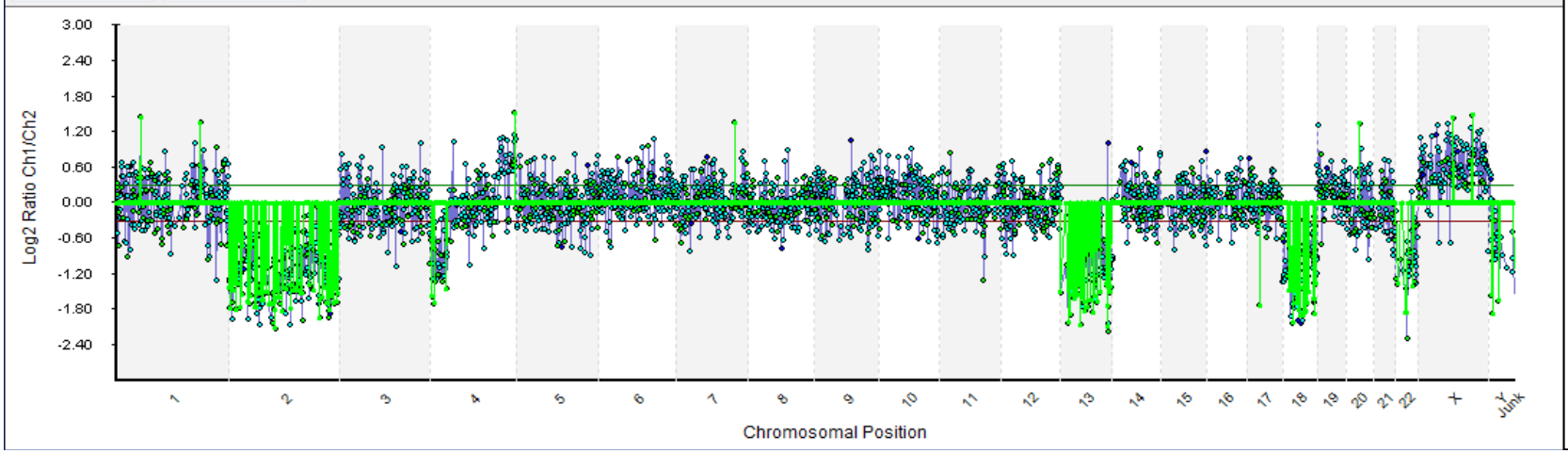
PGD for infertility

Santiago Munné



Reprogenetics

USA:
Livingston, NJ

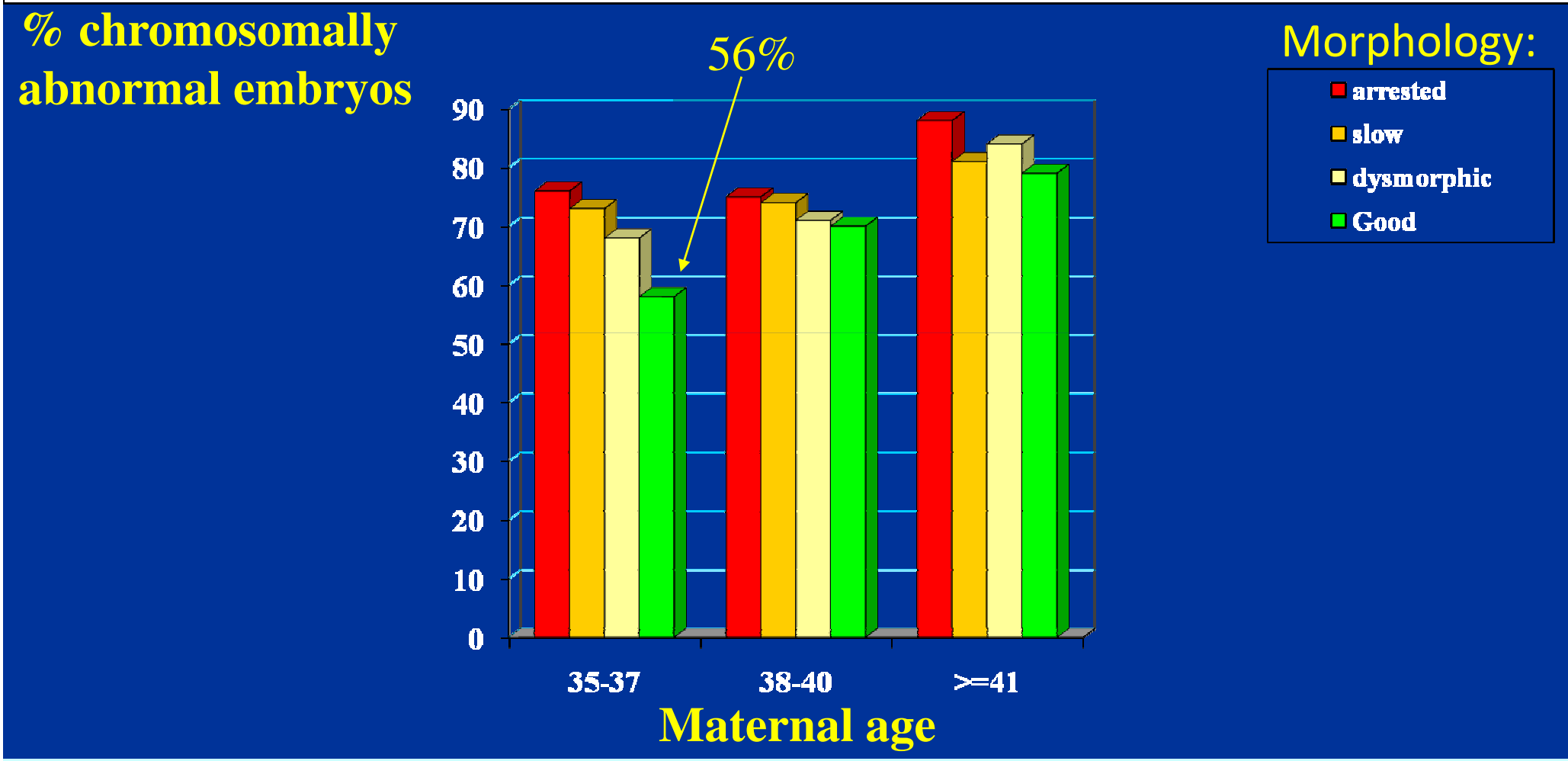
Europe:
Barcelona, Spain
Oxford, UK
Hamburg, Germany

Asia:
Kobe, Japan

South America:
Lima, Peru

The majority of embryos with 'good' morphology are chromosomally abnormal

Reprogenetics



embryos analyzed: 6054. Morphologically normal embryos: 3751. Source: Munné et al. 2007.
Similar results also found by Munne et al 1995, Marquez et al. 2000, Magli et al. 2007.

PGD Hypothesis

PGD may improve ART outcome in women of advanced maternal age Munné et al. (1993)

Despite large studies indicating the advantages of aneuploidy screening, the notion that PGS for infertility is beneficial is not shared uniformly.

Contradicting PGD results using day 3 biopsy and FISH

Reprogenetics

Positive effect

Gianaroli et al. 1999
Munne et al 1999
Gianaroli et al 2001a
Gianaroli et al. 2001b
Munne et al. 2003
Gianaroli et al. 2004
Munne et al. 2005
Munne et al 2006
Verlinsky et al. 2005
Colls et al. 2007
Garrisi et al. 2009
Rubio et al. 2009

No effect (small)

Werlin et al. 2003
Jansen et al. 2008
Mersereau et al. 2008
Scholcraft et al. 2009

No effect (Large)

Staessen et al. 2004
Platteau et al. 2005

Negative effect

Mastenbroek et al. 2007
Hardarson et al. 2008

Contradicting PGD results using day 3 biopsy and FISH

Reprogenetics

Proposed explanations:

1) Mosaicism and self-correction

2) Sub-optimal PGD and biopsy methods

Mosaicism

Mosaicism produces <7% misdiagnosis

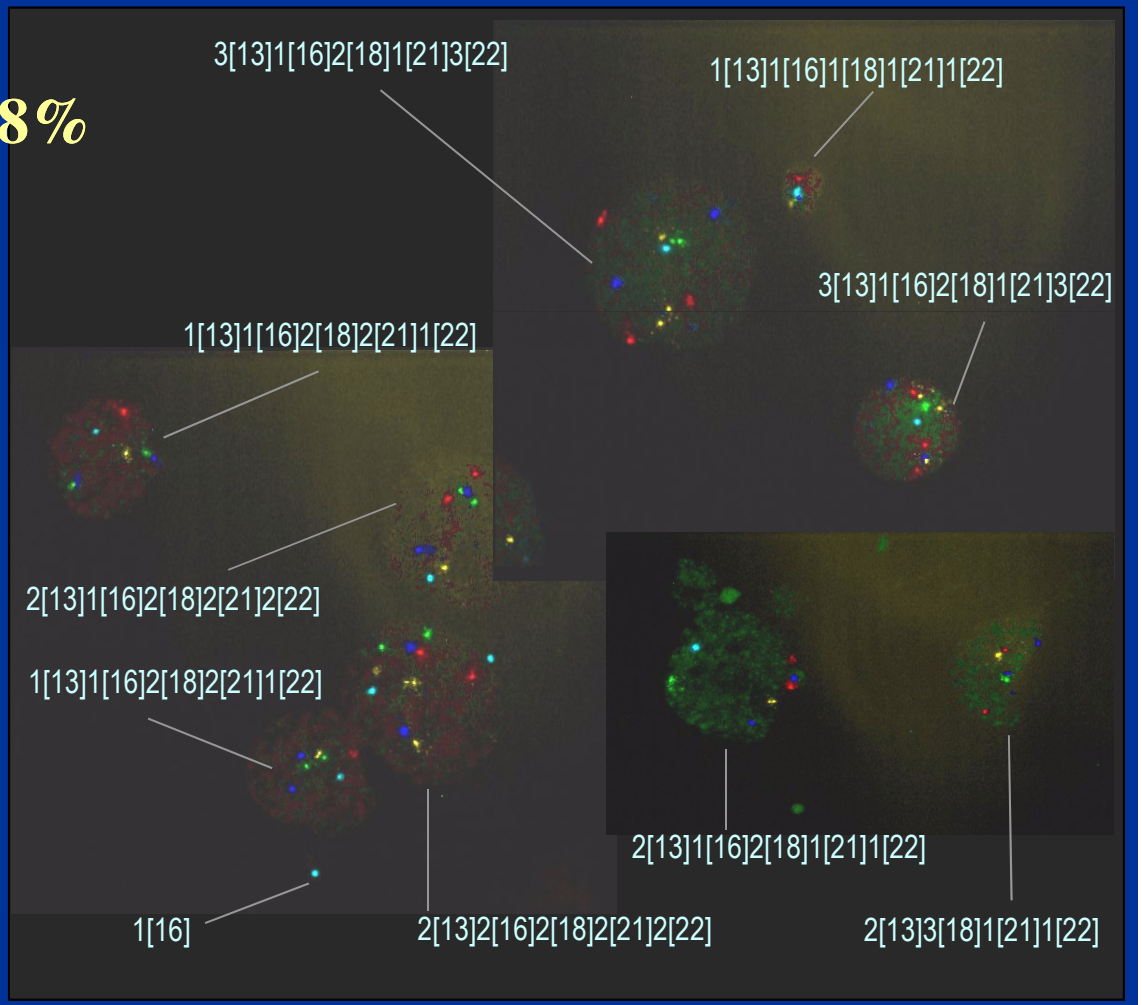
Reprogenetics

592 embryos found abnormal by PGD were reanalyzed and found to be:

normal	13
mosaic <49% abnormal	27
mosaic 50-99% abnormal	124
mosaic 100% abnormal	297
homogeneously abnormal	131

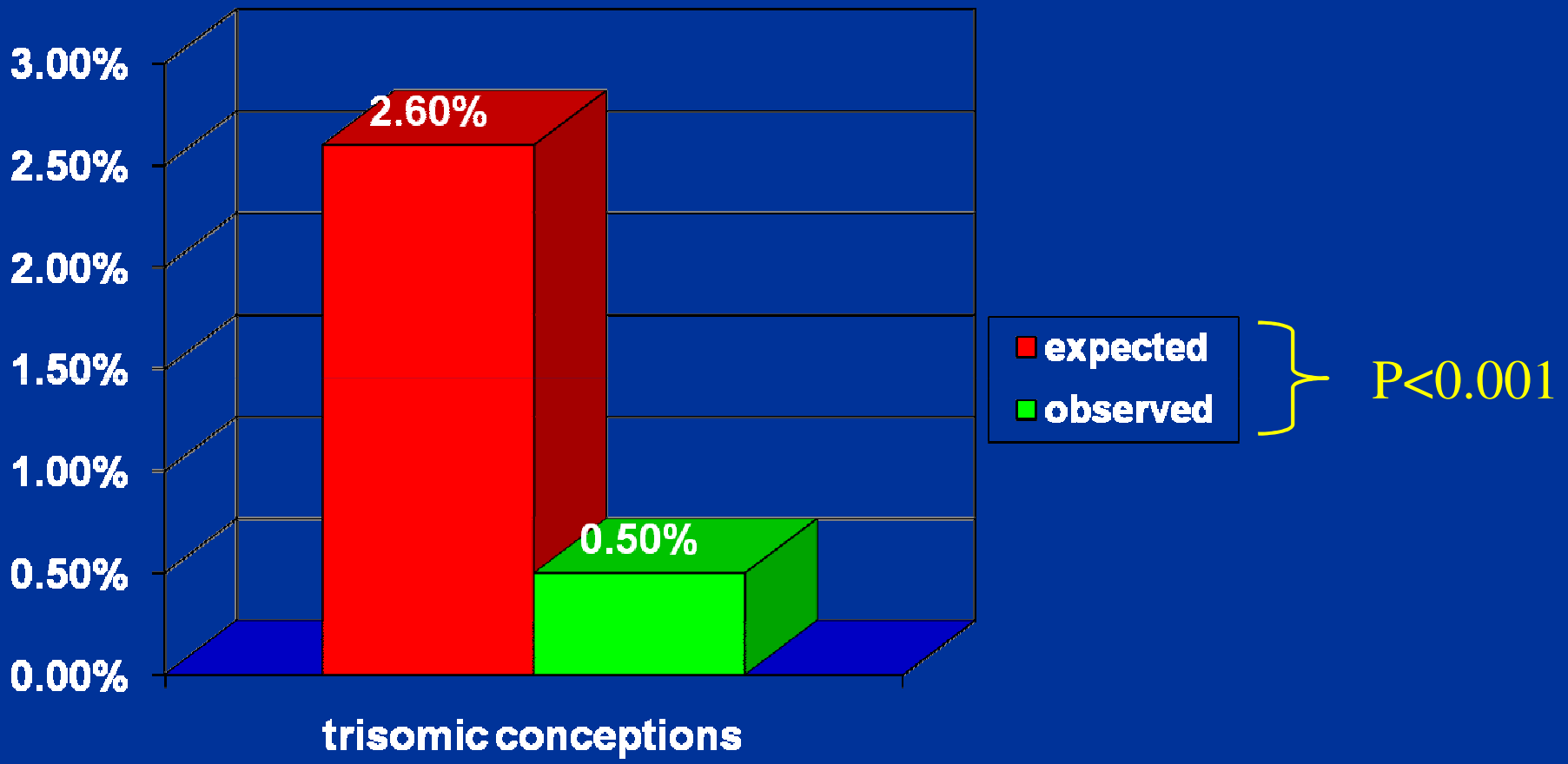
6.8%

Colls et al. (2007)



Negative predictive value

Reprogenetics



Aneuploidy rates for chromosomes X,Y,13,18,21. Munne et al. 2006 and Reprogenetics data up to 10/2007. Average age 37, Observed: Based on 2300 pregnancies after PGD, Expected: Eiben et al. 1994. Observed and expected adjusted by maternal ages

self-correction myth

Trisomy correction is rare: UPD evidence

Reprogenetics

UNIPARENTAL DISOMY:

Trisomy rescue: creates a zygote with 2 chromosomes from one parent and none from the other.

EXAMPLE TRISOMY 15:

Trisomy 15 in cleavage stage embryos:	1.874%	a
UPD-15 in newborns:	0.001%	b
Estimated correction of trisomy 15 to UDP:	1/3	c

Trisomy 15 day 3 embryos that self-corrected: a x b x c = 0.56%

a: Munne et al. (2004), b: From: OMIM, c: 1/3 of corrections will produce UPD

Fetus seldom self correct: it's the placenta that becomes abnormal

Reprogenetics

ELSEVIER

DEVELOPMENTAL BIOLOGY

Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype

Jingly F. Weier[a,b], Heinz-Ulrich G. Weier[b], Christine J. Jung[c], Matthew Gormley[a], Yan Zhou[c], Lisa W. Chu[b], Olga Genbacev[c], Alexi A. Wright[c], Susan J. Fisher[a,c,d,*]

2005, Dev. Biol. 279, 420-432

This work questions the assumption that placental confined mosaicism is the result of fetal self-correction. At the contrary, it suggests that normal fetuses may develop abnormal placenta.

Sub-optimizal methods (FISH studies, day 3 biopsy)

Optimal PGS methods

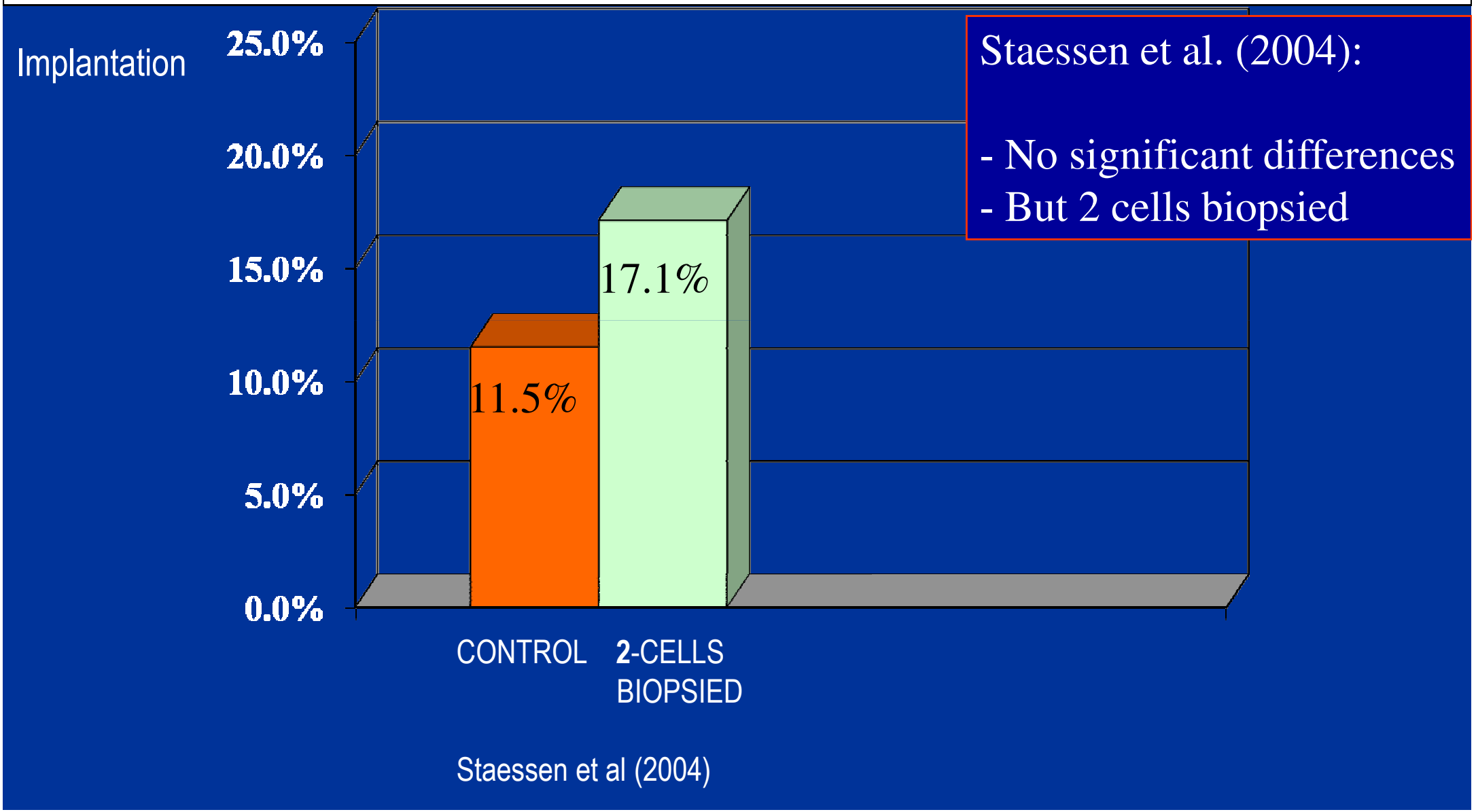
Reprogenetics

	Optimal PGS	Questionable PGS
Biopsy media	with aminoacids	simple media
Biopsy time / embryo	1 min	> 5 min
# cells biopsied	one	two
Fixation method	Carnoy's	Tween 20
# chromosomes tested	≥8	≤6
# analysts / case	2	1
Use of NRR*	yes	no
Large experience	yes	no
Error rate	<10%	10-50%
Number of zygotes	>5	≤ 5

**NRR: No result rescue, or re-testing of dubious chromosome with different probes.*

PGD for AMA: randomized studies

Reprogenetics



Staessen et al. (2004):

- No significant differences
- But 2 cells biopsied

Two cell biopsy is detrimental

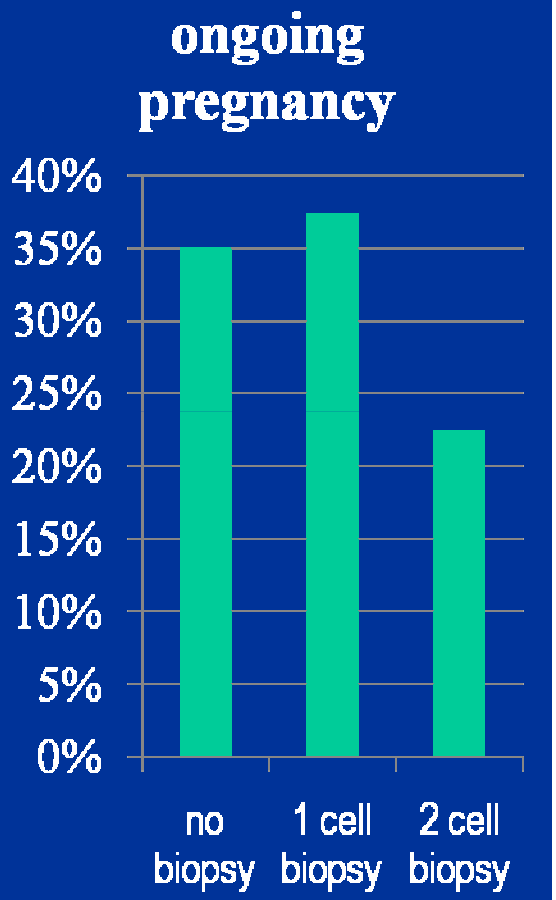
Reprogenetics

Hum. Reprod. Advance Access published September 21, 2009

human reproduction ORIGINAL ARTICLE *Embryology*

Impact of cleavage-stage embryo biopsy in view of PGD on human blastocyst implantation: a prospective cohort of single embryo transfers

A. De Vos[1,4], C. Staessen[2], M. De Rycke[2], W. Verpoest[1], P. Haentjens[3], P. Devroey[1], I. Liebaers[2], and H. Van de Velde[1]



P < 0.001

" The data presented here clearly indicates that two cell biopsy significantly impacts clinical outcome. Our previous report providing no arguments in favour of PGS (Staessen et al., 2004) was criticised by others arguing that PGS might have been beneficial if only one cell had been removed (Cohen et al., 2007). In respect to the present findings, this criticism seems justified".

PGD for AMA: randomized studies
Mastenbroek et al. (2007)

Reprogenetics

1) 20% of cycles undiagnosed (literature: 1-3% of embryos *)
2) 59% implantation reduction due to biopsy:

implantation

Control	14.7%
Biopsied, no PGD	**6.0%**
Biopsied and PGD	16.8%

59% reduction

3) Average number of embryos analyzed was only 5
4) Chromosomes 15 and 22 (21% abnormalities) not analyzed

** 1% Gianaroli et al. (2004), 3.1% Colls et al. (2007)*

Number of chromosomes analyzed

Reprogenetics

At least 9 chromosomes should be tested:

# chromosomes analyzed	% abnormal fetuses detectable
5	28%
6	47%
9	70%
12	80%
24	100%

Minimum number of embryos to do PGD

Reprogenetics

# 2pn's	Av. Age	Pregnancies
1-5	38	22%
6-9	38	36%
10-13	38	40%
≥ 14	38	48%

Data: Last 300 cases, to 7/2007, Saint Barnabas Medical center, unpublished

Error rate should be <10%

Reprogenetics

Analysis of remaining cells of embryos previously analyzed by PGD:

study	technique	error rate
Baart et al 2004	FISH	50.0%
Li et al. 2005	FISH	40.0%
Gleicher et al. 2009	FISH	15-20%
Munne et al. 2002	FISH-9	7.2%
Colls et al., 2007	FISH-9	4.7%
Magli et al. 2007	FISH-9	3.7%
Munne et al. 2010	array CGH	1.8%

PGD for Recurrent Pregnancy Loss (RPL)

All controlled PGD studies on idiopathic RPL show a decrease in miscarriages

Reprogenetics

Idiopathic RPL :

Werlin L, et al. (2003) Preimplantation genetic diagnosis (PGD) as both a therapeutic and diagnostic tool in assisted reproductive technology. Fertil Steril, 80:467

Munné et al. (2005) Preimplantation genetic diagnosis reduces pregnancy loss in women 35 and older with a history of recurrent miscarriages. Fertil Steril 84:331

Garrisi et al. (2009) Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil. Steril 92: 288

Rubio et al. (in press) Prognosis factors for Preimplantation Genetic Screening in repeated pregnancy loss. Reprod Biomed Online, in press

Reduction in miscarriages in RPL after PGD

Reprogenetics

PGD results according to previous number of miscarriages

# previous miscarriages	cycles	% loss expected	% loss after PGD	
2	90	29%	19%	N.S.
>2	190	38%	9%	p<0.00.1

Garrisi et al. (2009), and Reprogenetics, unpublished

Reduction in miscarriages in RPL after PGD

Reprogenetics

PGD results according to age when previous number of miscarriages is 3-5

maternal age	cycles	% loss expected	% loss after PGD	
<35	78	26%	10%	p<0.025
≥35	202	39%	13%	p<0.001

Garrisi et al. (2009), Reprogenetics, unpublished results

Reduction in miscarriages in RPL after PGD

Reprogenetics

PGD results according to fertility

method conception	cycles	% loss expected	% loss after PGD	p	% to term
IVF	115	35%	14%	p<0.01	34%
natural	124	41%	15%	p<0.005	37%

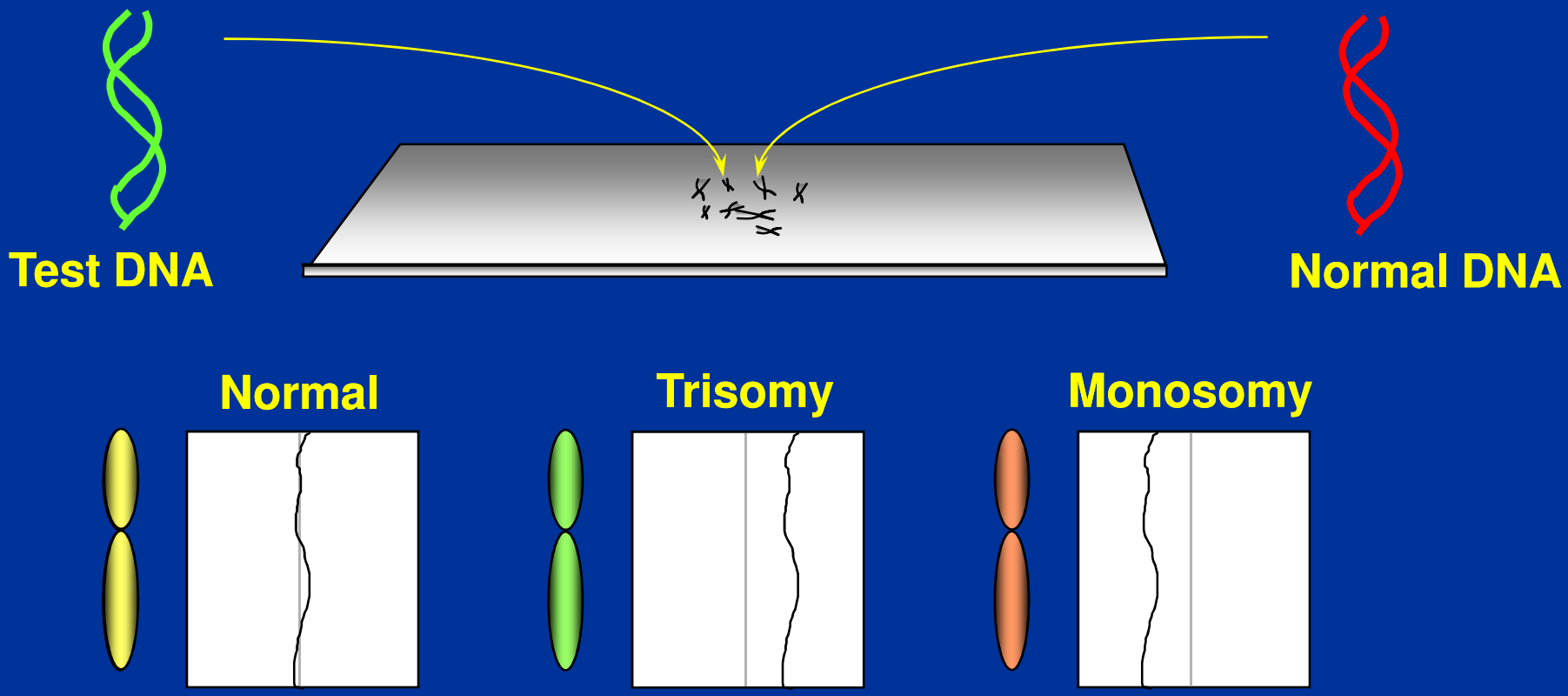
Average maternal age: 37.5
Garrisi et al. (2009)

New approach to PGD:

- 24 chromosome analysis by arrays
- Blastocyst biopsy and vitrification

Comparative Genome Hybridization (CGH)

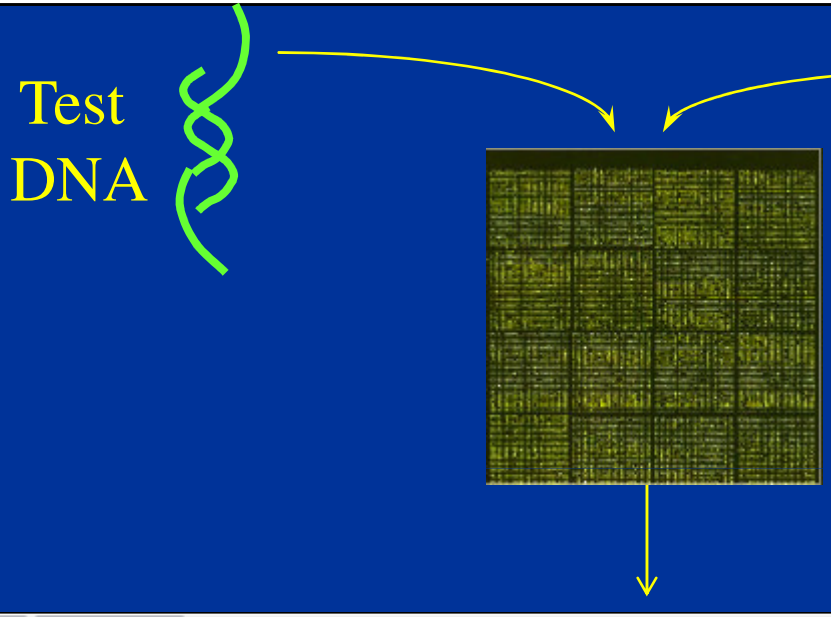
Reprogenetics



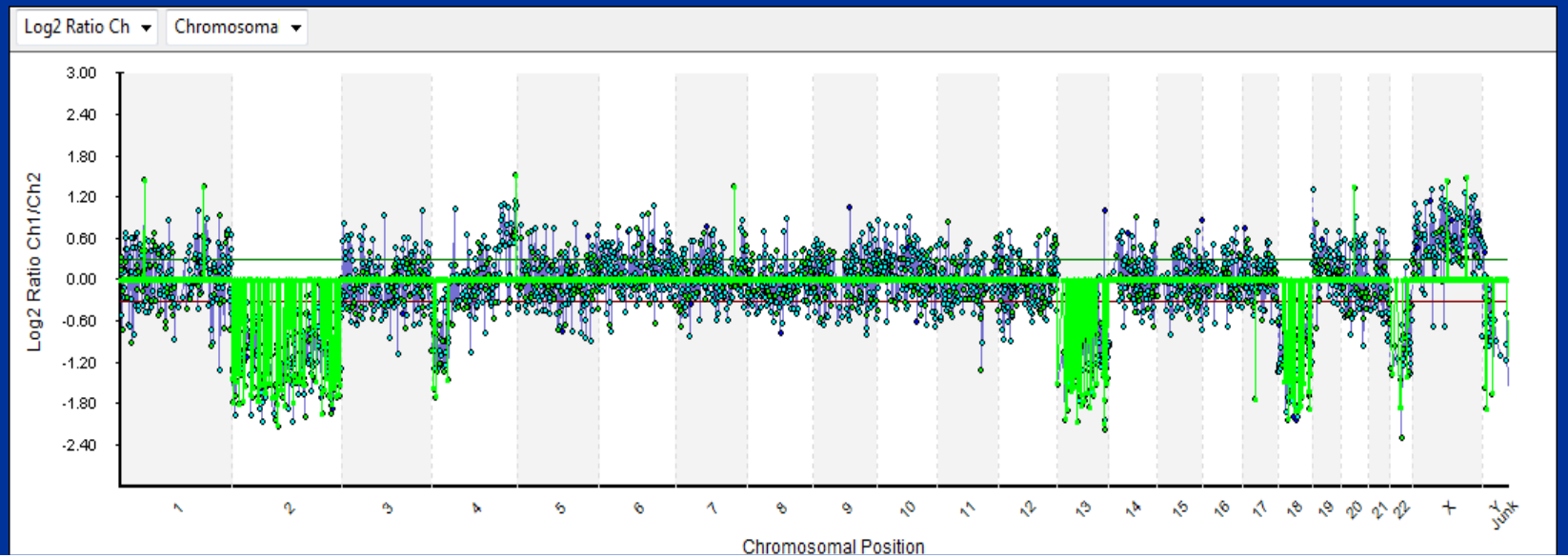
Kallioniemi et al. (1992), applied to single cells by Wells et al. (1999)

Array CGH

Reprogenetics



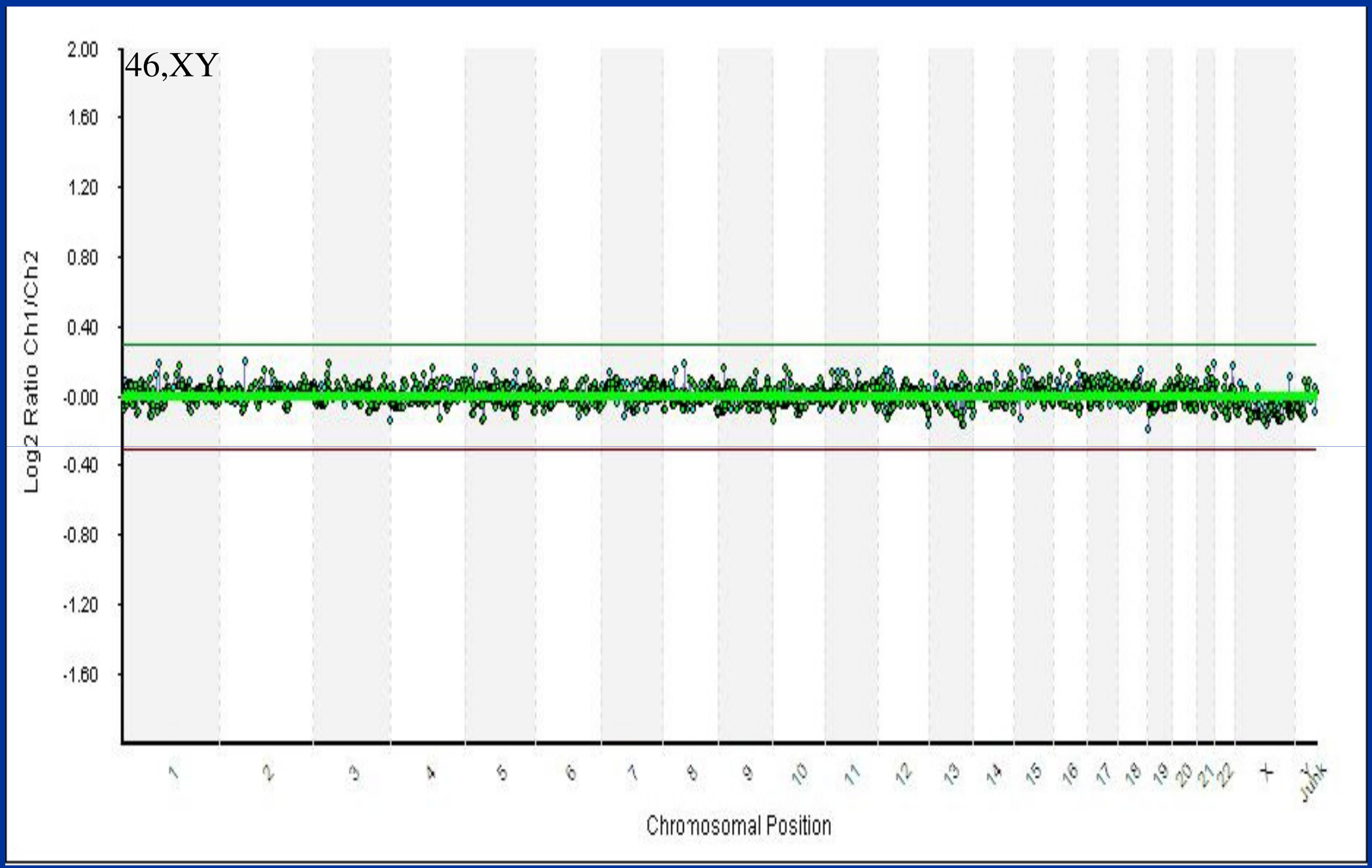
2700 probes
Same band resolution as karyotype

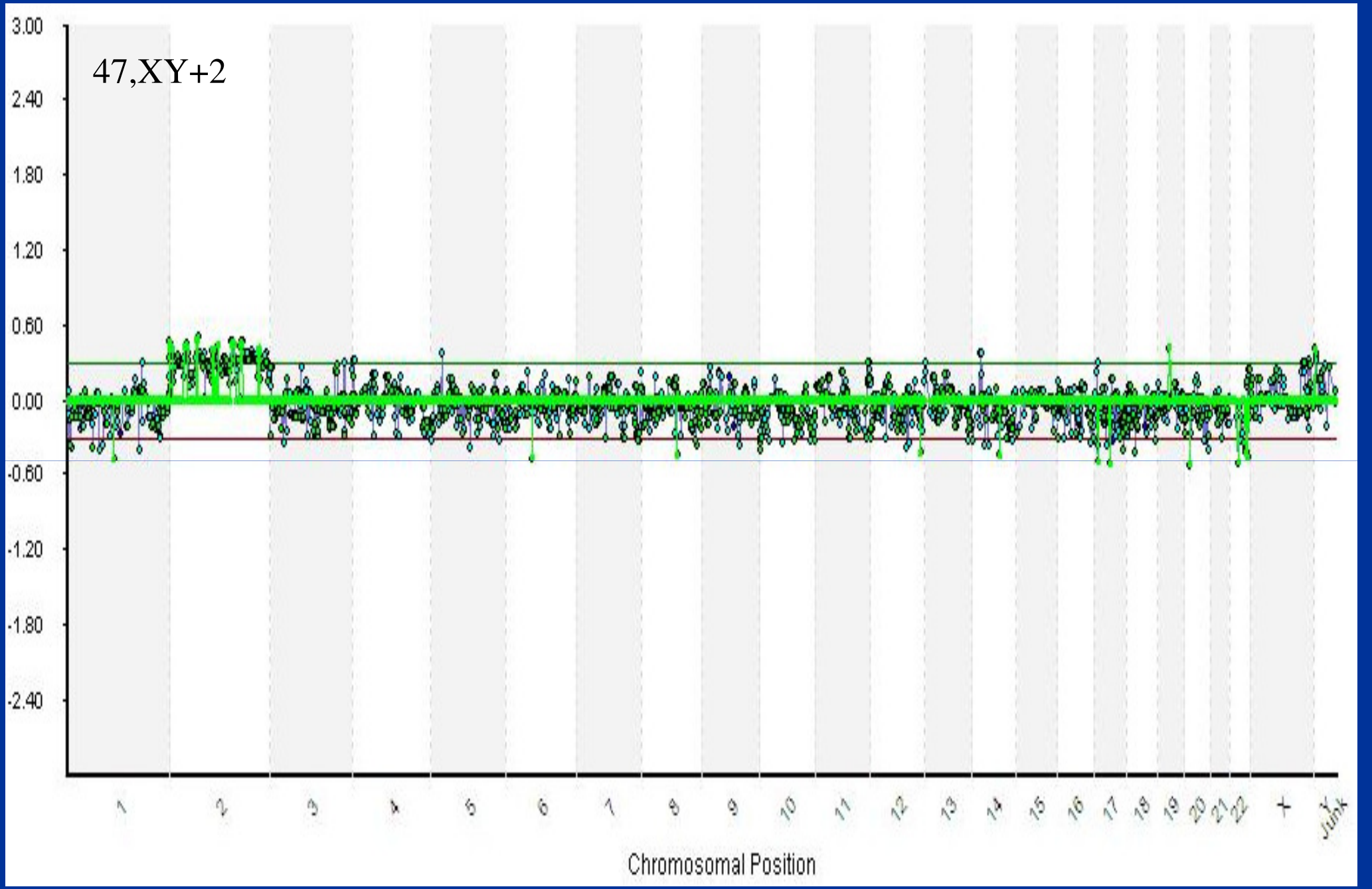


aCGH advantages

Reprogenetics

- All 24 chromosome type of aneuploidies detected
- Results in 24 hours; allows for PB or day 3 biopsy
- Parental DNA not required: ad hoc decisions possible
- Used in >15,000 patients with mental retardation


46,XY
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
Log2 Ratio Ch1/Ch2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Junk
Chromosomal Position


47,XY+2
3.00
2.40
1.80
1.20
0.60
0.00
-0.60
-1.20
-1.80
-2.40
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Junk
Chromosomal Position

aCGH validation: no results

Reprogenetics

Embryos undiagnosed:

biopsy on day **3**: 2% (16/724)

biopsy on day **5**: ≈0% (0/64)

Gutierrez-Mateo et al. (in press)

aCGH validation: error rate

Reprogenetics

- Validation method 1: single cells from cell lines analyzed*

  Error rate in euploid cell lines: 0/9
  Error rate in aneuploid cell lines: 0/42

- Validation method 2: Reanalysis of the rest of the embryo by FISH with 19 chromosomes probes**

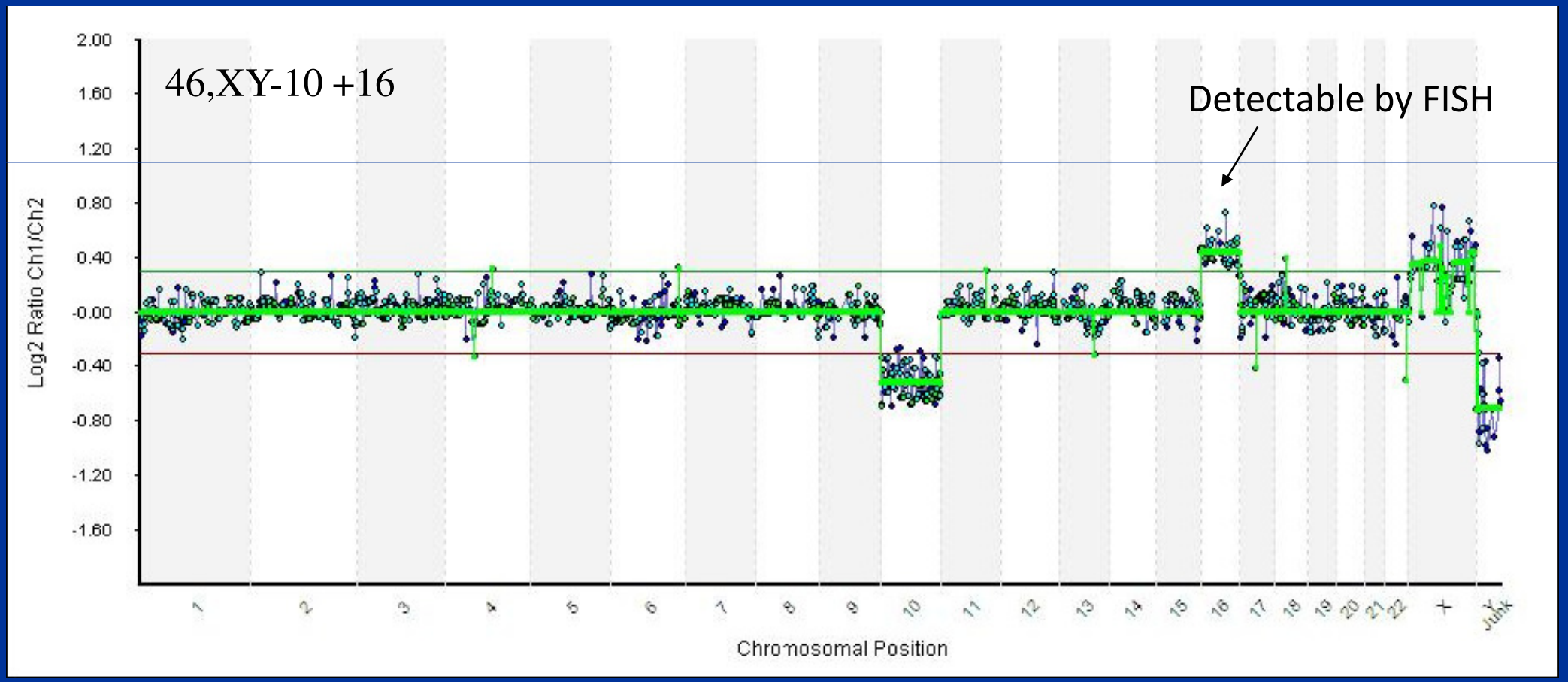
  Error rate from day 3 biopsies: 1.8% (1/56)

* Mamas et al (submitted), ** Gutierrez et al. (in press)

Detection of abnormalities: aCGH vs FISH-12

Reprogenetics

aCGH detected 50% more abnormalities than FISH-12 and 20% more abnormal embryos (Colls et al. 2009)



Day 3 biopsy, day 5 transfer and array CGH

Reprogenetics

Cycles performed: 219
Maternal age (av.) 37.5

	Pregnancy Rate		*Ongoing Pregnancy Rate*	
	Per Cycle	Per ET	Per Cycle	Per ET
Control	37%	37%	31%	31%
PGD	46%	60%	42%	55%
	NS	< 0.001	NS	< 0.001

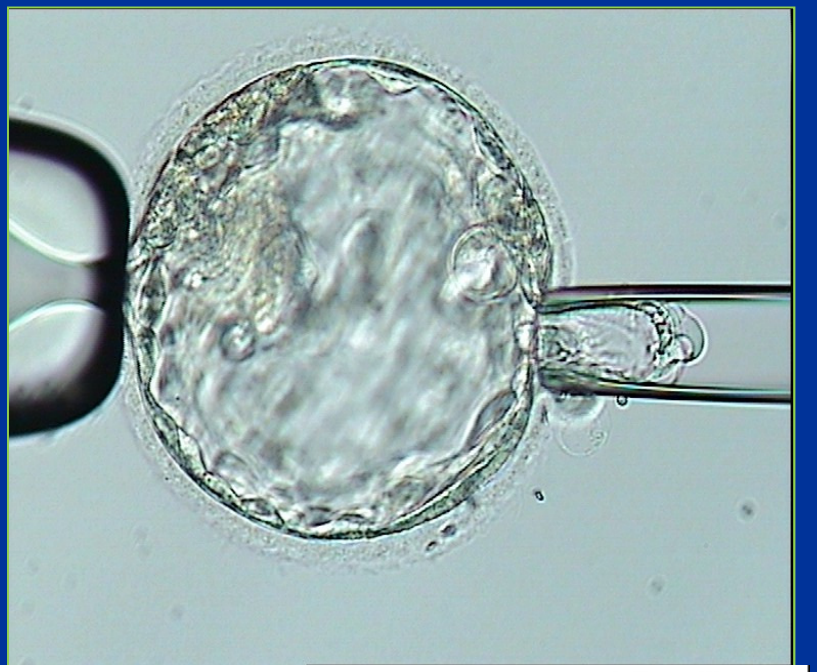
* Expected from each center SART data, controlled by age
Data from 24 centers. Munné et al. (2010) ESHRE, and unpublished data

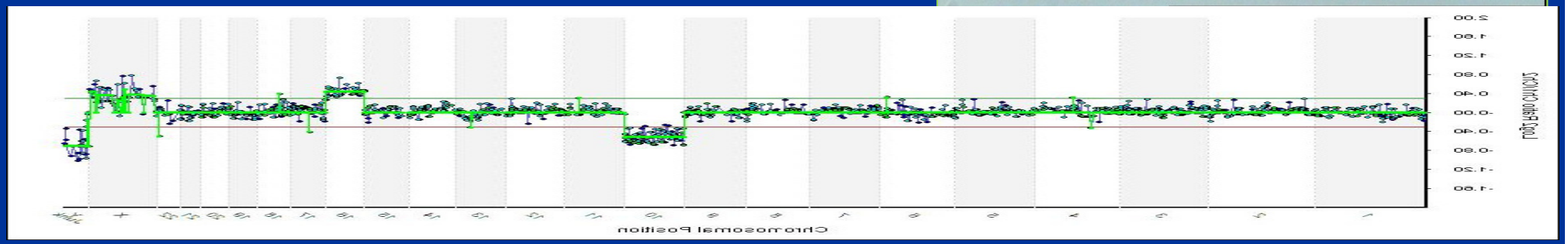
array CGH on blastocyst biopsies: Why?

Reprogenetics

Advantages:

1) More DNA: More robust diagnosis
2) Eliminates some mosaic embryos
3) Reduces error rate
4) Reduced impact of embryo biopsy
5) Less embryos to process
6) Facilitates single embryo transfer
7) Uterine environment optimized after thaw





CGH on blastocyst biopsies: clinical results

Reprogenetics

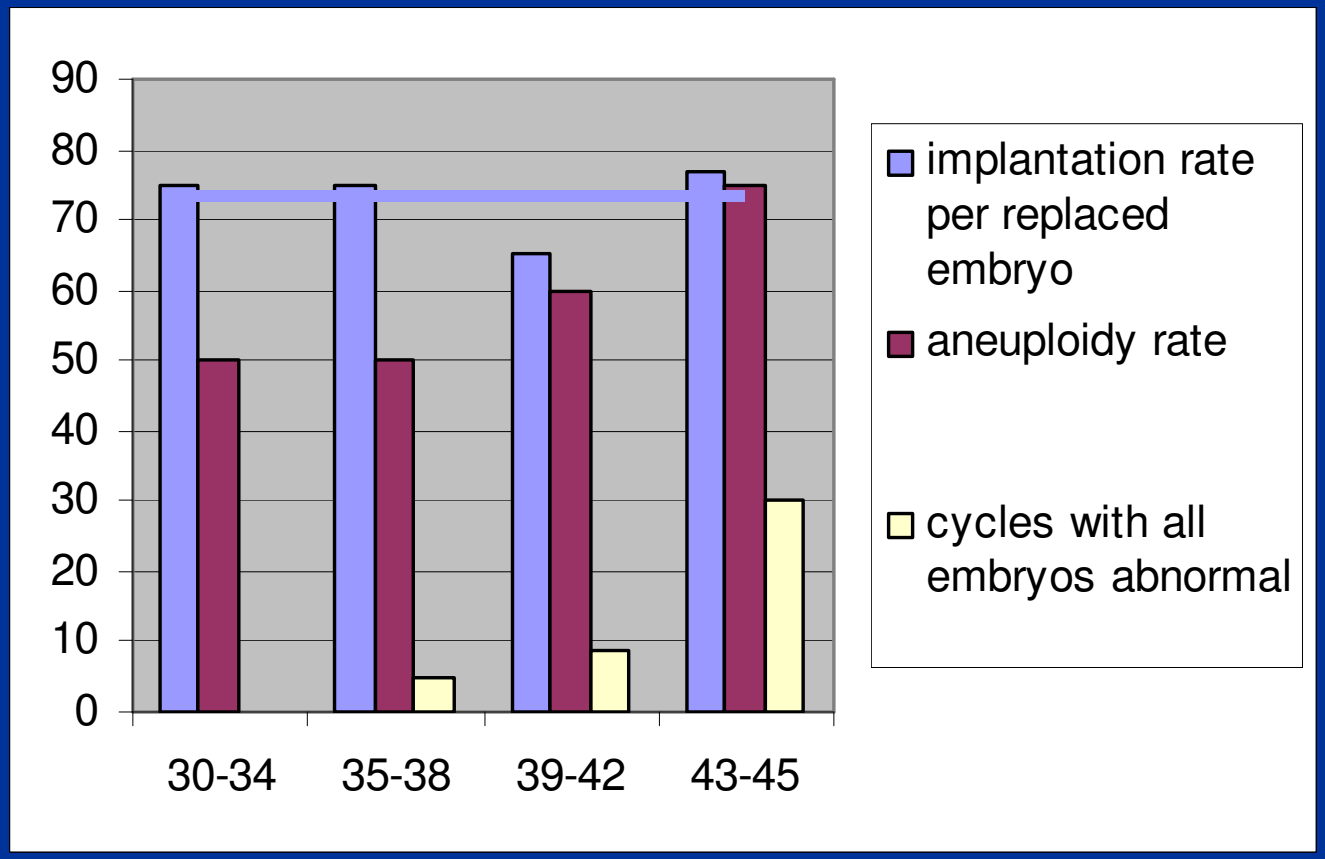
	Cycles	Mat. age	Prev. failed	embryos replaced cycles	implant. (+ sac)	ongoing preg.
CGH :	45	37.7	2.4	2.0	**67%**	**79%**
control :	113	37.1	1.2	2.7	**28%**	**60%**

p=0.0003

Schoolcraft et al. (in press)

CGH on blastocyst biopsies: Implantation is independent of age

Reprogenetics



Patients <43 who are eligible for blastocyst transfer have a >95% change of having normal embryos available for transfer

Results of aCGH in PB and day 3 biopsies

Reprogenetics

ESHRE study: PB data

Average age	40
Cycles	42
Embryo replaced	n/a
Implantation rate	n/a
Pregnancy rate	19%
Error rate	11%

Reprogenetics: data day 3 biopsy

Average age	40
Cycles	107
Av. Embryos replaced	1.0
Implantation rate	31%
Pregancy rate	26%
Error rate	2% *

** Gutierrez-Mateo et al., Fertil Steril, accepted*

SNP and CNP arrays:
For diagnosis of aneuploidy

aCGH vs. SNP arrays: Genome coverage

Reprogenetics

	# of probes		probe size		genome covered
aCGH	4,000	x	150,000 kb	=	600.0 Mb (25%)
SNPs	300,000	x	50 kb	=	1.5 Mb (>0.1%)

SNP arrays: Treff ' team validation

Comparison of implantation rates for those cases with mixed transfers:

- 33 transfers with a mix of SNP array normal and abnormal embryos
- 17 ongoing / delivered pregnancies
- 86 total embryos transferred:
  - 42 normal
  - 44 abnormal

	Embryo delivers	Failed Ongoing development
PGD normal	18	24
PGD abnormal	0	44

P<0.01

Scott, PCRS 2010

Slide adapted from R. Scott

SNP arrays: Treff ' team

Blastocyst biopsy, Cryopreservation, SNP array, transfer in thawed cycle

- N=368
- Two centers: RMANJ, CCRM
- Age = 38.2 years
- Number of prior attempts = 2.4
- Blastocysts transferred = 1.6

- Pregnancy rates:
  - clinical: 80%
  - ongoing past 1st trimester: 76%
  - sustained implantation rate: 60%
  - rates equivalent at the two centers (differ by < 1%)

Scott, PCRS 2010

Slide adapted from R. Scott

CONCLUSIONS

Conclusions: chromosome abnormalities

Reprogenetics

- Age and morphology are poor indicators of aneuploidy

- Less than 50% of good morphology day 3 embryos and less than 60% of blastocysts are normal in patients >35

- Selecting for euploid embryos should improve ART outcome

Conclusions: FISH studies

Reprogenetics

Studies with improved results differ from those that show no improvement in that:

1) Reduce biopsy damage (1 cell, experience, blast?)
2) Low error rate (fixation, NRR, 2 analyzers)
3) Analyze 16,15,21,22 chromosomes + ≥4 more
4) Extensive experience
5) Appropriate population (≥5 embryos, ≥ 35 y. old)

Conclusions: array CGH

Reprogenetics

- Blastocyst biopsy + CGH, SNP arrays + vitrification shows very high implantation rates (72%, av. Age 38).
- Array CGH and day 5 biopsy will produce same results
- Array CGH and day 3 biopsy improves results when normal embryos are available.
- Additional vitrification step may still be advantageous

Reprogenetics

Santiago Munné, PhD, Director
Jacques Cohen, PhD, Director

USA

Pere Colls, Ph.D
Dagan Wells, Ph.D
George Pieczenik, Ph.D
Cristina Gutiérrez, Ph.D
Jorge Sanchez, PhD
John Zheng, MD
Tomas Escudero, MS
Kelly Ketterson, MS
Jill Fischer, MS
Gary Harton, MS
Jessica Vega, MS
Tim Schimmel
Sasha Sadowy
Sophia Tormasi
N-neka Goodall
Renata Prates
Piedad Garzón
Laurie Ferrara
Bekka Sellon-Wright
Maria Feldhaus

munne@reprogenetics.com
www.reprogenetics.com

Spain
Mireia Sandalinas, MS
Carles Giménez, PhD
César Arjona, MS
Ana Jiménez, PhD
Elena Garcia, MS

Japan
Tetsuo Otani, MD
Muriel Roche
Miho Mizuike

UK
Dagan Wells, PhD
Elpida Fragouli, PhD
Samer Alfarawati, MS
Michalis Konstantinidis

South America
Paul Lopez
Luis Alberto Guzman

Germany
Karsten Held, MD