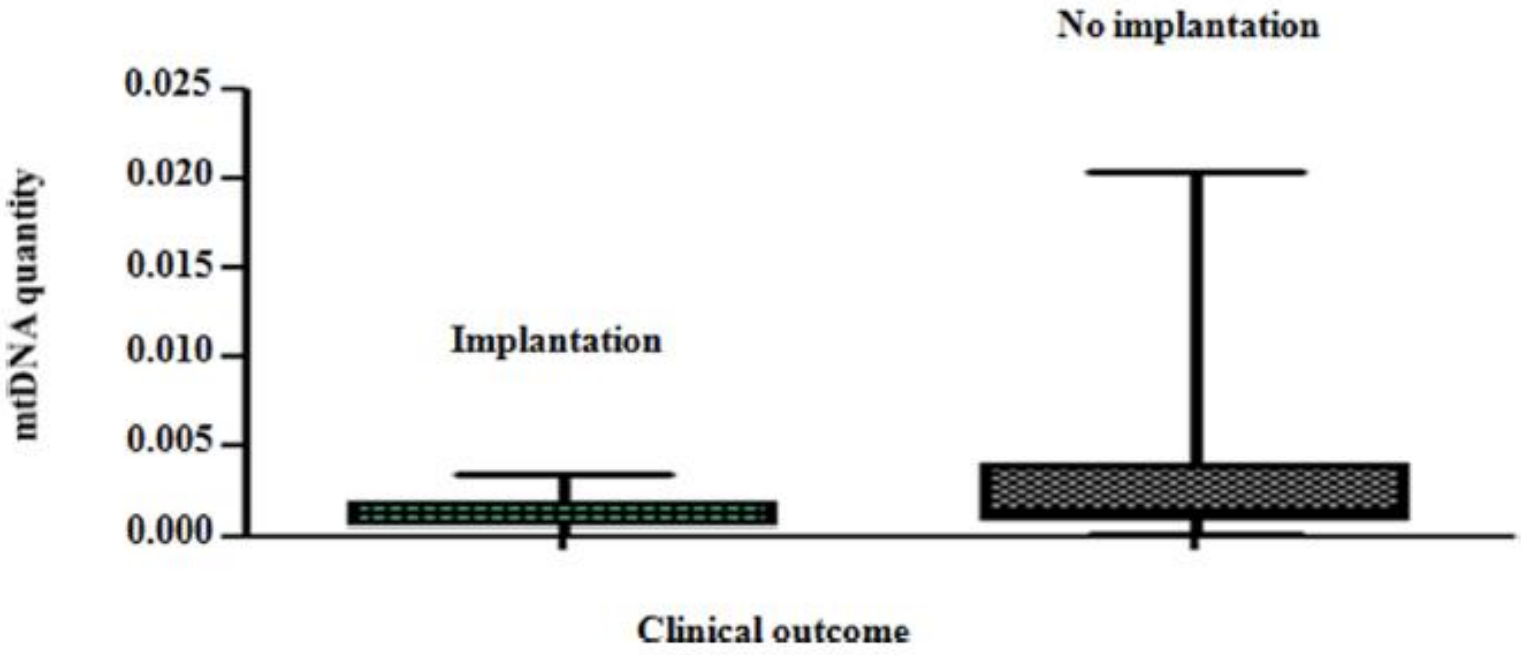
Wells D, *et al*. *J Med Genet* 2014;

Transferred Embryos Based on Euploidy Status by CGHa and NGS

Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential

June 3, 2015

Elpida Fragouli[1]*, Katharina Spath[2], Samer Alfarawati[1], Fiona Kaper[3], Andrew Craig[4], Claude-Edouard Michel[4], Felix Kokocinski[4], Jacques Cohen[5], Santiago Munne[5], Dagan Wells[1,2]



For Discussion

- Because of these pitfalls, who should we offer PGD to?
  - RPL?
  - Advanced Maternal Age?
  - Multiple Failed IVF cycles?
  - ALL?
  - With Mitochondrial DNA?

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