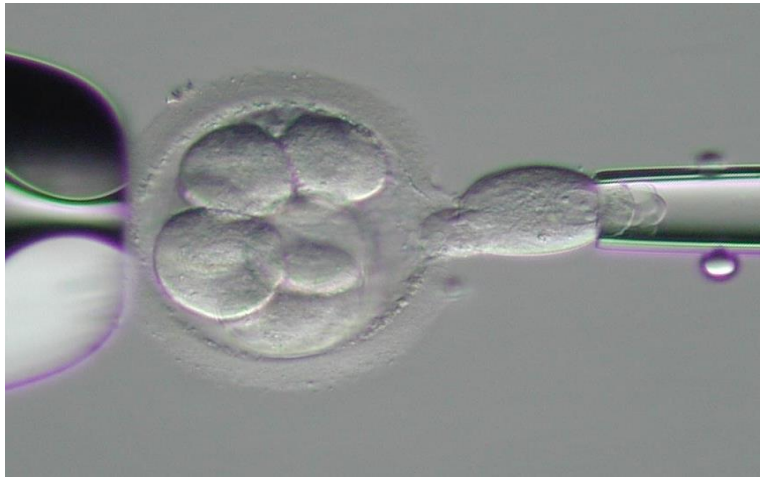
Preimplantation Genetic Screening

Dr Gülay Özgön
Nesiller Genetik Tanı Merkezi

Preimplantation Genetic Testing

Detection of genetic information in an embryo made by examining a representative sample taken at a preimplantation stage of development



Pre-implantation genetic screening (PGS)

- Screening for large chromosomal imbalances (implantation failure, early miscarriage, viable trisomies e.g. T21)
- Who is being offered this technology?
  - Recurrent pregnancy loss
  - Recurrent implantation failure
  - Advanced maternal age
  - ?Everyone
- What is the evidence to support use of PGS?

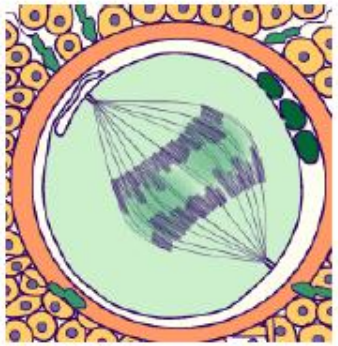
Accepted uses of PGS

Pre-implantation genetic screening (PGS) diagnosis of non-familial chromosomal disorders (aneuploidy testing) where:

- (i) the woman is of advanced reproductive age
- (ii) the woman has had recurrent implantation failure or recurrent miscarriage

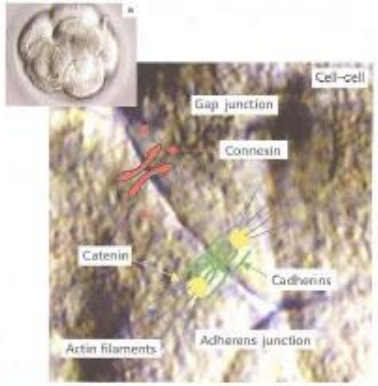
Embriyo Development

-DNA synthesis
-Nuclear membrane breakdown
-Chromosome alignment on the spindle apparatus at the equator



The zygote, the first cell with an individual genome (2n4C).

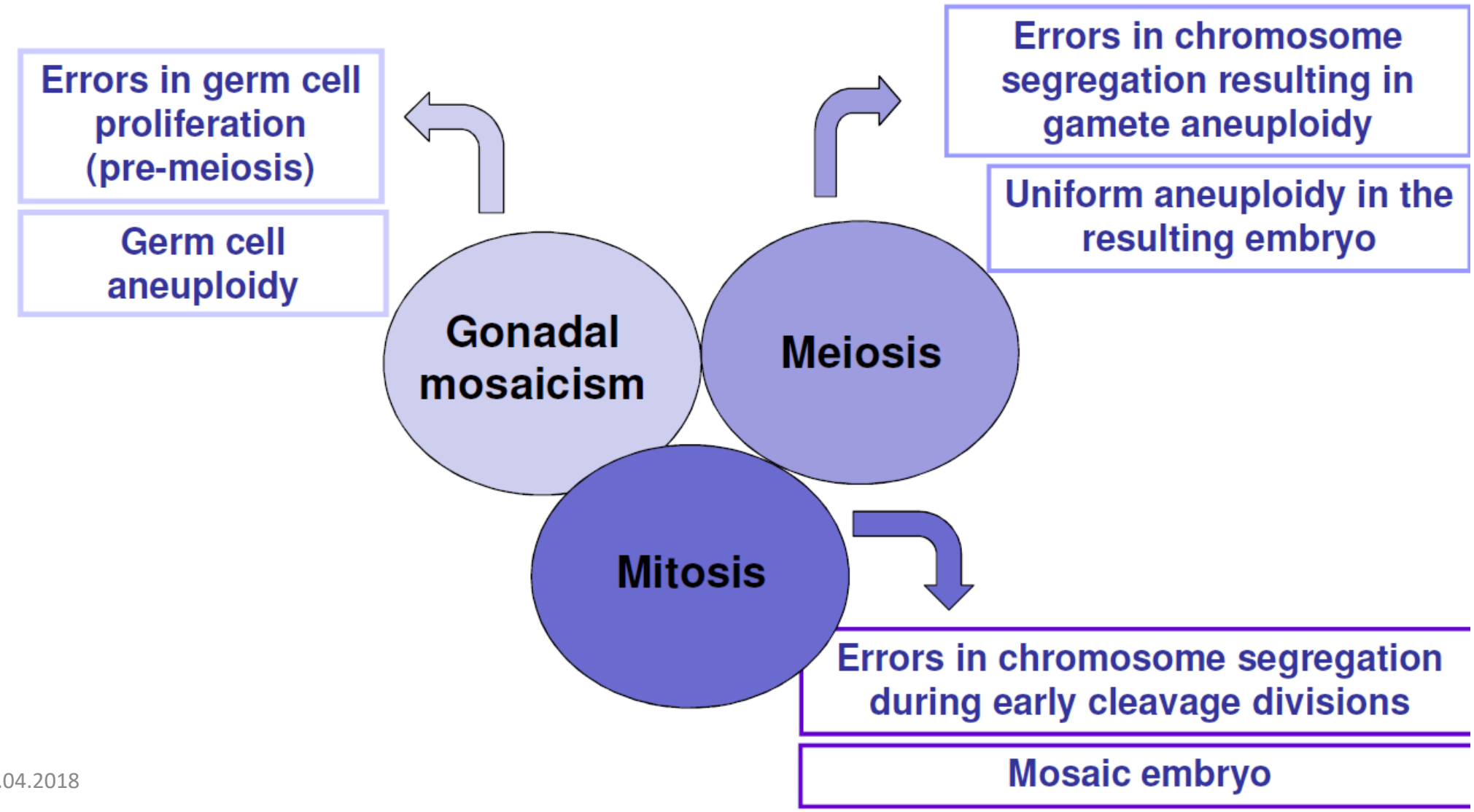
-Embryonic genome activation
-Blastomeres still totipotent



-Compaction
-Blastomeres start commitment to ICM or TE
-Gradual loss of totipotency
-Formation of:
•gap junctions
•adherens junctions
•tight junctions
•desmosomes

-Cavitation
-Formation of the blastocoelic fluid
-Differentiation into ICM or TE
-Zona thinning
→ blastocyst expansion
-Zona hatching
→ implantation

Origin of Aneuploidy



Origin of Aneuploidy

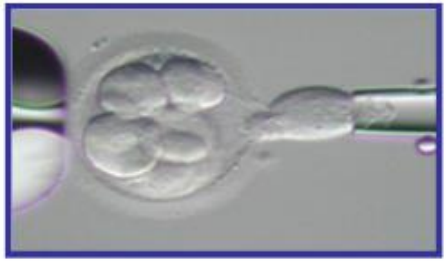
Polar bodies

Errors in chromosome segregation resulting in gamete aneuploidy

Uniform aneuploidy in the resulting embryo

Meiosis

Blastomere



Mitosis

Trophectoderm

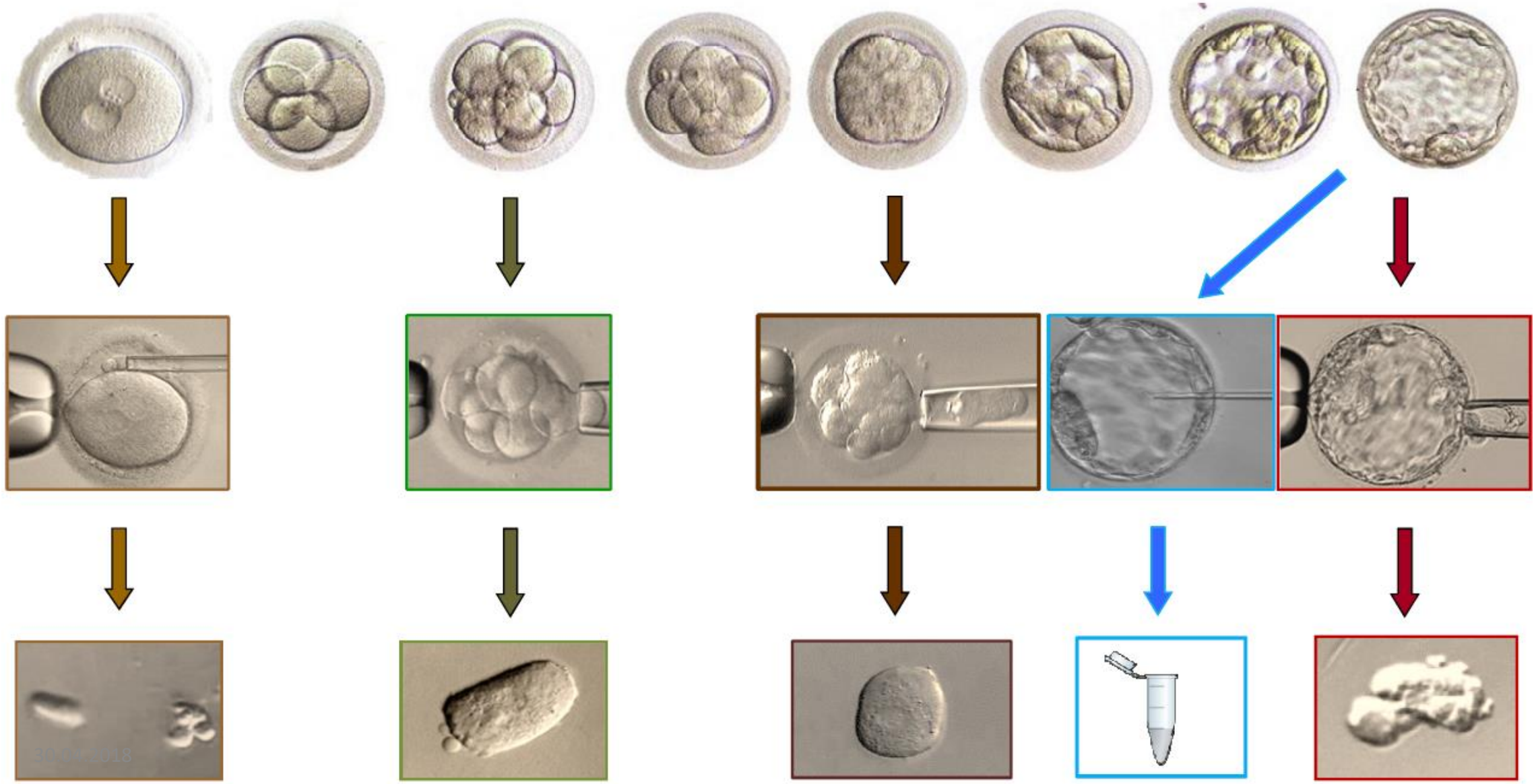
Errors in chromosome segregation during early cleavage divisions

Mosaic embryo

Errors in chromosome segregation during meiotic divisions in oogenesis and following mitotic divisions are a major cause of aneuploidy conceptions, leading to:

- implantation failure
- pregnancy loss
- congenital disorders

Which stage to biopsy

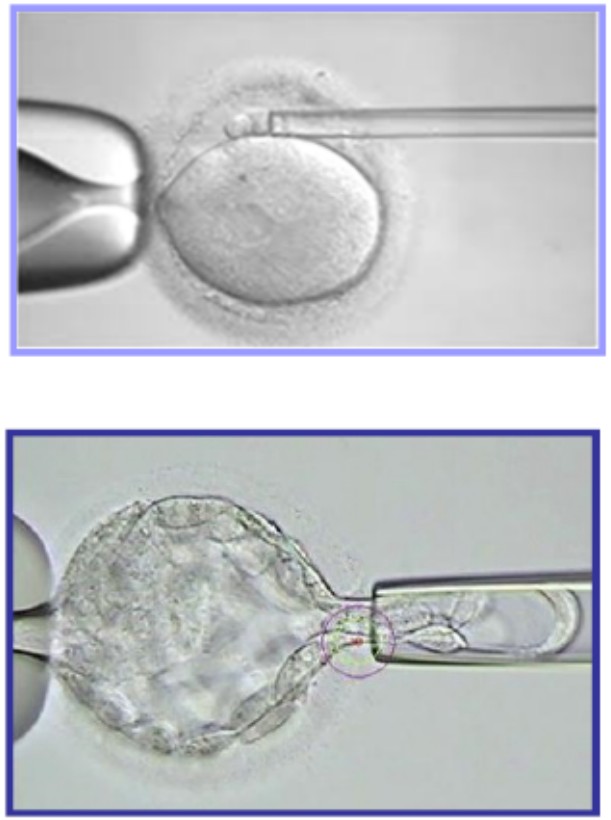


PB Testing

Pros	Cons – genetics lab	Cons – IVF lab
Less invasive	Informative only for the maternal counterpart	More biopsy procedures (oocytes>day3 embryos>blastocysts)
	Moderately predictive of the embryo status	Sequential biopsies – timing
	PB1 involved in both single chromatids and whole chromosomes copy number variations	

Data from the literature
Two registered ongoing RCTs (ESHRE ESTEEM; Weill Medical College, Cornell University)
Some studies report no negative effects on embryo development / implantation (Eldar-Geva et al., 2014; Hammoud et al., 2010; Montag et al., 2013)
Some studies report poor prediction of embryo ploidy (Capalbo et al., 2013; Salvaggio et al., 2014)

PB Testing



In regularly developing embryos, severe male factor patients excluded, PBs are highly predictive of the blastocysts chromosome status (94%).

Gianaroli et al., 2014; Magli et al., 2015

PB Testing

- It is not a common procedure

- No sufficiently powered controlled studies have been published so far, supporting a positive or negative impact of PB biopsy and testing on embryo developmental potential.

Testing on day 3

Pros	Cons – genetics lab	Cons – IVF lab
Paternal, maternal and mitotic contribution to aneuploidy	High level of mosaicism – only one cell analyzed – impact on accuracy	More biopsy procedures (day3 embryos>blastocysts)
Usually multiple embryos available	High level of chromosome instability – impact on accuracy	More risk of embryo damage – use of Ca/Mg-free medium, ZP opening ?
	Moderately predictive of the blastocyst chromosome status	

Testing on day 3 embrios, Mosaicism

The clinical consequences of mosaicism depend on 1) when during development the error occurs, and 2) on the proportion of cells that continue to propagate. At the cleavage-stage embryo, the consequences of chromosomal mosaicism are more severe then if it occurs at later stages.

Incidence of mosaicism

from 15 to 90% at the cleavage stage
from 15 to 30% at the blastocyst stage
from 1 to 2% in prenatal diagnosis

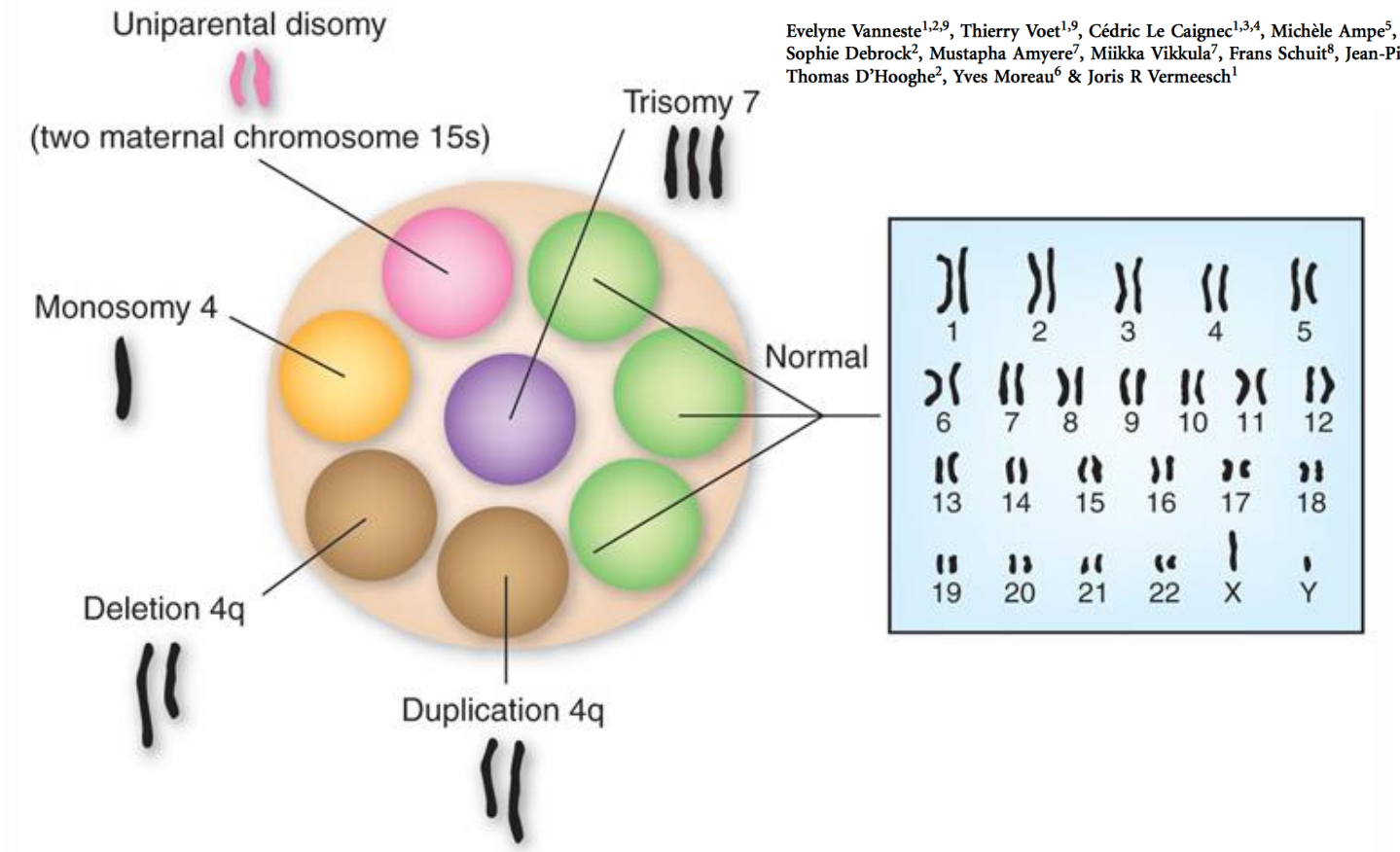
a selection mechanism against mosaicism / correction of aneuploidy in the later stages of development

Taylor et al., 2014

Cleavage stage mosaicism

Chromosome instability is common in human cleavage-stage embryos

Evelyne Vanneste[1,2,9], Thierry Voet[1,9], Cédric Le Caignec[1,3,4], Michèle Ampe[5], Peter Konings[6], Cindy Melotte[1], Sophie Debrock[2], Mustapha Amyere[7], Miikka Vikkula[7], Frans Schuit[8], Jean-Pierre Fryns[1], Geert Verbeke[5], Thomas D'Hooghe[2], Yves Moreau[6] & Joris R Vermeesch[1]



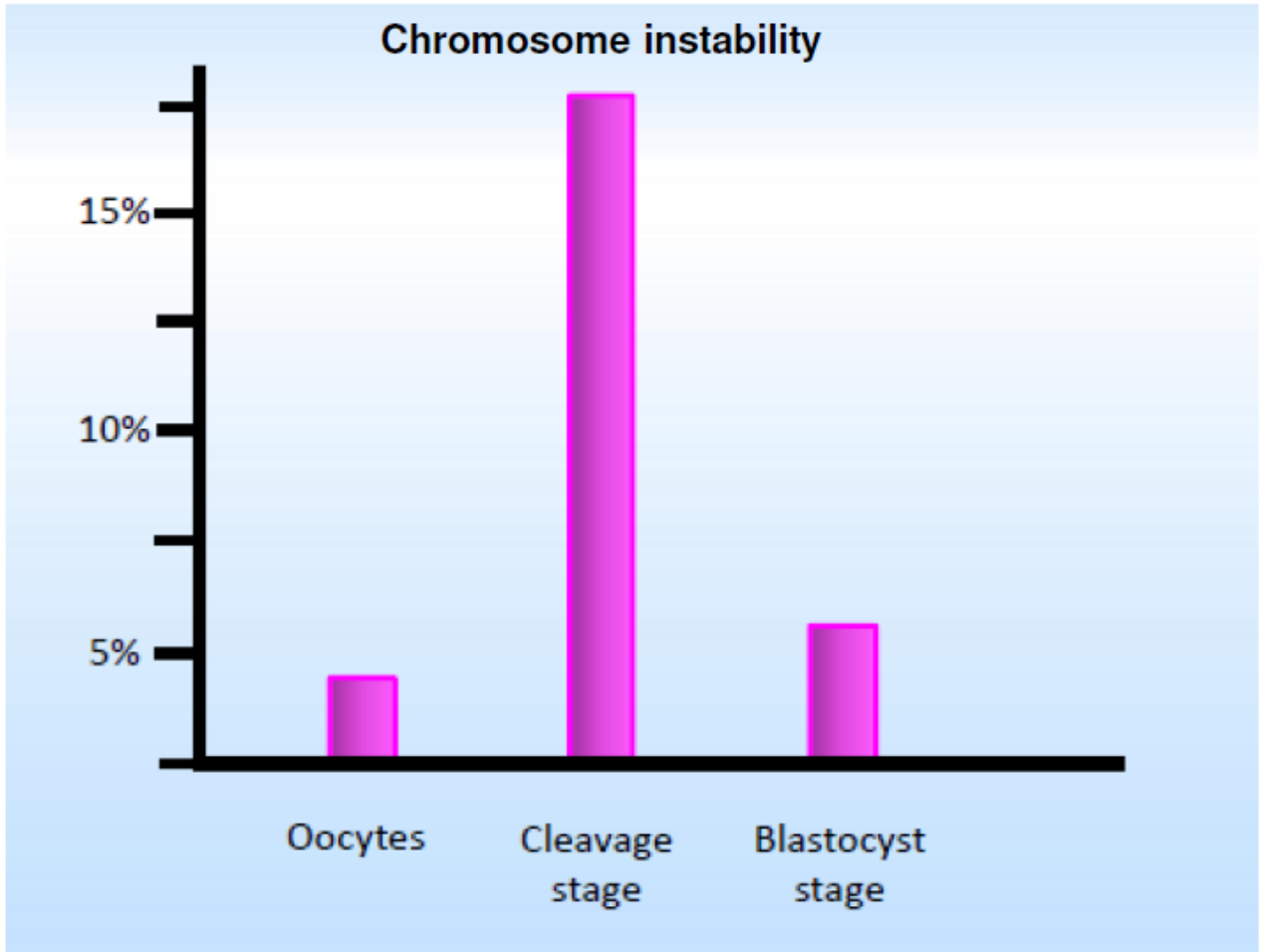
Chaos in the embryo

David H Ledbetter

The chromosomes of human embryos seem to be more unstable than previously thought. An analysis of embryos derived from *in vitro* fertilization reveals high rates of structural abnormalities (pages 577–583).

nature medicine
15, 490 - 491 (2009)

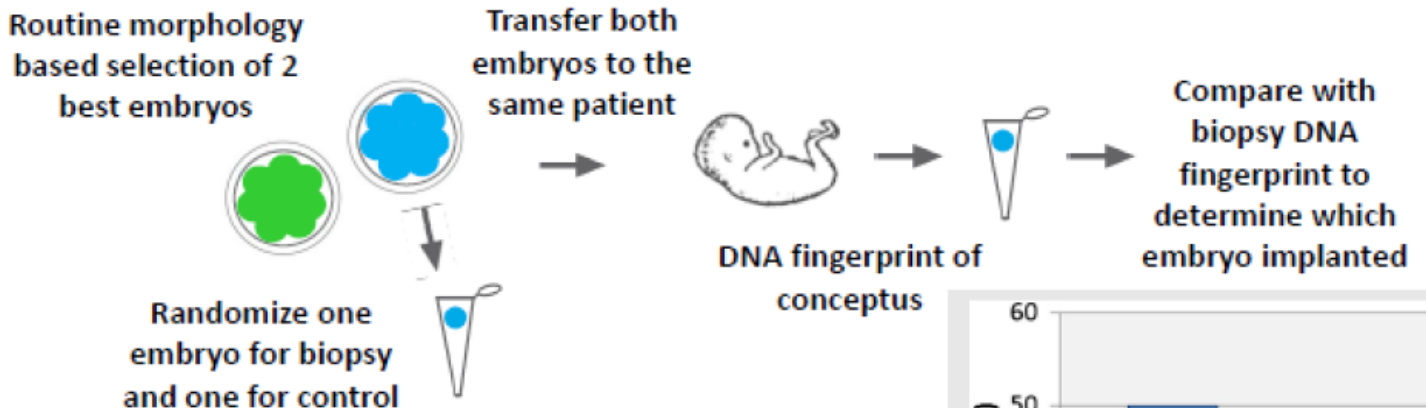
Testing on day 3 embrios



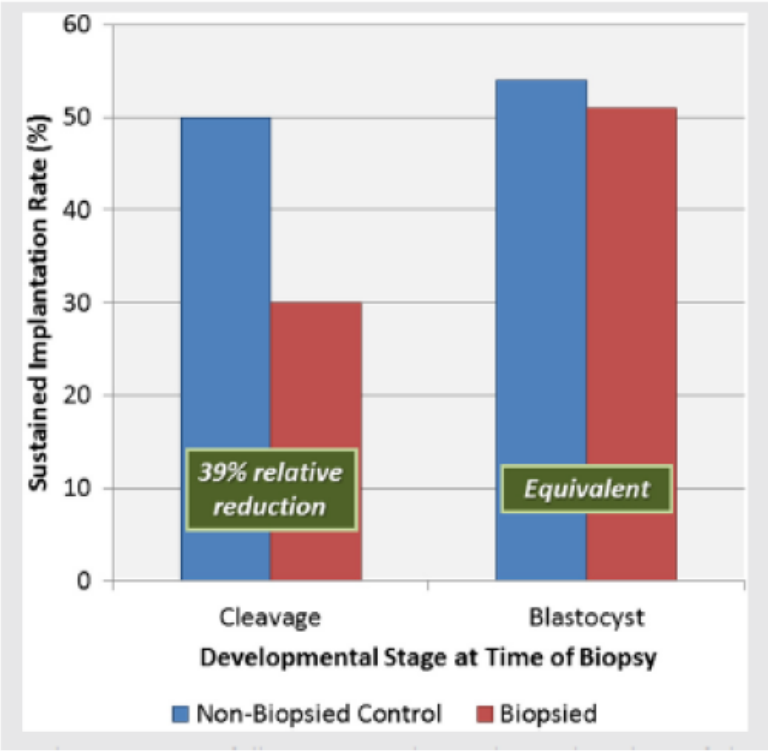
Frequent segmental deletions, duplications and amplifications, often reciprocal in sister blastomeres, implying the occurrence of breakage-fusion-bridge cycles.

Vanneste et al., 2009; Wells https://www.eshre.eu/Specialty-groups/Special-Interest-Groups/Reproductive-Genetics/Archive/Rome-2015.aspx

Testing on day 3 embrios



- **Cleavage-stage biopsy markedly reduced embryonic reproductive potential.**
- **Trophectoderm biopsy had no measurable impact on implantation**
  → may be used safely when embryo biopsy is indicated.



Scott et al., 2013

Testing on day 3 embrios

Data from the literature
A meta-analysis reports that day 3 biopsy analyzed by FISH has a negative outcome (Mastenbroek et al., 2011)
One published RCT reports a clinical advantage after day 3 biopsy analyzed by FISH (Rubio et al., 2013)
Some studies report reduced embryo developmental potential after blastomere biopsy (Kirkegaard et al., 2012; Scott et al., 2013)

Testing on day 3 embrios

- It is not a common procedure anymore.

- The high incidence of mosaicism and possible damage consequent to blastomere removal seem to be the reasons for a non-advantageous clinical outcome.

Testing on day 5 embrios

Pros	Cons – genetics lab	Cons – IVF lab
Paternal, maternal and mitotic contribution to aneuploidy	Short time for diagnosis (unless cryopreserving)	Fewer embryos available
More cells available: more robust diagnosis	Concordance between TE and ICM cells	Increased workload → cryopreservation and thawings
Low level of mosaicism		Concerns over extended embryo culture
Little (if any) impact on embryo – embryonic mass not reduced		

Which stage analyse for Aneuploidy Test

1. Aneuploidy in embryos can be generated by

   - gonadal mosaicism, meiotic and mitotic errors

   - sperm contribution to aneuploidy is increased in patients with severe male factor ➔ meiotic aneuploidy
     ➔ centrosome defects ➔ mitotic errors

2. The chromosome analysis of oocytes has revealed that
   - more net errors in the aneuploid zygotes occur in meiosis II
   - premature chromatid separation is the prevalent form of errors at meiosis I

3. The chromosome analysis of embryos has revealed
   - high level of mosaicism at the cleavage stage
   - low level of mosaicism at the blastocyst stage

Which stage analyse for Aneuploidy Test

4. The clinical outcome resulting from the testing of aneuploidy in preimplantation embryos is reported to be beneficial
   ➔ a small number of RCTs supports blastocyst biopsy – TE biopsy may be ideal, in experienced hands other stages also have value

5. Mosaicism can cause misdiagnosis
   ➔ incidence <6% at the cleavage stage

6. In regularly developing embryos, biopsy at previous stages are highly predictive of the blastocyst chromosome condition

7. Polar bodies in AMA patients could be useful for patients with poor response to ovarian stimulation

24-chromosome copy number analysis: a comparison of available technologies

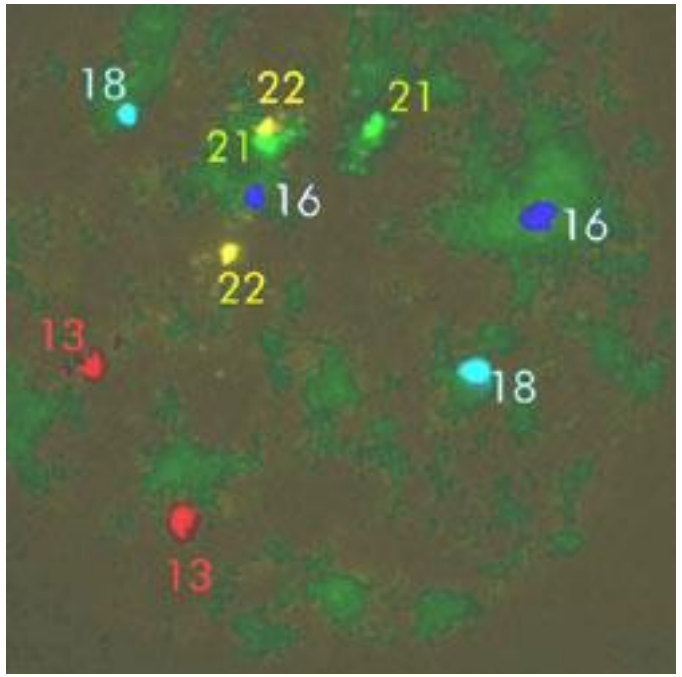
Alan H. Handyside, M.A., Ph.D.

Bluegnome, Fulbourn, Cambridge; and Institute of Integrative and Comparative Biology, University of Leeds, Leeds, United Kingdom

Method Selection

- preferences for biopsy method
- fresh versus frozen transfer
- the turnaround time of the test
- the clinic wishes to set up an in-house facility or outsource to a service lab.

FISH



Positive outcome after preimplantation diagnosis of aneuploidy in human embryos *

FREE

Santiago Munné, Cristina Magli, Jacques Cohen, Paula Morton, Sasha Sadowy, Luca Gianaroli, Michael Tucker, Carmen Márquez, David Sable, Anna Pia Ferraretti, ...
Show more

Hum Reprod (1999) 14 (9): 2191-2199. **DOI:** https://doi.org/10.1093/humrep/14.9.2191

ELSEVIER

Reproductive BioMedicine Online

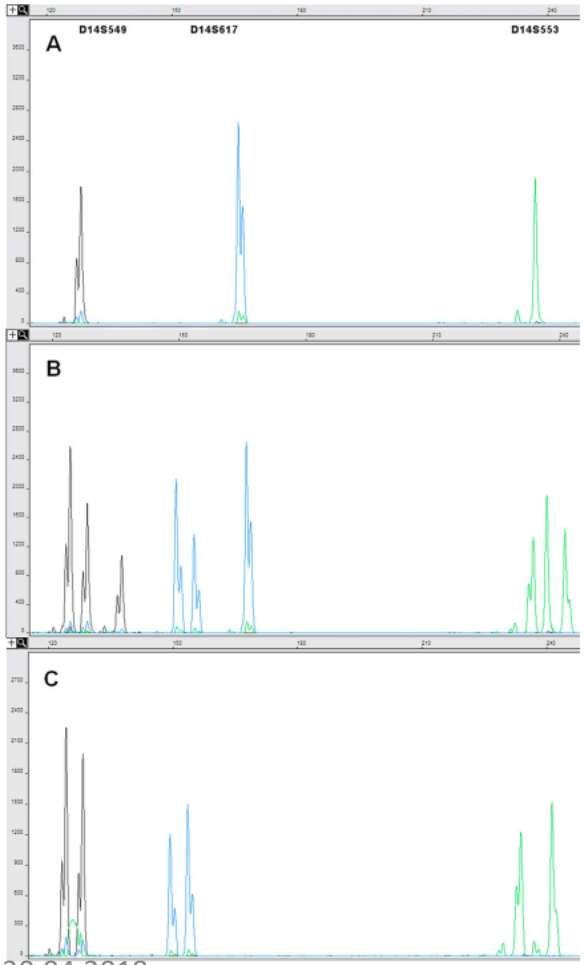
Volume 7, Issue 1, 2003, Pages 91-97

Article

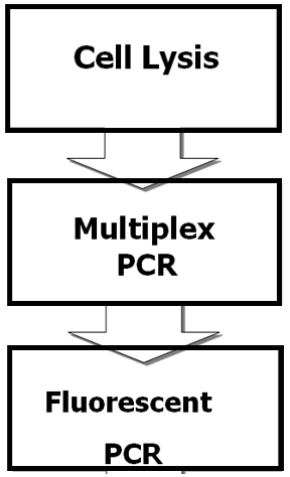
Improved implantation after preimplantation genetic diagnosis of aneuploidy

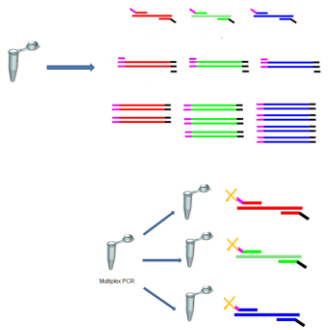
Santiago Munné✉, Mireia Sandalinas, Tomas Escudero, Esther Velilla, Renee Walmsley, Sasha Sadowy, Jacques Cohen, David Sable

PCR



Fiorentino. A PCR-based PGD protocol for chromosomal translocation. Fertil Steril 2010.





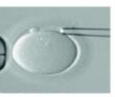
GENETICS

Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations

Francesco Fiorentino, Ph.D.,[a] Georgia Kokkali, M.Sc.,[b] Anil Biricik, M.Sc.,[a] Dimitri Stavrou, M.D.,[b] Bahar Ismailoglu, B.Sc.,[a] Rosangela De Palma, M.D., Ph.D.,[a] Lucia Arizzi, B.Sc.,[a] Gary Harton, B.Sc.,[c] Mariateresa Sessa, Ph.D.,[a] and Kostantinos Pantos, M.D., Ph.D.[b]

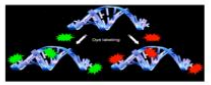
[a] "GENOMA," Molecular Genetics Laboratory, Rome, Italy; [b] Centre for Human Reproduction, Genesis Athens Hospital, Athens, Greece; and [c] Genetics & IVF Institute, Fairfax, Virginia

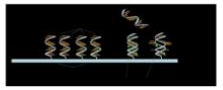
ArrayCGH



Sample preparation and amplification

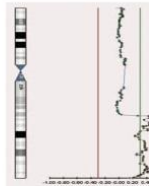
Labelling





Hybridization

Scanning and Report



ELSEVIER

Fertility and Sterility

Volume 94, Issue 4, September 2010, Pages 1173-1177

Modern trends

The use of arrays in preimplantation genetic diagnosis and screening

Joyce C. Harper Ph.D.[a], Gary Harton B.S.[b]

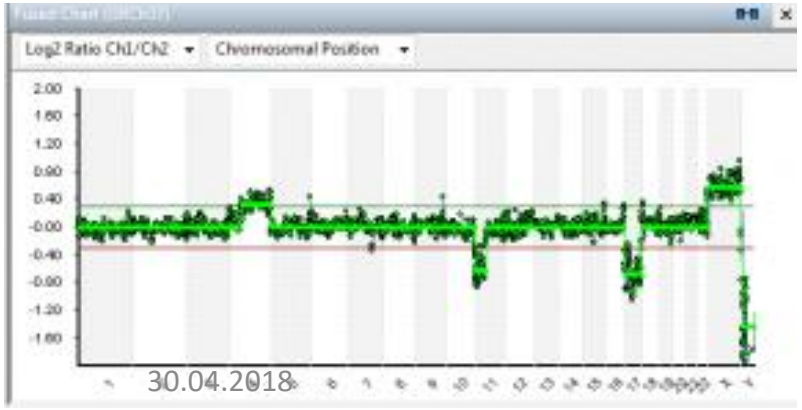
Current Genomics, 2012, *13*, 463-470 463

Preimplantation Genetic Diagnosis for Aneuploidy and Translocations Using Array Comparative Genomic Hybridization

Santiago Munné*

Reprogenetics, 3 Regent Street, Suite 301, Livingston, NJ 07078, USA

Abstract: At least 50% of human embryos are abnormal, and that increases to 80% in women 40 years or older. These abnormalities result in low implantation rates in embryos transferred during *in vitro* fertilization procedures, from 30% in women <35 years to 6% in women 40 years or older. Thus selecting normal embryos for transfer should improve pregnancy results. The genetic analysis of embryos is called Preimplantation Genetic Diagnosis (PGD) and for chromosome analysis it was first performed using FISH with up to 12 probes analyzed simultaneously on single cells. However, suboptimal utilization of the technique and the complexity of fixing single cells produced conflicting results. PGD has been invigorated by the introduction of microarray testing which allows for the analysis of all 24 chromosome types in one test



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Reproductive BioMedicine Online

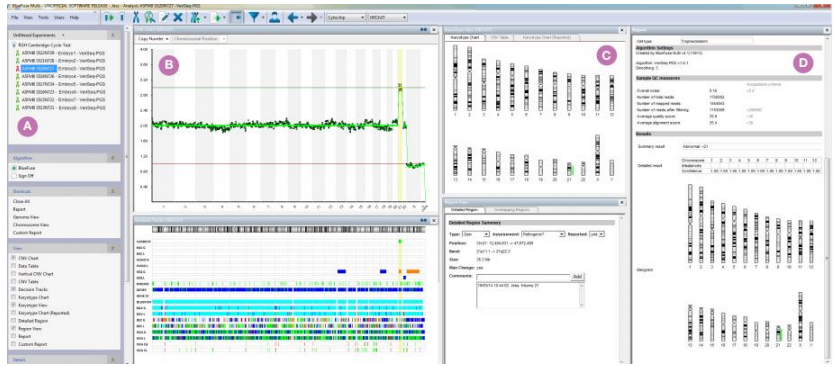
Volume 17, Issue 6, 2008, Pages 841-847

Article

Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening

Ali Hellani[a], Khaled Abu-Amero[b], Joseph Azouri[c], Siham El-Akoum[a]

NGS



Yang *et al. BMC Medical Genomics* (2015) 8:30
DOI 10.1186/s12920-015-0110-4

BMC Medical Genomics

RESEARCH ARTICLE Open Access

Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study

Zhihong Yang[1,2,5*], James Lin[2], John Zhang[3], Wai Ieng Fong[4], Pei Li[5], Rong Zhao[5], Xiaohong Liu[5], William Podevin[6], Yanping Kuang[7] and Jiaen Liu[5]

ORIGINAL ARTICLE: GENETICS

Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos

Francesco Fiorentino, Ph.D.,[a] Anil Biricik, M.Sc.,[a] Sara Bono, B.Sc.,[a] Letizia Spizzichino, B.Sc.,[a] Ettore Cotroneo, B.Sc.,[a] Giuliano Cottone, B.Sc.,[a] Felix Kokocinski, Ph.D.,[b] and Claude-Edouard Michel, Ph.D.[b]

[a] Genoma Molecular Genetics Laboratory, Rome, Italy; and [b] Bluegnome, Cambridge, United Kingdom

Similar Clinical Data NGS vs arrayCGH

Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study

Zhihong Yang[1,2,5*], James Lin[2], John Zhang[3], Wai leng Fong[4], Pei Li[5], Rong Zhao[5], Xiaohong Liu[5], William Podevin[6], Yanping Kuang[7] and Jiaen Liu[5]

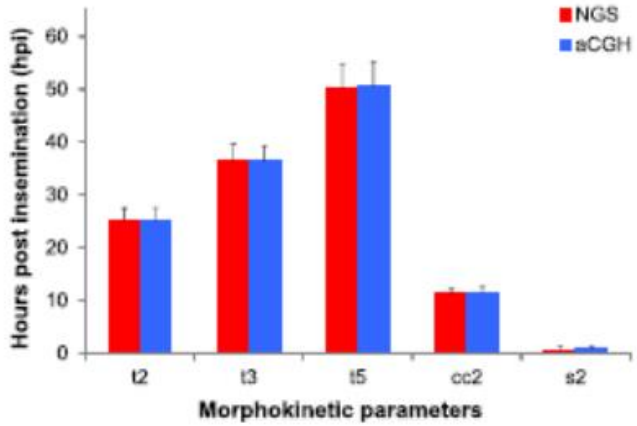


Table 7 Comparison of pregnancy and implantation outcomes between NGS (Group A) and aCGH (Group B) in Phase II study

Parameters	NGS	aCGH	*p*
Patients with SET	27	23	
Patients with DET	52	55	
Clinical pregnancy rate with SET % (n)	62.9 % (17)	60.9 % (14)	0.879[a]
Clinical pregnancies rate with DET % (n)	82.2 % (43)	76.4 % (42)	0.568[a]
Overall clinical pregnancy rate % (n)	75.9 % (60)	71.8 % (56)	0.681[a]
Overall implantation rate % (n)	70.5 % (92)	66.2 % (88)	0.564[a]
Overall ongoing pregnancy rate % (n)	74.7 % (59)	69.2 % (54)	0.560[a]
Overall miscarriage rate % (n)	1.3 % (1)	2.6 % (2)	0.620[b]

SET single embryo transfer, *DET* double embryo transfer
[a]by Chi-square analysis
[b]by Fisher's exact test

24-chromosome copy number analysis: a comparison of available technologies

Alan H. Handyside, M.A., Ph.D.

Bluegnome, Fulbourn, Cambridge; and Institute of Integrative and Comparative Biology, University of Leeds, Leeds, United Kingdom

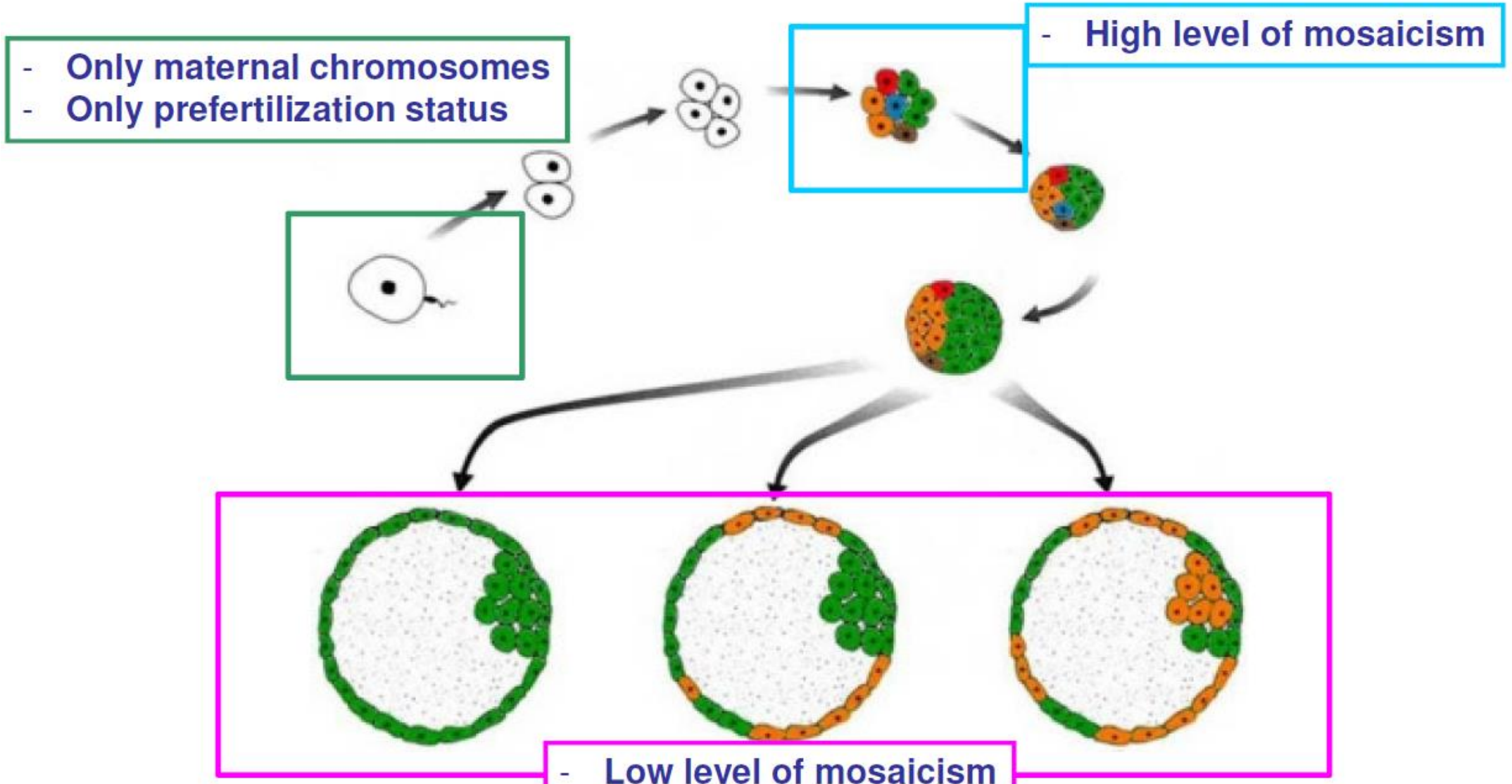
Comparison of available technologies for 24-chromosome copy number analysis.

Method	Duration of test	Complexity	Equipment cost	Reagent cost	Resolution	Pros and Cons
CGH	12–72 h	Medium	Medium	Low	Low	Low cost Skilled Labor intensive
Array CGH	12–24 h	Medium	Medium	Medium	Medium	Robust Scalable
Digital PCR	8 h	Medium	Medium	Low	Low	Low cost Scalable Rapid Polar body analysis only
Real-time quantitative PCR	4 h	Medium	Medium	Low	Low	Low cost Not scalable without additional equipment Multiple cell samples only
SNP microarray	16–72 h	High	High	Medium	High	Genome-wide analysis Quantitative and marker analysis Parental origin
Next-generation sequencing	15 h	High	High	Medium	Low	Scalable with multiplexing

Note: CGH = comparative genomic hybridization; PCR = polymerase chain reaction; SNP = single-nucleotide polymorphism.

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

Which stage analyse for Aneuploidy Test



Robberecht et al., 2010

Preimplantation Genetic Screening (PGS)

- Conflicting evidence
- Improves implantation rate and live birth rate (Dahdouh, 2015. Fertil Steril 2015; 104:1503–1512. Meta-analysis 3 trials included
- Intention to treat analysis. Among all attempts at PGS or expectant management among recurrent pregnancy loss (RPL) patients, clinical outcomes including pregnancy rate, live birth (LB) rate and clinical miscarriage (CM) rate similar. (Murugappan 2016; Human Reproduction 31:1668–1674)
- PGS decreased chances of live birth in association with IVF. National improvements in live birth and miscarriage rates reported with PGS in older women are likely the consequence of favorable patient selection biases. (Kushnir 2016. Fertil Steril 106: 75–9)
- Concern of accuracy of diagnosis and high rate of false-positives. Gleicher 2016. Reprod Biol Endocrinol doi 10.1186/s12958-016-0193-6

Limitations

- Mosaicism – some embryos considered unsuitable for transfer develop into healthy pregnancies (Greco 2015. NEJM 373:2089–90).
- ?Couples choice to transfer non-euploid embryo
- Pre and post test counseling essential
- Different platforms – inconsistent results. Discordance in results seen in published reports (Tortoriello 2016. J Assist Reprod Genet 33:1467–1471)

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CORRESPONDENCE

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

N Engl J Med 2015; 373:2089-2090 | November 19, 2015 | DOI: 10.1056/NEJMc1500421

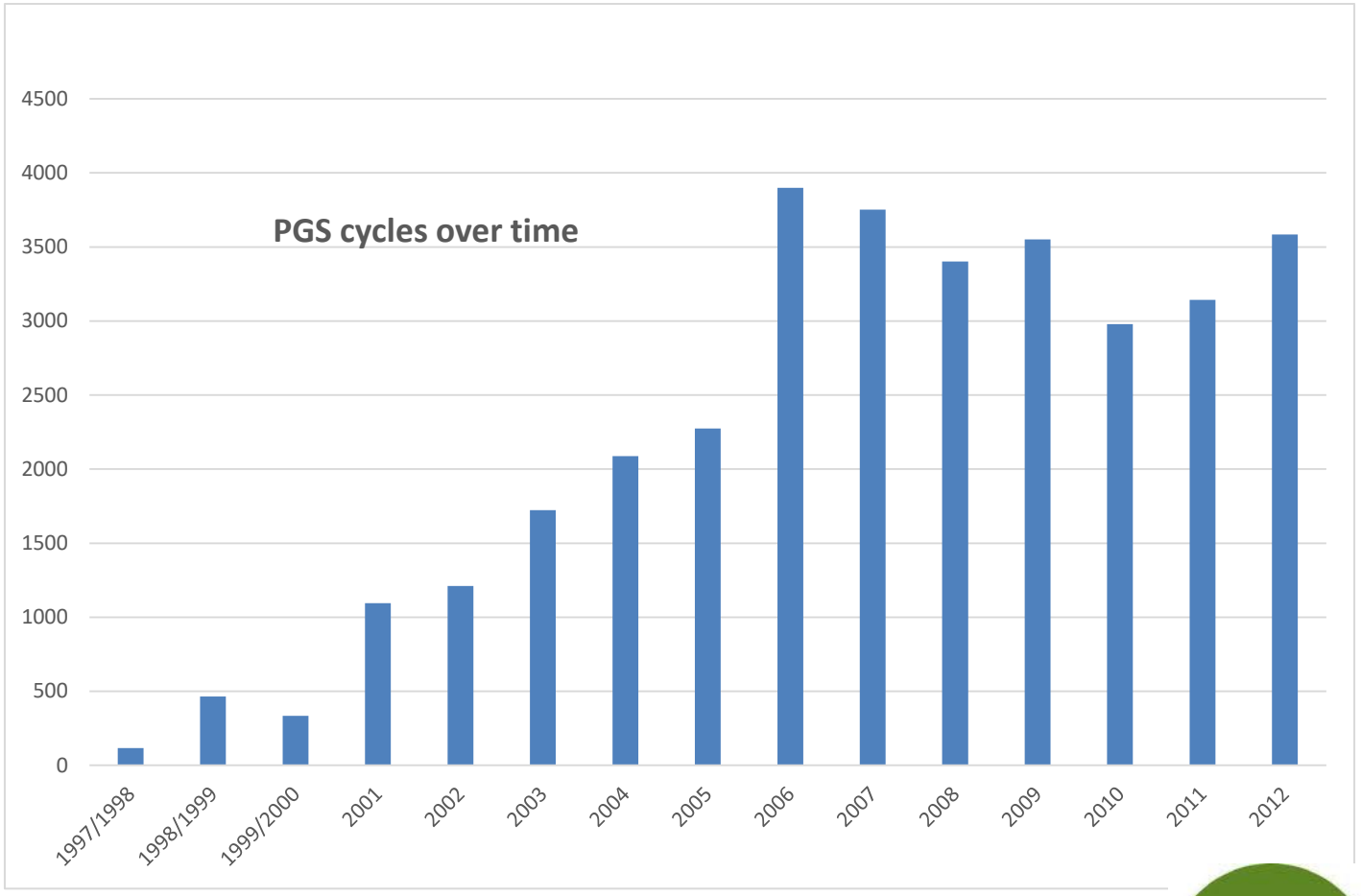
Ermanno Greco, M.D.
Maria Giulia Minasi, M.Sc.
Francesco Fiorentino, PhD.
European Hospital & Genoma Molecular Genetics Laboratory
Rome, Italy

“Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts” NEJM 373:21

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

Number of PGS cycles reaching a plateau?



ESHRE 2015 Coonen: Unpublished and still to be verified data

"Rather than improving outcome for childless couples, PGS encourages the waste of healthy embryos which are excluded from transfer to the uterus."

"The procedure just appears to increase costs and complexities of IVF. Its utilization, at present, should therefore be acknowledged as highly experimental and refuted in routine IVF care."