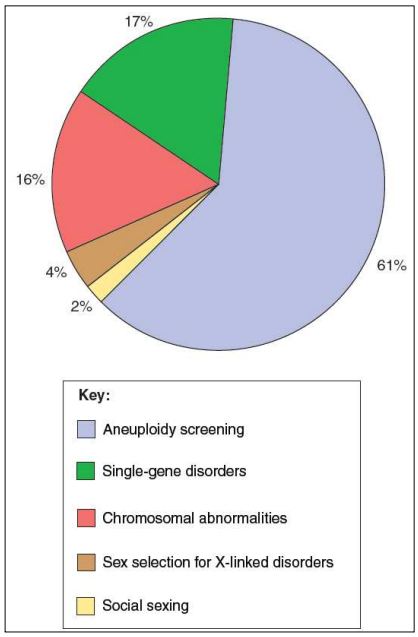
New perspectives on embryo biopsy, not how, but when and why

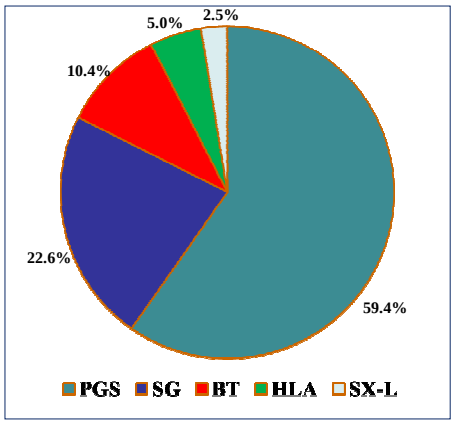
PGS

Kangpu Xu, PhD
Director, Laboratory of Preimplantation Genetics
Center for Reproductive Medicine
Weill Cornell Medical College of Cornell University

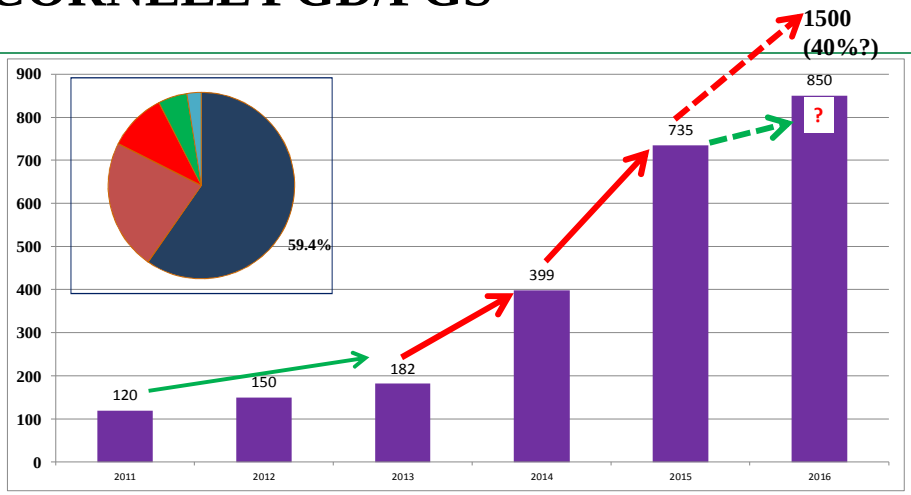
"ESHRE 10 Years", Harper et al., HRU 2012; 18:234



CRM-PGD, 1992 to 2015



CORNELL PGD/PGS



FISH -> Array
D3 – D5/6

~20% of IVF Patients
~4000 IVF Cycles

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A Statement on the use of Preimplantation Genetic Screening (PGS) of chromosomes for IVF patients

CONSENSUS STATEMENT ON PGS

For all practitioners of IVF there is the clinical imperative

- to achieve the highest chance of a live birth per single attempt, reducing the time to delivery for each patient;
- to reduce the incidence of miscarriage; reduce the number of multiple pregnancies;
- decrease the number of non-viable embryo transfers ('unnecessary IVF transfer cycles');
- eliminate the freezing of embryos that are chromosomally abnormal;
- to diagnose patients with no chance to deliver with IVF; and,
- given the high incidence of embryo aneuploidy in all IVF cycles, to minimize the chance of transferring an aneuploid embryo.

- **The Undersigned have issued the Statement below and welcome debate and comment in this forum.**

http://www.ivf-worldwide.com/cogen/general/cogen-statement.html

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Contradictions in Recent Literature

Outcomes of in vitro fertilization with preimplantation genetic diagnosis: an analysis of the United States Assisted Reproductive Technology Surveillance Data, 2011–2012

Jeani Chang, M.P.H., Sheree L. Boulet, Dr.P.H., Gary Jeng, Ph.D., Lisa Flowers, M.P.A., and Dmitry M. Kissin, M.D., M.P.H.

Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

man Reproduction, Vol.30, No.2 pp. 473–483, 2015
vanced Access publication on November 28, 2014 doi:10.1093/humrep/deu303

human reproduction REVIEW *Reproductive genetics*

The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review

Evelyn Lee[1,*], Peter Illingworth[2], Leeanda Wilton[3], and Georgina Mary Chambers[1]

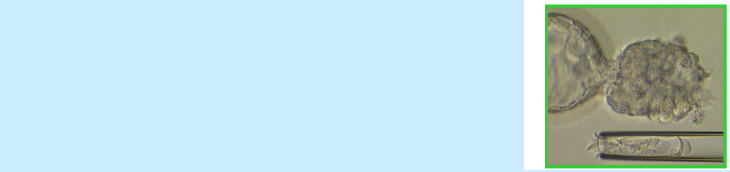
[1]National Perinatal Epidemiology and Statistics Unit, School of Women's and Children's Health, University of New South Wales (UNSW), Level 2, McKeon Dickson Building, Randwick Hospitals Campus, Sydney 2031, Australia [2]IVF Australia Pty Ltd, 176 Pacific Highway, Greenwich, Sydney 2065, Australia [3]Melbourne IVF, Victoria Parade, East Melbourne, VIC 3002, Australia

- Aneuploidy screening was the most common indication for PGD.
- Use of PGD was not observed to be associated with an increased odds of clinical pregnancy or live birth for women <35 years.
- PGD for aneuploidy was associated with a decreased odds of miscarriage for women >35 years, but an increased odds of a live-birth and a multiple live-birth delivery among women >37 years.
- The three RCTs demonstrated benefit in young and good prognosis patients in terms of clinical pregnancy rates and the use of single embryo transfer.
- However, studies relating to patients of advanced maternal age, recurrent miscarriage and implantation failure were restricted to matched cohort studies, limiting the ability to draw meaningful conclusions.

Technical advancement & limitations

- Biopsy from D5/6 embryos,
- Specimens undergone WGA (noise and background) and WGA products subject to array or NGS to obtain chromosome copy number analysis
- WGA products subject to array or NGS to obtain chromosome copy number analysis
- Software makes "Call" or "Not to Call", "A SPECILIST" will make the final "CALL" and prepare the report.
- Variations of unknown significance

Biopsy for PGS - When



Vitrification

ET

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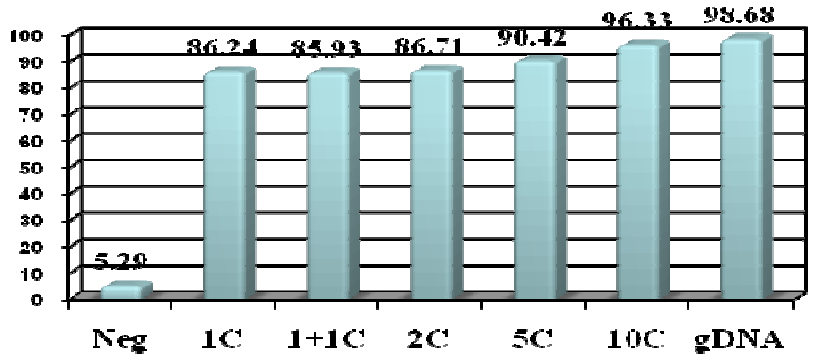
Use of SNP Array for few cells (2009)

Molecular Human Reproduction, Vol.15, No.11 pp. 739–747, 2009
Advanced Access publication on August 11, 2009 doi:10.1093/molehr/gap066
MHR ORIGINAL RESEARCH

SNP Calling from WGA (MDA) 1, 2, 5, 10 cells, Affy 10K SNP array



The SNP call rate from 1C, 1+1C and 2C groups showed no significant difference ($p>0.05$), but when the cell number increased to 5-10 cells, the call rate presented significant difference ($p<0.05$).

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Detection of Mosaicism

Chromosome complements of the blastomeres analyzed by aCGH

Human Reproduction, Vol.28, No.1 pp. 256–264, 2013
Advanced Access publication on October 9, 2012 doi:10.1093/humrep/des362

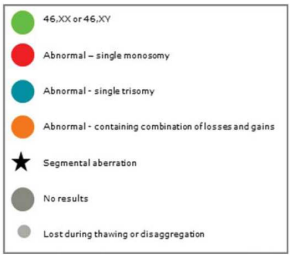
human reproduction ORIGINAL ARTICLE *Reproductive genetics*

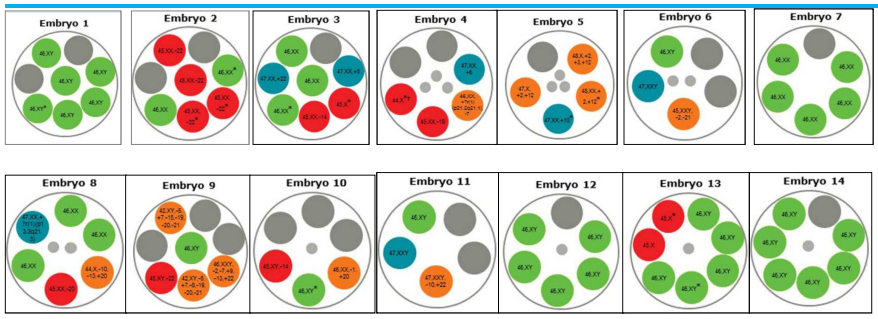
Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos

A. Mertzanidou[1,†], L. Wilton[2,†], J. Cheng[3,4,†], C. Spits[1], E. Vanneste[5], Y. Moreau[3,4], J.R. Vermeesch[5], and K. Sermon[1,*]

[1]Faculty of Medicine and Pharmacy, Research Group Reproduction & Genetics, Vrije Universiteit Brussel (VUB), 1090 Brussels, Belgium [2]Preimplantation Genetics, Melbourne IVF, Suite 10/320 Victoria Parade, Victoria, Australia [3]Department of Electrical Engineering, Esat-SCD, Katholieke Universiteit Leuven, 3001 Leuven, Belgium [4]IBBT-K.U.Leuven Future Health Department, 3001 Leuven, Belgium [5]Centre for Human Genetics, University Hospital Gasthuisberg, B-3000 Leuven, Belgium

*Correspondence address. Tel: +32-2-477-46-35; E-mail: karen.sermon@uzbrussel.be





Mertzanidou et al., 2013

D5/6 Mosaicism

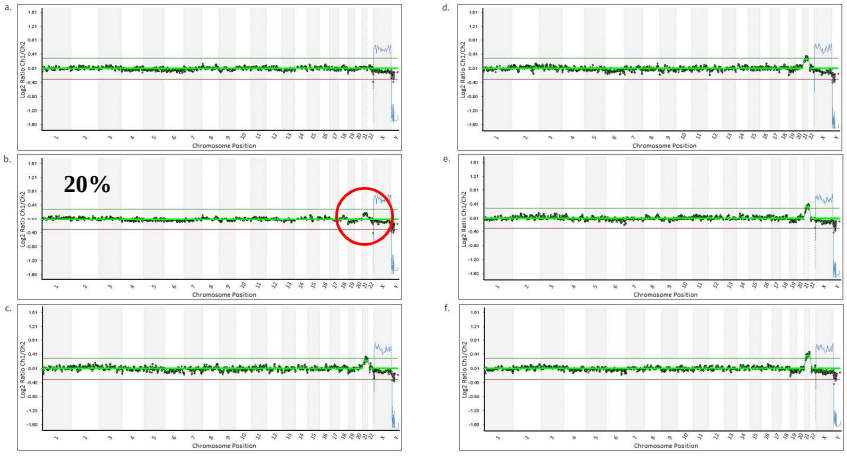
1) Can mosaicism be detected in the biopsied specimens with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?

Mix of 46,XX and 47,XX,+21, aCGH

(a) G1, 0% trisomic
(b) G2, 20% trisomic
(c) G3, 40% trisomic
(d) G4, 60% trisomic
(e) G5, 80% trisomic
(f) G6, 100% trisomic



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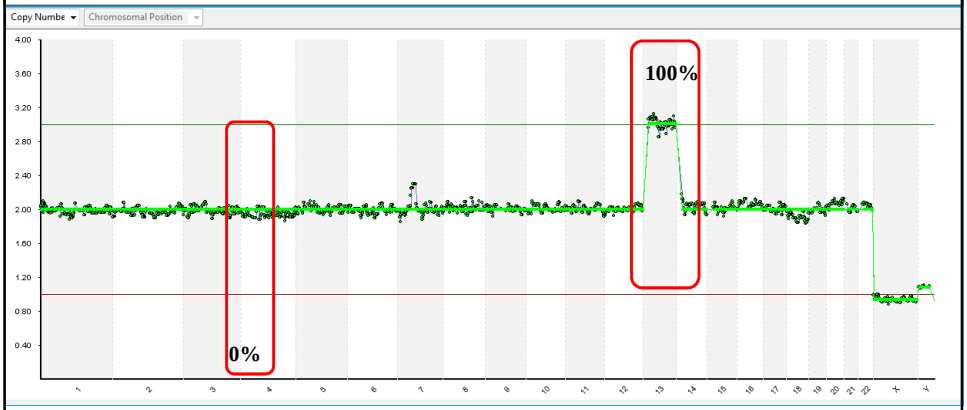
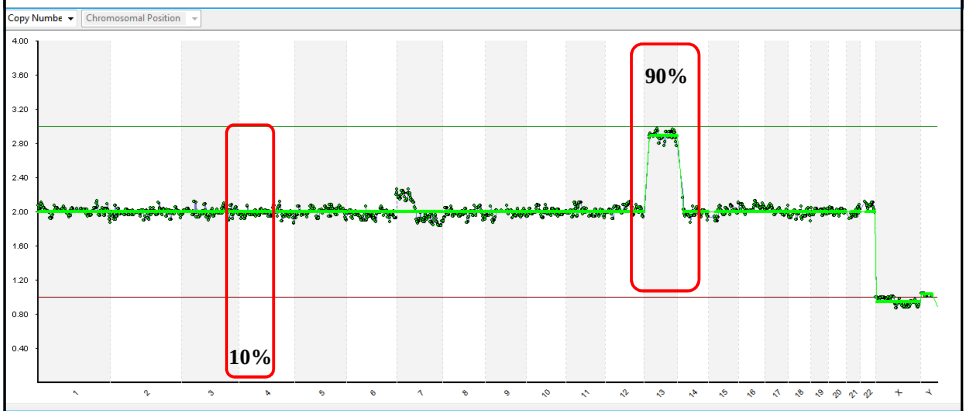
NGS Cell Mix Validation Test

46,XY, del(4p) : 47,XY,+13 (0 : 10)

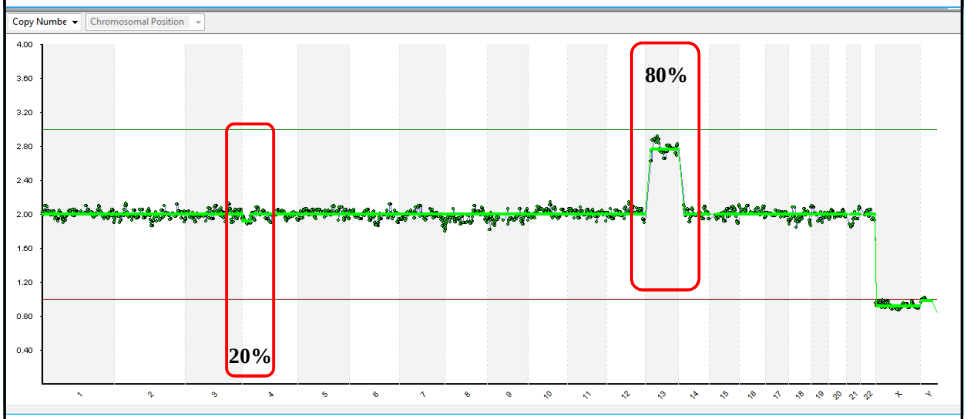


Wolf Hirschhorn Syndrome, WHS (~26MB)

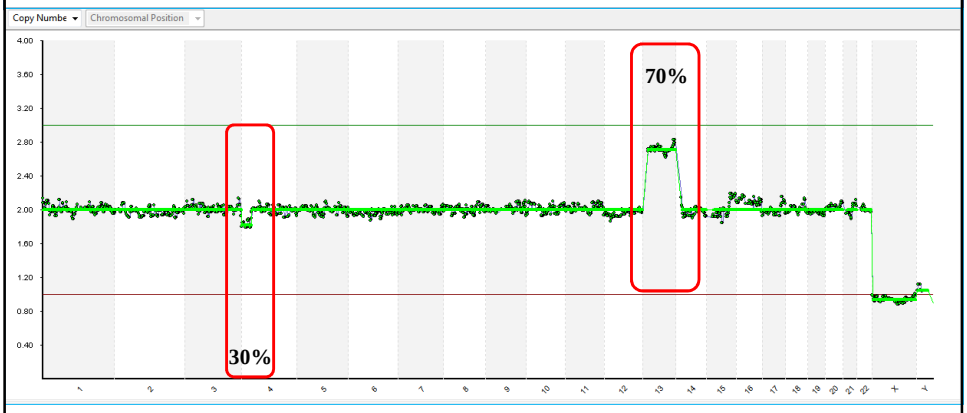
46,XY, del(4p) : 47,XY,+13 (1 : 9)



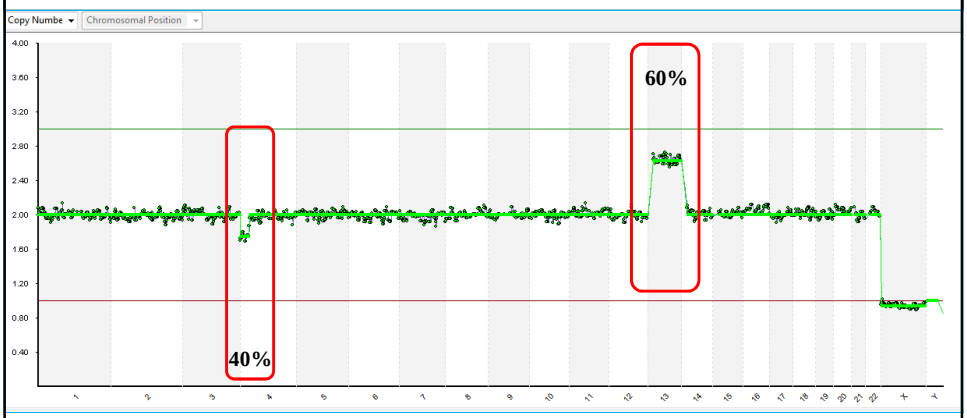
46,XY, del(4p) : 47,XY,+13 (2 : 8)



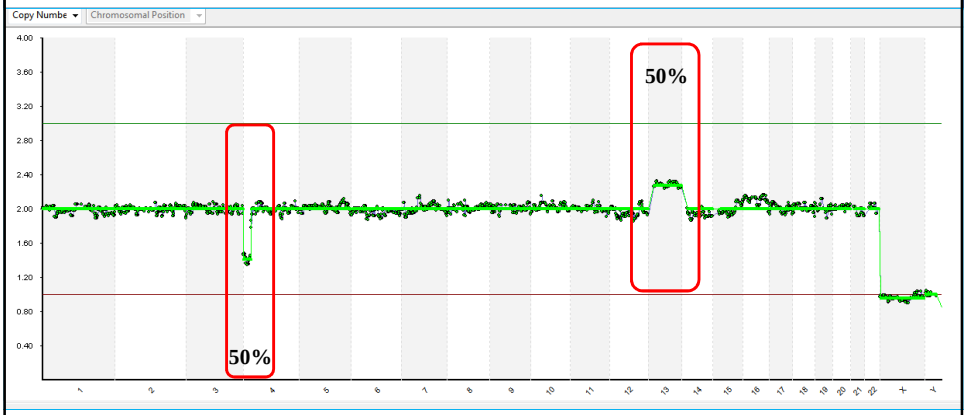
46,XY, del(4p) : 47,XY,+13 (3 : 7)



46,XY, del(4p) : 47,XY,+13 (4 : 6)



46,XY, del(4p) : 47,XY,+13 (5 : 5)



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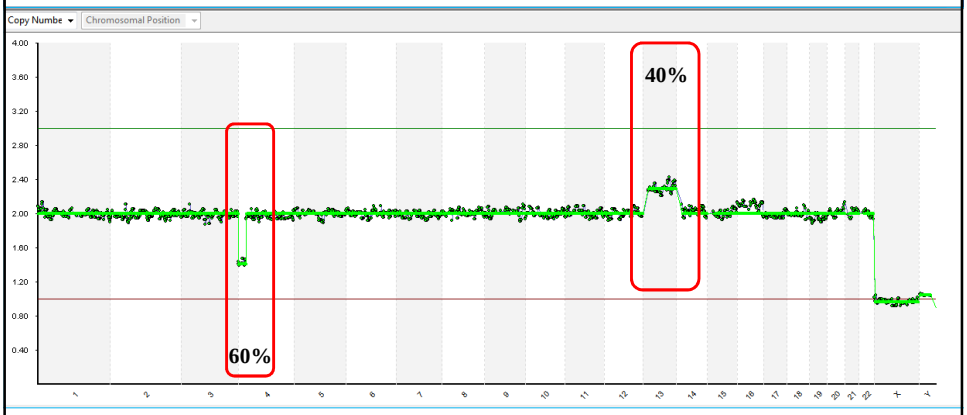
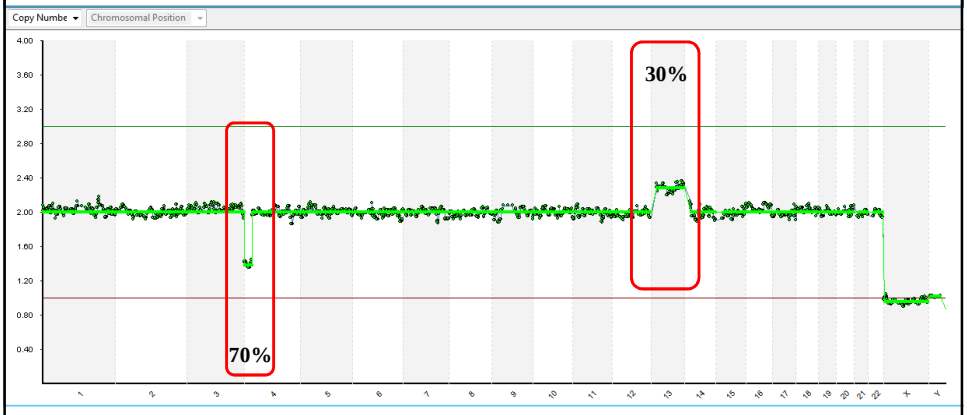
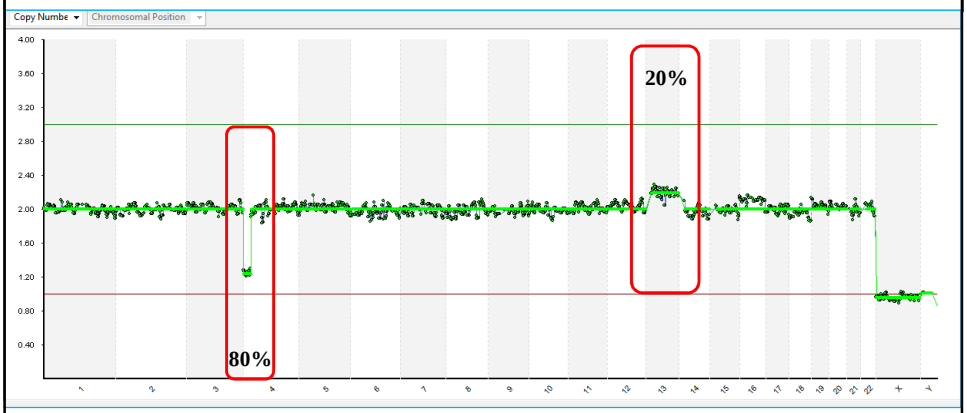


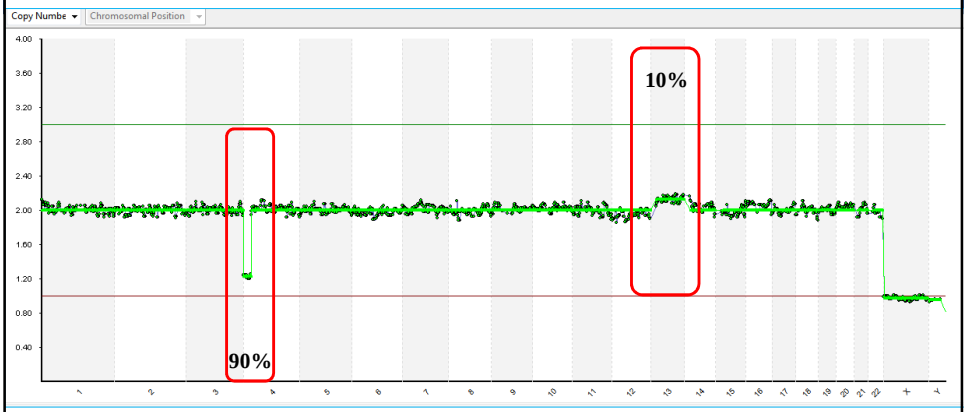
46,XY, del(4p) : 47,XY,+13 (7 : 3)



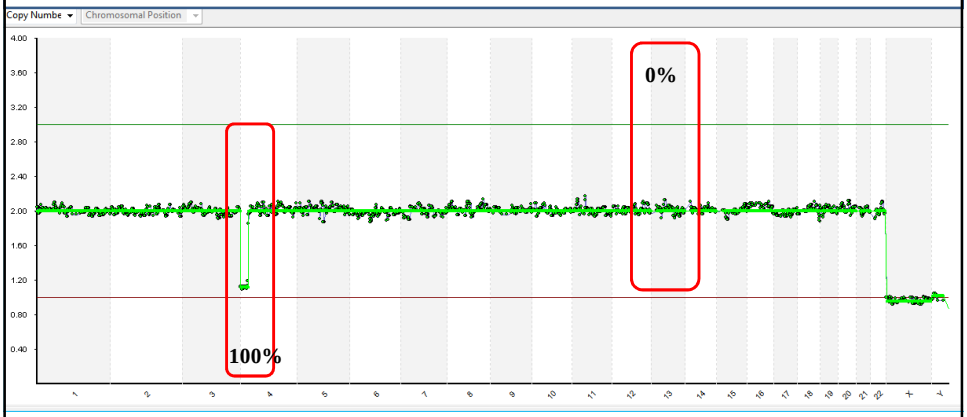
46,XY, del(4p) : 47,XY,+13 (8 : 2)



46,XY, del(4p) : 47,XY,+13 (9 : 1)



46,XY, del(4p) : 47,XY,+13 (10 : 0)



D5/6 Mosaicism

1) Can mosaicism be detected with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?

Incidence of mosaicism: 4%, 16%, 21%, 33% or 69%

3.9%
Johnson et al., (Mol. Hum. Reprod., 2010) observed 49/51 (96.1%) ICM samples were concordant with TE biopsies derived from the same embryos.

~16%
Northrop et al. (Mol. Hum. Reprod., 2010) found 16% of embryos are mosaic.

21.2%
Capalbo et al., (Hum. Reprod. 2013), by FISH reanalysis of previously aCGH-screened blastocysts, a total of 66 aneuploidies were scored, 52 (78.8%) observed in all cells and 14 (21.2%) mosaic.

~33%
Fragouli et al., (Hum. Reprod., 2011) demonstrated that about one-third of all blastocysts are mosaic.

69%
Liu et al. (Biol. Reprod., 2012) reported 69% of abnormal blastocysts from women of advanced age are mosaic.

D5/6 Mosaicism

1) Can mosaicism be detected with current array or NGS platform?

2) What do we know in the literature?

3) Are the rates we detected in the biopsied (TE) specimens truly reflecting what is a) in the whole embryo, b) in ICM?

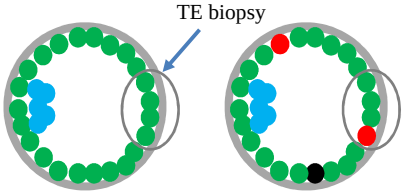
Mosaic patterns and risk of misdiagnosis

D3, cleavage stage

What is the incidence of mosaicism that may cause false positives?

D5/6, blastocysts

TE biopsy



TE = 25 Cells, ICM = 5 Cells

Correct diagnosis

Correct diagnosis(?)

Low rate of mosaicism

False negative

Likely, will not implant

False positive?

20% Diploid/aneuploid

Diploid ICM

Diploid (euploid) TE

Aneuploid cells

Should we transfer mosaic embryos?

Transfer of Mosaic (monosomic) Embryos

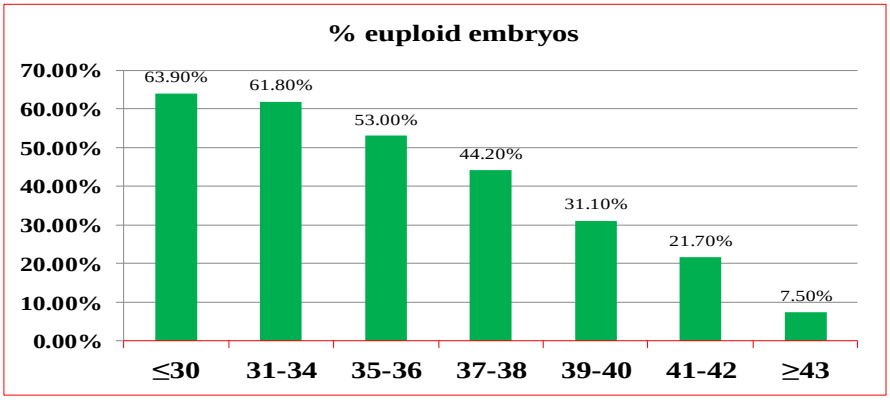
Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

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

Our study shows that mosaic embryos can develop into healthy euploid newborns. These findings have implications for women who undergo IVF resulting in mosaic embryos but no euploid embryos.

Greco et al., , New Engl. J. Med., 2016.

Euploidy with age (PGS 24 chromosomes)



Total# **3507**

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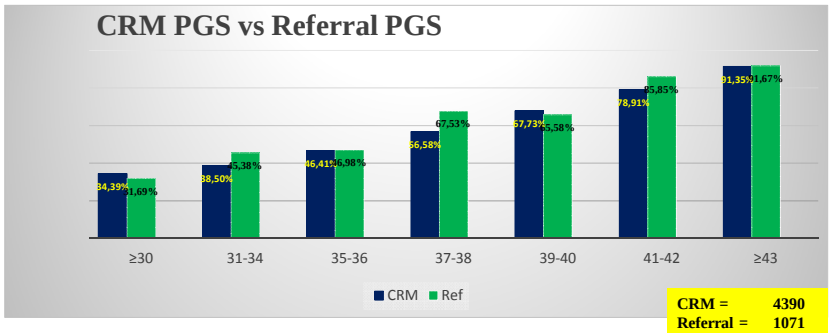
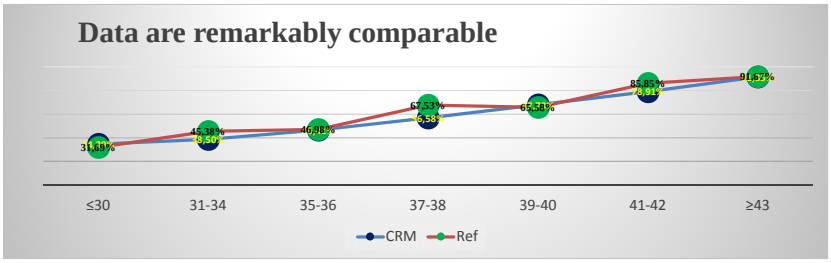
Aneuploidy detected by CRM and Referral Labs (D5/6)



CRM = **4390**
Referral = **1071**



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Update: 2016-03-26

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Preimplantation genetic screening: who benefits?

Fert, Steril, 2016; Article in press.

Hey-Joo Kang, M.D., Alexis P. Melnick, M.D., Joshua D. Stewart, M.D., Kangpu Xu, Ph.D., D.V.M., and Zev Rosenwaks, M.D.

The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York

Table 1

Demographic characteristics of PGS and control cycles.

Characteristic	Age, ≤37 y			Age, >37 y		
	PGS (n = 99)	Controls (n = 677)	*P*	PGS (n = 60)	Controls (n = 216)	*P*
Age, y	33.9 ± 2.8	32.4 ± 3.2	<.01	40.6 ± 1.5	39.7 ± 1.3	<.01
BMI, kg/m^2	22.2 ± 6.1	23.1 ± 4.6	.13	23.2 ± 8.3	23.5 ± 4.3	.72
Gravidity	2.1 ± 1.8	0.94 ± 1.3	<.001	2.75 ± 1.54	1.5 ± 1.92	<.001
Parity	0.47 ± 0.85	0.27 ± 0.59	.11	0.40 ± 0.59	0.43 ± 0.76	.79
Previous IVF	1.81 ± 1.76	0.71 ± 1.27	<.001	3 ± 2.82	0.93 ± 1.51	<.001
Previous abortion	0.95 ± 1.42	0.28 ± 0.61	<.001	1.35 ± 1.57	0.45 ± 0.82	<.001

Note: Data are presented as mean ± SD.

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Preimplantation genetic screening: who benefits?

Fert, Steril, 2016; Article in press.

Hey-Joo Kang, M.D., Alexis P. Melnick, M.D., Joshua D. Stewart, M.D., Kangpu Xu, Ph.D., D.V.M., and Zev Rosenwaks, M.D.

The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York

Table 2

IVF cycle outcomes in women ≤37 years old.

Outcome	SET			Double ET		
	PGS (n = 62)	Controls (n = 161)	*P*	PGS (n = 37)	Controls (n = 516)	*P*
Age (y)	33.9 ± 2.8	32.3 ± 3.0	<.01	33.9 ± 2.9	32.4 ± 3.3	<.01
Implantation rate	0.54 ± 0.5	0.54 ± 0.52	.92	0.47 ± 0.40	0.52 ± 0.44	.52
Biochemical pregnancies	6 (9.7)	18 (11.2)	.75	5 (13.5)	62 (11.8)	.79
Clinical intrauterine gestations	34 (54.8)	85 (52.8)	.78	23 (62)	334 (64.7)	.75
Missed/spontaneous abortions	3 (4.8)	9 (5.6)	.82	0 (0)	25 (4.8)	.17
Live births	31 (50)	76 (47)	.71	23 (62)	309 (59.9)	.79
Twins	0	2 (1.2)	1	10 (27)	158 (31)	.65

Note: Data are presented as mean ± SD and n (%).

Table 3

IVF cycle outcomes in women >37 years old.

Outcome	SET			Double ET		
	PGS (n = 45)	Controls (n = 27)	*P*	PGS (n = 15)	Controls (n = 189)	*P*
Age (y)	40.8 ± 1.7	39.3 ± 1.5	<.01	40 ± 0.85	39.7 ± 1.3	.37
Implantation rate	0.62 ± 0.49	0.37 ± 0.49	.04	0.53 ± 0.30	0.37 ± 0.42	.18
Biochemical pregnancies	5 (11)	2 (7.4)	.61	1 (6.7)	23 (12.2)	1.0
Clinical intrauterine gestations	28 (62.2)	10 (37.0)	.04	13 (86.7)	97 (51.3)	<.01
Missed/spontaneous abortions	2 (4.4)	5 (18.5)	.051	1 (6.7)	16 (8.5)	1.0
Live Births	26 (57.8)	5 (18.5)	<.01	12 (80)	81 (42.9)	<.01
Twins	0	0		5 (33.3)	29 (15.3)	.14

Note: Data are presented as mean ± SD and n (%).

- **Among patients ≤37, IVF-PGS does not improve CIG, LB, and miscarriage rates.**
- **IVF-PGS in women >37 improved CIG and LB rates.**
- **However, per cycle, the PGS advantage in this age group does not persist.**

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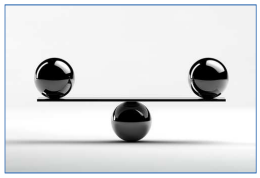
Discussions

Aneuploid embryos can be identified accurately when gain or loss in one or more chromosomes are involved.
Although mosaicism can be accurately detected in a model system, it is difficult to know exact number of cells biopsied, therefore, the extent of mosaicism in the specimen can only be estimated.
Knowledge of mosaicism on D5/6 embryos is limited.

Present views on PGS are controversial.
With PGS, improvement of overall IVF outcome, particularly for woman of advanced age, is not yet clear.
Specific indications need to be identified, discussed with patients, number of "BIOPSIABLE" should be evaluated for each individual patient/PGS-Cycle.



PGS may not be applied for "ALL" patients

Rebiopsy maybe considered when there is a doubt on the results and the embryo quality appears to be "good".

Research on D5/6 mosaicism is urgently needed.

Artificial gametogenesis will address the issues.

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Acknowledgement (PGD is a Team Effort)

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