PRE-CONGRESS COURSE 12

The current status of PGD and PGS

Special Interest Group Reproductive Genetics
Munich - Germany, 29 June 2014

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The current status of PGD and PGS

Munich, Germany
29 June 2014

Organised by
The ESHRE Special Interest Group Reproductive Genetics

Contents

Course coordinators

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Course description

There have been many new developments in the field of PGD. For almost 20 years, cleavage stage biopsy has been the main type of biopsy but there has been an increase in the number of blastocyst and polar body biopsies. New developments in genetic analysis enable screening of all chromosomes from a single cell and more efficient methods of detection of single gene defects. Next generation sequencing is fast approaching the PGD and PGS arena. Data from randomised controlled trials for PGS will hopefully shed light on the use of this technique to improve IVF outcome. With the increase in the amount of information we can gather from a single cell, many ethical concerns arise.

Target audience

IVF and PGD scientists and medics, anyone interested in PGD and PGS

Learning objectives

At the conclusion of this course, the participant should be informed about:
- New methods to detect single gene defects, aneuploidy and mitochondrial disorders
- Outcome and implications of PGD (report PGD Consortium)
- Results of RCTs on PGS
- PGS and Mechanisms responsible for aneuploidy
- Health of children from PGD/PGS cycles
- Ethical implications and dilemmas in PGD/PGS

Educational needs

New developments in genetic analysis enable screening of all chromosomes from a single cell and more efficient methods of detection of single gene defects. Next generation sequencing is fast approaching the PGD and PGS arena. There is a need to inform geneticists, embryologists, specialist in the area and the public on these new technologies, their use and application in treatment and counselling.

Expected outcomes

At the conclusion of the course the participant will have learned about new methods to detect single gene defects, aneuploidy and mitochondrial disorders, outcome and implications of PGD from the ESHRE PGD Consortium, results of RCTs on PGS, and ethical implications and dilemmas in PGD/PGS.

Scientific programme

Chairmen: Joep Geraedts - The Netherlands and Tania Milachich - Bulgaria

09:00 - 09:30	An update on embryo biopsy ***Georgia Kokkali - Greece***
09:30 - 09:45	Discussion
09:45 - 10:15	An update of the ESHRE PGD Consortium ***Joanne Traeger-Synodinos - Greece***
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break

Chairmen: Ursula Eichenlaub-Ritter – Germany and Georgia Kokkali - Greece

11:00 - 11:30	RCT results for PGS ***Joep Geraedts - The Netherlands***
11:30 - 11:45	Discussion
11:45 - 12:15	The biology of aneuploidy in preimplantation embryos and implications for PGD/PGS ***Laura Francesca Rienzi - Italy***
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch

Chairmen: Joanne Traeger-Synodinos – Greece and Hubert Smeets - The Netherlands

13:30 - 14:00	Paediatric follow up of children born by PGD/PGS ***Maryse Bonduelle - Belgium***
14:00 - 14:15	Discussion
14:15 - 14:45	Next generation sequencing ***Rossa Chiu - China***
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break

Chairmen: Claudia Spits – Belgium and Edith Coonen - The Netherlands

15:30 - 16:00	PGD in mitochondrial DNA disorders ***Hubert Smeets - The Netherlands***
16:00 - 16:15	Discussion
16:15 - 16:45	Ethical dilemmas in PGD/PGS ***Guido De Wert - The Netherlands***
16:45 - 17:00	Discussion
17:00 - 18:00	Business meeting SIG Reproductive Genetics

European Society of Human Reproduction and Embryology

The current status of PGD and PGS

An update on embryo biopsy

Georgia Kokkali, Ph.D.
Genesis Athens Clinic, Greece

European Society of Human Reproduction and Embryology

No commercial relationships or conflict of interest to declare

Lecture overview - learning objectives

- Stages at which genetic material can be sampled
- Different protocols available for biopsy procedures
- Pros and cons of biopsy at different stages
- Update of use of different biopsy methods in clinical application

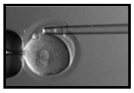
Preimplantation Genetic Diagnosis

- Inheritable diseases
  - Monogenic
    - Autosomal dominant/ autosomal recessive
    - X-linked
  - Triplet repeat disorders
    - Expansion of a triplet repeat of bases on a chromosome
- Chromosome abnormalities
    - Numerical
    - Structural

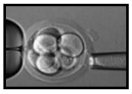
Preimplantation Genetic Diagnosis

- Other indications involve:
  - Human Leukocyte antigen (HLA) typing
  - Adult-onset Mendelian diseases
  - Cancer predisposition syndromes
  - Mitochondrial disorders

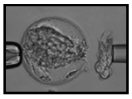
Potential sources of genetic material



- First and/or second polar body
  Verlinsky et al., (1990) Human Reproduction 5: 826-829



- Blastomeres
  Handyside et al., (1990) Nature 344: 768-770



- Trophectoderm cells

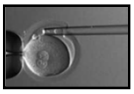
  Kokkali et al, (2005) Human Reproduction 20:1855-1859
  McArthur et al., (2005) Fertility and Sterility 84(6):1628-36

PGD Consortium recommendations for biopsy procedures

- Ensure all micromanipulation equipment is installed correctly, calibrated and maintained per written procedures
- Ensure the appropriate reagents and micromanipulation tools are available, sterile and within their expiration date
- Ensure that biopsy is performed by a suitably qualified person who is trained to a written procedure and adheres to that procedure (Human Fertilisation and Embryology Authority, 2003)
- Embryo biopsy dishes should be made up before the procedure, and clearly labelled with the patient name and embryo numbers
- Embryo biopsy dishes should contain a drop of biopsy medium of sufficient size to maintain pH, osmolality and temperature during the procedure
- Sufficient rinse drops comprising culture medium should be available to rinse embryos after the biopsy procedure

Harton et al., HR, 2011

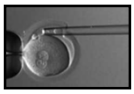
Polar Body Biopsy



- First reported by Verlinsky and colleagues, HR, 1990
- Originally 1st polar body biopsy (preconception diagnosis)

Indications	Single gene disorders (maternal only) Autosomal Recessive or Dominant X-linked Chromosomal rearrangements (maternal only) PGS meiotic errors (maternal only)

Polar body biopsy strategies



	Simultaneously	**Sequential**
Timing of biopsy	one-step biopsy few manipulations required polar body identity is not clear	two-step biopsy more manipulations required polar body identity is clear
Biopsy technique	**Piercing and aspiration** Bevelled pipette No drilling required	**Drilling and aspiration** Mechanical (PZD microneedle) Laser (non contact diode)

Polar body biopsy

- video

Polar Body Biopsy

Advantages	•Ethically/legally acceptable •No misdiagnosis due to mosaicism •Allows long time for genetic testing •Biopsy has little/no impact to the embryo •Applicable to virtually all patients

Patients who generate low number of oocytes may not produce blastocysts in vitro while may become pregnant with day 3 embryo transfer

However, prospective randomized studies show that in selected groups of patients, SBT resulted in significantly higher pregnancy rates and delivery rates compared with eSET on Day 3.

Zech et al., FS, 2007; Papanikolaou et al.,NEJM, 2006

Prospective study: eSET (D2) vs SBT (D5/6)

	sSET	SBT	P-value
Number of transfers (%)	243 (100%)	218 (93)	
Number of clinical pregnancies	71	92	
Rate per transfer	29.2%	42.2%	<0.006
Rate per oocyte retrieval	29.2%	39.1%	<0.03
Clinical implantation rate	29.6%	43.6%	<0.004
Number of deliveries	61	80	
Rate per transfer	25.1%	36.7%	<0.01
Rate per oocyte retrieval	25.1%	34.0%	<0.05

Adopted from *Guerif et al., HR, 2009*

Polar Body Biopsy

Advantages	•Ethically/legally acceptable •No misdiagnosis due to mosaicism •Biopsy has little/no impact to the embryo •Allows long time for genetic testing •Applicable to virtually all patients
Disadvantages	•No mitotic errors detected •No paternal mutations detected •Fragility of polar bodies •Labor intensive and expensive

Polar Body biopsy for PGD

Number of PGD cycles	Type of Mendelian disorder	Number of different disorders tested
504	Autosomal recessive	81
270	X-linked	24
164	Autosomal dominant of maternal origin	41
151	Maternally derived de novo mutations of dominant origin	

Kuliev and Rechitsky, MHR, 2011

Polar Body biopsy for PGS

ESHRE-sponsored proof-of principle study

Randomized controlled trial: First and second polar bodies analysed by array-based technology for the complete chromosome analysis with the aim to examine whether ART clinical outcome can be improved
(*Geraedts et al., HR, 2010)*

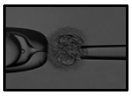
The euploid/aneuploid status of the polar bodies was highly concordant (94%) with the status of the corresponding, mainly aneuploid, zygotes and 98.5% of aneuploid cleavage stage embryos
(*Geraedts et al., HR, 2011; Christopikou et al., HR, 2013*)

Some controversy concerning accuracy *(Capalbo et al., HR, 2013)*

Important aspects to consider:
Polar body biopsy

- Valid alternative to couples with ethical objections to embryo biopsy or countries with legal restrictions
- Biopsy of both polar bodies is required
- Expertise required to overcome technical difficulties
- No mosaicism issues - the method of choice for PGS

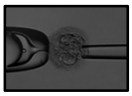
Cleavage stage biopsy



- First clinical application by Handyside and colleagues 1990
- Most widely practiced

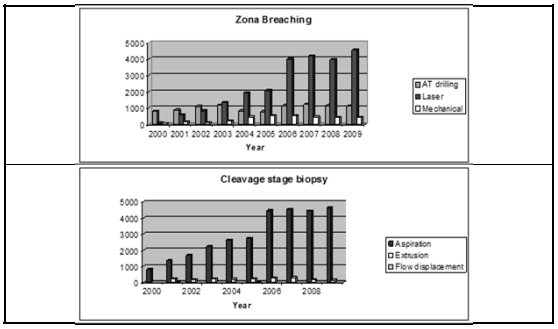
Indications	Single gene disorders Chromosomal rearrangements PGS meiotic and mitotic errors HLA typing

Cleavage stage biopsy strategies



- Zona drilling
  - Mechanical (PZD microneedle)
  - Chemical (Acid Tyrodes solution pH 2.2)
  - Laser (non contact diode laser)
- Blastomere removal
  - Aspiration
  - Extrusion
  - Displacement
- Biopsy media :Ca^{2+} Mg^{2+} free
  - Loosen Gap junctions

Cleavage stage strategies



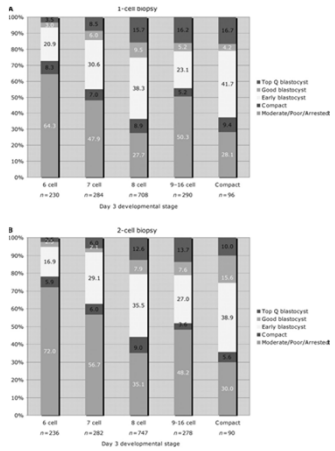
Adopted from PGD Consortium Data Collections II-XII

Cleavage stage (blastomere) biopsy

- video

Cleavage stage biopsy

Advantages	Applicable for all PGD indications Male and post-fertilisation errors are detected Applicable for most patiens Sufficient time for genetic testing Multiple cells for accuracy?



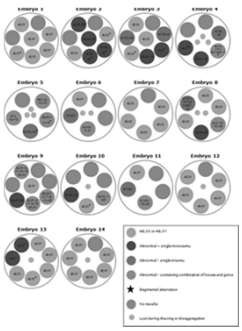
Impact of 1 or 2 cell cleavage stage biopsy on blastocyst development

Goossens et al., HR, 2008

Cleavage stage biopsy

Advantages	Applicable for all PGD indications Male and post-fertilisation errors are detected Applicable for most patiens Sufficient time for genetic testing Multiple cells for accuracy?
Disadvantages	**Mosaicism is common**

Mosaicism in cleavage stage embryos using array-CGH



Only 48% of blastomeres were euploid
Johnson et al., 2010

65% of tested blastomeres were abnormal
Gutiérrez-Mateo et al., 2011

73% of the embryos were mosaic, 22% diploid and 5% with other abnormalities
vanEchten-Arends et al., 2011

31–70% of the embryos carried structural deletions, duplications or amplifications
Vanneste et al., 2009

Is there the minimum required number of karyotypically normal cells in an embryo to be viable?

Mertzanidou et al., HR, 2013

The high level of mosaicism in cleavage stage embryos may be related to:

- Embryo self-correction via preferential growth of euploid cells
- Embryo self correction via preferential allocation of euploid cells to ICM
- Non-full activation of the embryo genome with possible depletion of maternal mRNAs responsible for cell cycle control
  Wells and Delhanty, MHR, 2000; Fragouli et al., HR, 2008; Barbash-Hazan et al., FS, 2009, Vassena et al., Dev, 2011
- S-phase DNA replication which produces artefacts
  Van der Aa et al., NAR, 2013

The cleavage stage is a genetically unusual and transient time
Embryos somehow "sort themselves out" by blastocyst stage
Trisomy rescue?
Random loss?
Selective survival of euploid lines?

Cleavage stage biopsy

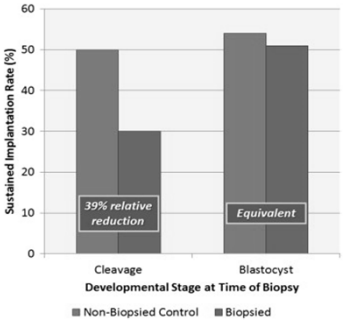
Advantages	Applicable for all PGD indications Male and post-fertilisation errors are detected Sufficient time for genetic testing Multiple cells for accuracy?
Disadvantages	Mosaicism is common **Concerns over damage to the embryo and its implantation rate**

Blastocyst biopsy versus cleavage stage biopsy for PGD of monogenic diseases

	D3 Biopsy	D5 Biopsy	P value
Cycles	10	10	
2pn	131	128	
Biopsied	101	53	
Diagnosed	76	50	0.002
Unaffected	47	26	
For ET	35/47	26/26	
Blastocyst Not Diagnosed	12	3	
Blastocysts Affected	19	14	
Blastocyst Develop. Rate	50%	47%	0.329
Implantation Rate	26.7%	47.6%	0.107
Clinical Pregnancy	6	6	
Pregnancies to Term	4	5	
Babies Born	5	8	

Kokkali et al, HR, 2007

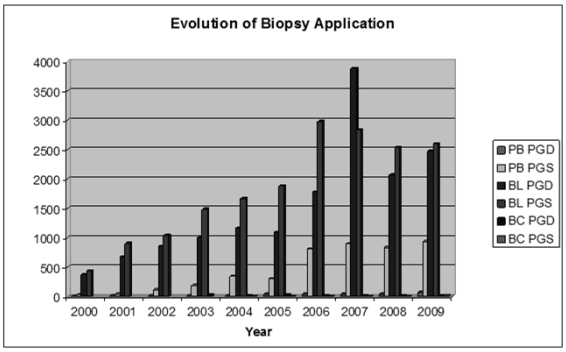
Impact of blastomere biopsy



- 46 D3 ETs
- 67 D5 ETs

- Aneuploidy rates were similar in both groups

Scott et al., FS, 2013



Adopted from PGD Consortium Data Collections II-XII

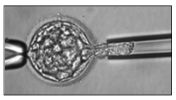
Important aspects to consider: cleavage stage biopsy

- Number of biopsied blastomeres
- Chromosomal mosaicism
- Impact of biopsy on implantation potential

Why biopsy at the blastocyst stage?

- Allows biopsy of embryos demonstrated to be competent to have undergone embryonic genome activation
- Allows the biopsy of cells that are not involved in the formation of the embryo proper rather than cells that may be committed to forming the ICM
- For routine PGD the removal of 5 TE cells represents less than 5% of the embryo compared to 13 - 25% when 1-2 blastomeres are removed on day 3

Blastocyst stage biopsy



1. Removal of 2-10 TE cells for PGD/PGS

First pregnancies reported in the literature in 2005, following blastocyst laser biopsy for PGD/PGS

Kokkali et al., 2005; McArthur et al., 2005; Kokkali et al., 2007

First pregnancy reported in the literature from the US in 2008, following blastocyst laser biopsy of cryopreserved - thawed blastocysts for PGS

Lathi and Behr, 2009

2. Removal of 15-20 TE cells for multiple analyses

First pregnancies reported in the literature from Australia/Greece in 2008, following blastocyst laser biopsy for DNA fingerprinting, cDNA libraries, microarray gene expression analysis

Jones & Cram et al., 2008

Blastocyst stage biopsy

Indications	PGD for single gene disorders Chromosomal rearrangements PGS for meiotic and mitotic errors HLA typing Combination of all of the above

Blastocyst biopsy strategies:
Pre-Clinical applications

- Utilized mechanical, chemical and laser methods
- All techniques compatible with survival and growth in vitro
- No biopsied blastocysts transferred to evaluate implantation potential

Dokras et al, 1990,1991; Muggleton-Harris & Findlay, 1991; Pickering & Muggleton-Harris, 1995; Muggleton-Harris et al, 1993, 1995; Veiga et al, 1997

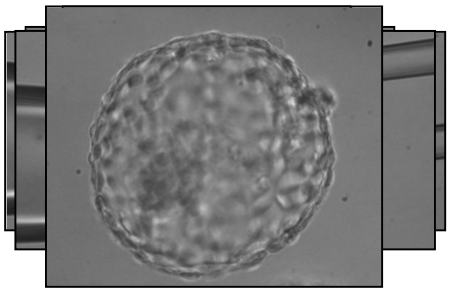
Blastocyst Biopsy strategies

- Laser Assisted Hatching
- Trophectoderm cell biopsy:
  - Dissection of 2-10 TE cells for PGD
  - Dissection of 10-20 TE cells for multiple molecular analyses
- Further incubation and transfer of biopsied blastocyst to the uterus or vitrification

Blastocyst Biopsy strategies

- Laser Assisted Hatching
  - D3/D4 – prior to blastocyst development
    - Advantage: the TE will herniate as the blastocyst develops perhaps allowing earlier biopsy on D5
    - Disadvantage: a proportion of blastocysts will have the hole located at, or close to, the ICM disallowing biopsy of TE in the absence of more invasive manipulation i.e. to rotate the blastocyst within the zona
  - D5 – once the ICM can be clearly identified
    - Advantage: the hole is made at the opposite pole to the ICM guaranteeing that every blastocyst can be biopsied without further manipulation
    - Disadvantage: Pre-incubation period is required to allow TE to herniate through the hole

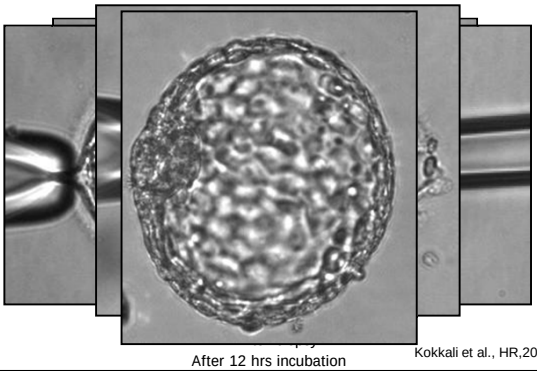
Blastocyst Biopsy: LAH on D3



Following 6hrs incubation post-biopsy

Following 24hrs incubation post-biopsy

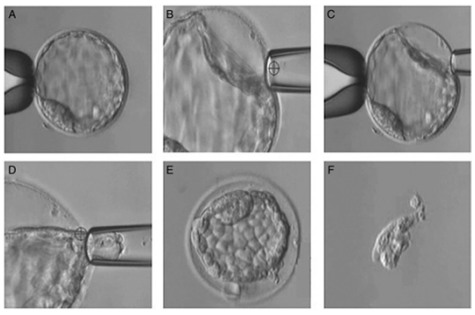
Blastocyst Biopsy – LAH on D5



After 12 hrs incubation

Kokkali et al., HR,2007

Blastocyst biopsy: LAH on D5



Capalbo et al., HR, 2014

Blastocyst stage biopsy

Advantages	More DNA = less no results Less mosaicism = less error rate Reduced/ no impact of embryo biopsy Less embryos to process = decreased workload, decreased costs Facilitates single embryo transfer Compatible with fresh embryo transfer or vitrification Re-biopsy option for failed polar body or cleavage stage PGD analysis
Disadvantages	Not all embryos reach blastocyst the same day

Blastocyst biopsy: clinical application for PGD/PGS

Clinical application: Blastocyst biopsy for PGD SGD

	D5 biopsy/ D6 transfer	D5 biopsy/ Vitrification	D5 biopsy vitrified/ D6 transfer
Cycles treated	177	40	13
diagnosed	93%	90%	92%
Cycles to transfer	113	34	13
Implantation rate	48.8%	50%	46%
Pregnancies/ transfer	51.3%	70.5%	63%
	McArthur *et al* . (2008) Prenat Diagn	Chang *et al.* (2013) Hum Reprod	Lathi *et al*. (2012) RBMOnline

Clinical application:

Blastocyst biopsy, vitrification for PGD SGD with CGH

	Mean Age	Cycles	Ongoing pregnancy rate	Implantation rate
Forman et al., (2012) Fertil Steril	34.8	48	56%	51%
Kokkali et al., (2011) RCOG	35.4	34	50%	48%

Blastocyst biopsy for PGS (CGH-Array)

Clinical application:

Blastocyst biopsy, CGH and vitrification

	Cycles	Mat. Age	Prev. Failed Cycles	Embryos Replaced	Implantation (+sac)
CGH:	45	37.7	2.4	2.0	72%
Control:	113	37.1	1.2	2.7	46%
					p=0.03

Schoolcraft et al., FS, 2010

Randomised Trial:
<35, blastocyst biopsy, CGH, fresh transfer

	Control	CGH	
Patients	48	55	
Maternal age	<35	<35	
Biopsy on D5	No	Yes	
Transfer on	Day 6	Day 6	
Embryos euploid (N)	n/a	53.2% (425)	
Embryos replaced (aver)	48 (1)	55 (1)	
Pregnancy rate (sac)	45.8%	70.9%	p=0.017
Ongoing pregnancy rate	41.7%	69.1%	p=0.009
Multiple pregnancies	0	0	

Yang et al., Molec Reprod, 2012

RCT:
Blastocyst biopsy, q-PCR CGH & fresh transfer

	Control	CGH	
Patients	83	72	
Maternal age	32.4	32.2	
Biopsy on D5	No	Yes	
Transfer on	Day 5	Day 6	
Embryos euploid (N)	n/a	69.9%	
Embryos replaced (aver)	163 (2)	134 (1.86)	p=0.0004
Delivery rate	47.9%	66.4%	p=0.001
Implantation rate	63.2%	79.8%	p=0.002

Scott et al., FS, 2013

RCT: CGH, blastocyst biopsy vs control fresh transfer versus frozen transfer

	1 euploid blastocyst	2 untested blastocysts	
Fresh transfer	65%	70%	NS
Frozen transfer	55%	52%	NS
	NS	NS	

Forman et al., FS, 2013

Important aspects to consider: Blastocyst stage biopsy

- Implantation potential not compromised
- Robust genetic analysis
- Low mosaicism

Next Generation Sequencing

- Next Generation Sequencing can test simultaneously for chromosome abnormalities, mitochondria mutations, known gene defects and fingerprinting
- Preferably achieved by blastocyst biopsy

Yin *et al.,* (2013) Biol Reprod; Treff *et al.,* (2013) Fertil Steril; Abou Tayoun *et al.,* (2013) Clin Chem; Ellard *et al.,* (2013) Hum Mutat

Take home message

- The hallmark of a successful ART program is a consistent and sustainable high pregnancy rate balanced by a low incidence of multiple gestations
- PGD/PGS requires biopsy method, an invasive manipulation that requires high technical standards:
  - Optimal in vitro culture conditions
  - Well-trained embryologists
  - Appropriate timing in fresh transfers
  - 99% survival rate vitrification system
- Benefits of genetic analysis in PGD/PGS should overcome the negative aspects of biopsy

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An update of the ESHRE PGD Consortium

Jan Traeger-Synodinos, DPhil (Oxon)
Associate Professor of Genetics,
Medical Genetics, Athens University
PGD Consortium Chair 2012-2014

- No conflicts of interest to report

Teaching objectives

An overview of PGD Consortium activities to understand the importance of:

- Relevance of data collections
- Quality assurance and audit in PGD/PGS
- The introduction of new technologies in PGD/PGS
- Exchange and dissemination of information/knowledge amongst PGD/PGS centres

What is Preimplantation Genetic Diagnosis (PGD)

•PGD was initially developed as an alternative to conventional prenatal diagnosis to preclude the need to terminate an affected on-going pregnancy.

•It is appropriate for couples who have a known and high-risk of transmitting a genetic condition to their off-spring.

•The first clinical pregnancies were reported in 1990, following embryo sexing using Y-specific PCR
Handyside et al, Nature, 1990

What is Preimplantation Genetic Screening (PGS)

- PGS aims to detect embryos with normal chromosome complement for embryo transfer in an assisted reproduction technique (ART) cycle and exclude the transfer of aneuploid embryos
- PGS is used as part of ART to improve pregnancy rates

- Both PGD and PGS involve ART so that genetic analysis can be based on biopsied material from oocytes or embryos

PGD Consortium - founded in 1997

Catherine Staessen, Joep Geraedts, Karen Sermon,
Joyce Harper, Stephane Viville, Inge Liebaers, Alan Handyside

ESHRE PGD Consortium – Aims

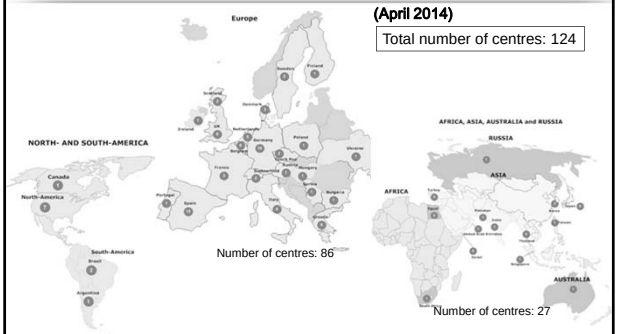
(revised Bylaws 2013)

- To collect prospectively and retrospectively data on the accuracy, reliability, effectiveness and safety of PGD.
- To survey the availability of PGD for different conditions facilitating cross referral of patients.
- To establish minimal standards and to promote best practice.
- To ensure the exchange of views/ideas and to network with other members of the PGD Consortium.

Consortium members by country

(April 2014)

Total number of centres: 124



Number of centres: 86

Number of centres: 11

Number of centres: 27

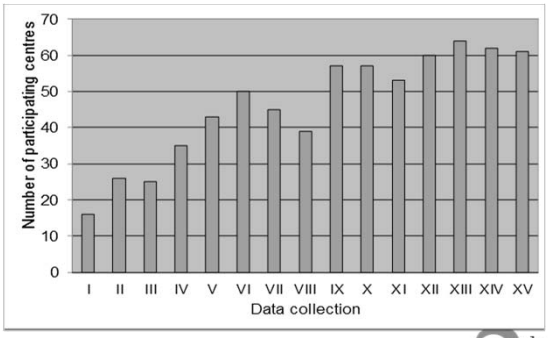
The PGD Consortium data collections

Data Collection – 15 years

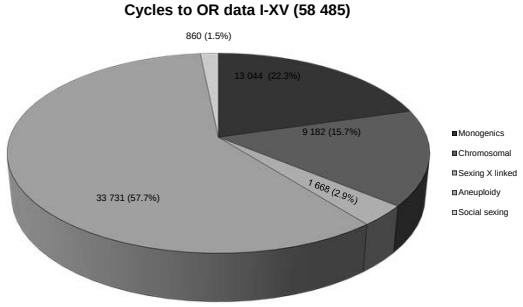
Data reports published by the PGD Consortium in Human Reproduction:

- ESHRE PGDConsortium (1997-1999)
- ESHRE PGD Consortium: data collection II (1999-2000)
- ESHRE PGD Consortium: data collection III (2000-2001)
- ESHRE PGD Consortium data collection IV (2001)
- ESHRE PGD Consortium data collection V (2002)
- ESHRE PGD Consortium data collection VI (2003)
- ESHRE PGD Consortium data collection VII (2004)
- ESHRE PGD Consortium data collection VIII (2005)
- ESHRE PGD Consortium data collection IX (2006)
- ESHRE PGD Consortium data collection X (2007)
- ESHRE PGD Consortium data collection XI (2008)
- ESHRE PGD Consortium data collection XII (2009)
- Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, Pehlivan Budak T, Renwick P, De Rycke M, Geraedts JP, Harton G. The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update. 2012 May-Jun;18(3):234-47

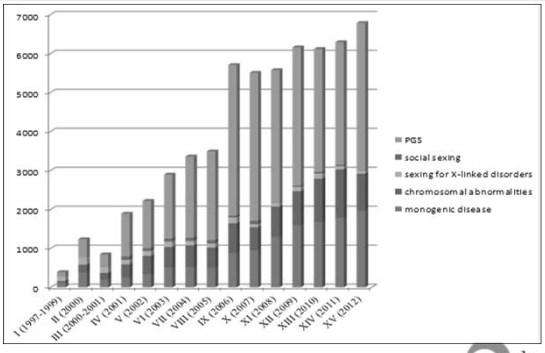
Data collections and number of participating centres



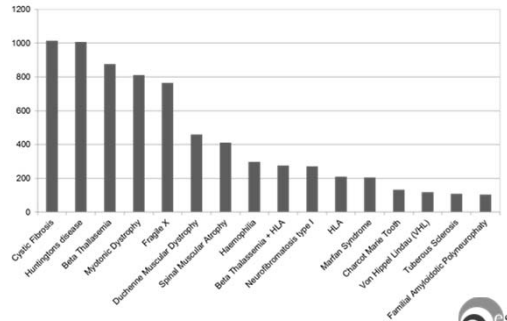
Overview of cycles Data I-XV



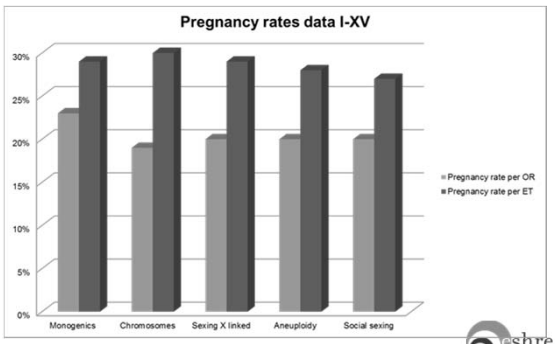
Evolution of cycle indications Data I-XV



Cumulative numbers of cycles per monogenic disorder (Data I-XV)

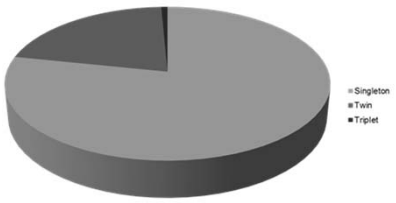


Cumulative Pregnancy rates Data I-XV



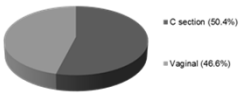
Cumulative data on deliveries (I-XV)

- 8966 deliveries:

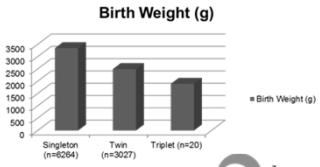


Cumulative data on deliveries & birth weights (Data I-XV)

- 8966 deliveries (with information)
  - 24 % pre-term (mainly twins & triplets)



- Mean birth weights of PGD babies are comparable to data from IVF baby birth weights:



Trends from data I to data XV (1)

Data I	Data XV
16 centres	60 centres
366 cycles	6782 cycles
82 pregnancies	1394 pregnancies
63 deliveries	1158 deliveries

Trends from data I to data XV (2)

	Data I	Data XV
Monogenic disorders	33%	29%
Chromosomal disorders	10%	14%
Sexing only	25%	1%
Social sexing	0%	0.1%
PGS	32%	56%

Current status of data collections

- Consortium Data XII recently published (Hum Reprod. 2014 Mar 11).
- The evaluation, correction and calculations for data collections XIII, XIV and XV are on course.
- The data collections are an extremely valuable resource for monitoring accuracy, reliability, effectiveness and safety of PGD/PGS, but they are a massive undertaking.

Statistical analysis of PGD Consortium data

The “big” data provides potential to investigate:

- Reproductive outcome of PGD
- Evolution of PGD/PGS cycles e.g. per year, per centre
- Multivariate analysis of success rates in relation to factors, including: female age, indication, ART method, number of oocytes, biopsy method & strategy, number of embryos analysed, transferrable, transferred……..

Veerle Goossens (ESHRE), Martine De Rycke (Belgium), Céline Moutou (France).

Database merging – an ongoing project

- ✓ Merged data IV to XI (**29 786 cycles)**
- ✓ Remove cycles cancelled before ART
- ✓ Add missing fields when possible
- ✓ Correction and complete missing data (when possible)
- ✓ Delete double entries

Cycles remaining: 29 307 cycles

Data analysis steps yet to do:

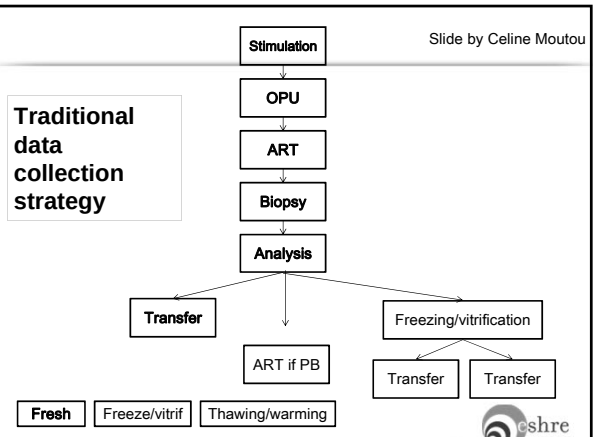
- Deliveries
- Create codes for all data
- Encode all data
- Statistical analysis

On-line data collection database

- Céline Moutou and Martine de Rycke are working on finding a suitable online database which we hope will be available for the next data collection at the end of this year.
- The aim is to simplify the data collection for submitting centres and for data analysis.
- However, the continuous developments in PGD/PGS practices has changed the association between “cycles” and “embryos”, and requires a modified approach.

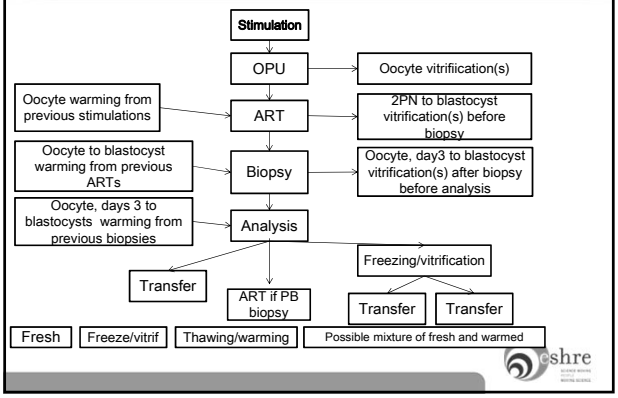
Slide by Celine Moutou

Traditional data collection strategy



Now & future

Slide by Celine Moutou



Proposal for new data collection : Based around Analysis

- Biopsies : for each biopsy : date, stage & number
- Analysis : Number analysed and results
- Transfer after analysis and follow-up (pregnancies-babies)
- Cryopreservation : Number, embryos stage, method
- Other transfers and follow-up (pregnancies-babies)

Slide by Celine Moutou

Other working groups of the PGD Consortium

Over the years various working groups have been formed to carry out activities in addition to the annual data collections.

Working groups focus on issues related to monitoring quality of PGD practices, supporting centres to ensure quality of services, promoting dissemination of knowledge and facilitating exchange of views and ideas.

All these aspects are important in supporting the generation of quality data for the data collections!

Quality assurance in PGD

Quality assurance - Guidelines

ESHRE PGD consortium best practice guidelines for organization of a PGD centre for PGD/preimplantation genetic screening†

G. Harton[1,2,5], P. Braude[2], A. Lashwood[2], A. Schmutzler[3], J. Traeger-Synodinos[4], L. Wilton[5], and J.C. Harper[6,7]

ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS)†

G.L. Harton[1,*], M.C. Magli[2], K. Lundin[3,4], M. Montag[5], J. Lemmen[6], and J.C. Harper[7,8]

ESHRE PGD consortium best practice guidelines for amplification-based PGD†

G.L. Harton[1,2,*], M. De Rycke[3], F. Fiorentino[4], C. Moutou[5], S. SenGupta[6], J. Traeger-Synodinos[7], and J.C. Harper[8,9]

ESHRE PGD consortium best practice guidelines for fluorescence *in situ* hybridization-based PGD†

G.L. Harton[1,2,*], J.C. Harper[2,3], E. Coonen[4], T. Pehlivan[5], K. Vesela[6], and L. Wilton[7]

http://www.eshre.eu/ESHRE/English/page.aspx/217

Quality assurance - Accreditation

- Running Workshops on Accreditation
  - 2008 – Brno
  - 2010 - London,
  - 2011 – Athens (in collaboration with Eurogentest)
- Publications:
  - Harper, JC, Sengupta, S, Vesela, K, Thornhill, A, Dequeker, E, Coonen, E, Morris, MA (2010) Accreditation of the PGD laboratory. Hum. Reprod.

Quality assurance - EQA Schemes for PGD

- Molecular Based Diagnosis
  - United Kingdom National External Quality Assessment Service (UKNEQAS)

UK NEQAS

- FISH Based Diagnosis
  - Cytogenetic European Quality Assessment (CEQA)

CEQA Cytogenetic European Quality Assessment

- Array Based Diagnosis (Pilot)
  - UKNEQAS and CEQA

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PGD-EQA Specialist Advisory Group

- Dr Sandi Deans (Scheme Organiser) , UK NEQAS Molecular Genetics Scheme Director
- Dr Ros Hastings (Scheme Organiser), CEQA Scheme Director
- Dr Sioban SenGupta (Chair), UCL, London, UK
- Dr Martine De Rycke, UZ Brussels, Belgium
- Dr Dagan Wells, Reprogenetics, UK
- Dr Elpida Fragouli, Reprogenetics, UK
- Dr Francesco Fiorentino, Genoma, Rome, Italy
- Dr Tina Buchholz, Munchen, Germany
- Dr Céline Moutou, Strasbourg, France
- Dr Pamela Renwick, Guys, London, UK
- Dr Leeanda Wilton, Melbourne, Australia
- Dr Edith Coonen, Maastricht, Netherlands
- Dr Jan Traeger-Synodinos, Athens, Greece
- Mrs Veerle Goossens (ESHRE link)
- Dr Gary Harton (company representative)

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SCIENCE MOVING PEOPLE MOVING SCIENCE

WG on misdiagnosis monitoring & audit

WG on Misdiagnosis monitoring & audit

Re-analysis studies of untransferred / supernumerary embryos

1. PCR- based PGD
   (Jan Traeger-Synodinos)

2. FISH-based PGD
   (Tugce Pehlivan)

Up to data XII, misdiagnosis include:

12/7759 (0.15%) PCR based cycles

19/30965 (0.06%) FISH-based PGD cycles

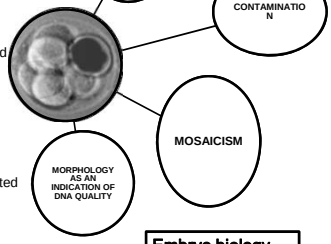
Implications and potential sources of misdiagnosis

Adverse : affected embryo genotyped as unaffected

Benign : embryo genotyped as unaffected (wild type) is a heterozygote for the condition

or

Unaffected embryo not transferred due to affected genotype at PGD



ADO

Inherent risks due to PGD protocol and/or laboratory conditions

CONTAMINATION

MOSAICISM

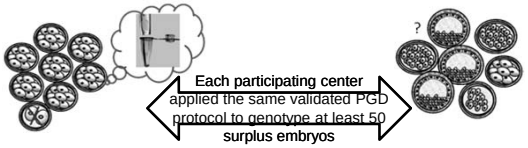
MORPHOLOGY AS AN INDICATION OF DNA QUALITY

Embryo biology

PGD Consortium Embryo-Follow up studies

Multi-centre studies - prospective & retrospective evaluation of the diagnostic accuracy & efficiency of PCR-based or FISH-based PGD.

Surplus embryos, (genotyped at a clinical PGD cycle but unsuitable for transfer or cryopreservation) to confirm the PGD genotype (reanalysis).



Each participating center applied the same validated PGD protocol to genotype at least 50 surplus embryos

TEST GENOTYPE

GENOTYPE @ PGD

1cell or 2 cells analyzed

TRUE GENOTYPE

GENOTYPE @ Reanalysis

whole embryo analyzed

Statistics for diagnostic test performance

TEST \ DISEASE	Affected/Aberrant genotype at embryo reanalysis (R=2/3)	Unaffected genotype at embryo reanalysis (R=1)	TOTAL
Affected/Aberrant genotype at PGD (PGD=2/3)	TRUE +VE (TP) (a)	FALSE +VE (FP) (b)	(a+b)
Unaffected genotype at PGD (PGD=1)	FALSE –VE (FN) (c)	TRUE –VE (TN) (d)	(c+d)
TOTAL	(a+c)	(b+d)	N

Sensitivity (Se); Proportion a/(a+c)
Specificity (Sp); Proportion d/(b+d)
False Negative (FN); Proportion c/(a+c)
False positive (FP); Proportion b/(b+d)

Negative predictive value; Proportion d/(c+d)
Positive predictive value; Proportion a/(a+b)
Odds ratio diagnostic test ; Proportion axd/bxc

DREESEN J. et al. Molecular Human Reproduction Vol.14, No.10 pp. 573–579, 2008

Desirable parameters for a diagnostic test

Sensitivity ⇒ 

↑Se: ↑ Probability that affected embryos will not be transferred

False Negative Rate ⇒ 

↓FN: ↓% of affected embryos will be transferred

NPV ⇒ 

↑NPV: ↑Probability that when PGD result is –ve for the disease the embryos are truly unaffected

Odds ratio (OR) ⇒ 

↑ OR: ↑ Discriminatory test performance

Data: re-analysis of PCR-based PGD

EVOLUTION OF DATA COLLECTION

In 2009- Invitation sent to all PGD consortium members to participate

Database 1
- 24 Centers initially showed interest
- 8 submitted data by deadline
- 1752 reanalyzed samples entered in database

Database 2
- Data curation
- Centers were asked to clarify omissions and discrepancies
- 1352 entries in curated database

Database 2a
- One center failed to reply to queries
- One center with high number of false negative results due to systematic design errors was excluded
- 940 entries

STATISTICAL ANALYSIS

Conclusions: re-analysis of PCR-based PGD

- Diagnostic outcomes were better for multiplex assays versus singleplex (OR 2116 versus 154), and for two-cell versus one-cell biopsy (OR 1036 vs 407).
- However, Sensitivity and NPV of singleplex/multiplex assays compared to one- or two-cell biopsy were not significantly different, indicating that 2-cell biopsy is not essential for more accurate clinical results.
- Inherent risks of PCR based PGD methods (ADO, contamination) accounted for 40.68% of discordant results, whereas mocaisism (biological risk) accounted for 57.63%.
- This study demonstrates the validity, robustness and high diagnostic value of PCR-based PGD.

European Journal of Human Genetics (2013), 1–7

www.nature.com/ejhg

ARTICLE

Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study

Jos Dreesen[1,11], Aspasia Destouni[2,11], Georgia Kourlaba[3], Birte Degn[4], Wulf Christensen Mette[4], Filipa Carvalho[5], Celine Moutou[6], Sioban Sengupta[7], Seema Dhanjal[7], Pamela Renwick[8], Steven Davies[9], Emmanouel Kanavakis[2], Gary Harton[10,12] and Joanne Traeger-Synodinos*[,2]

[1]Departments of Clinical Genetics and School for Oncology and Developmental Biology, GROW, Maastricht University Medical Centre, Maastricht, The Netherlands; [2]Laboratory of Medical Genetics, University of Athens, St Sophia's Children's Hospital, Athens, Greece; [3]The Stavros Niarchos Foundation-Collaborative Center for Clinical Epidemiology and Outcomes Research (CLEO), First and Second University Department of Pediatrics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece; [4]Fertility Clinic, Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejby DK-8200 Aarhus N, Denmark; [5]Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal; [6]Université de Strasbourg FRANCE & HUS – Service de la Biologie de la Reproduction, CMCO, 19, Rue Louis Pasteur, BP120, Schiltigheim, France; [7]UCL Centre for PG&D, Institute for Women's Health, London, UK; [8]Guy's & St Thomas' Centre for Preimplantation Genetic Diagnosis and Genetics, Guy's & St Thomas' NHS Foundation Trust, London, UK; [9]Embryogenesis Assisted Conception Unit, Athens, Greece; [10]Reprogenetics, 3 Regent Street, Suite 301, Livingston, NJ, USA

Re-analysis FISH-based PGD

Co-ordinated by Tugce Pehlivan

- Initially 18 centers showed interest to complete database (23 fields)
- Number of participating centers: 9 (10)
- Number embryos: 1012 (1042)
- Data analysis much more complicated than PCR-based re-analysis data.
- **From 1012 initial cases 380 cases were left**

Data: re-analysis of FISH-based PGD (1)

Results at PGD/ PGS analysis	Result at re-analysis	Category
Euploid (embryo not transferred, not cryopreserved)	All cells euploid	Concordant
	Mosaic - euploid >aneuploid	Concordant
	Mosaic - euploid <aneuploid	Discordant
	Mosaic – aneuploid	Discordant
	All cells same aneuploidy	Discordant
Aneuploid	All cells euploid	Discordant
	Mosaic - euploid >aneuploid	Discordant
	Mosaic - euploid <aneuploid	Concordant
	Mosaic aneuploid - identical to biopsy >other aneuploidies	Concordant
	Mosaic aneuploid - identical to biopsy <other aneuploidies	Discordant
	Chaotic	Concordant
	All cells other aneuploidy	Discordant

Table 1. Concordance criteria.

Data: re-analysis of FISH-based PGD (2)

PGD/PGS analysis	Embryo reanalysis	Result 1-cell biopsy	Result 2-cell biopsy
Aneuploid	Aneuploid	Concordant 212/255 (83.1%)	Concordant 76/87 (87.4%)
Aneuploid	Euploid	Discordant 43/255 (16.9%)	Discordant 11/87 (12.6%)
Euploid	Euploid	Concordant 29/35 (82.9%)	Concordant 1/2 (50%)
Euploid	Aneuploid	Discordant 6/35 (17.1%)	Discordant 1/2 (50%)

Table 2. Impact of number of cells biopsied on level of concordance.

Data: re-analysis of FISH-based PGD (3)

PGD/PGS analysis	Embryo re-analysis	Result grade 1 embryos	Result grade 2 embryos	Result grade 3 embryos
Aneuploid	Aneuploid	Concordant 184/218 (84.4%)	Concordant 86/104 (82.7%)	Concordant 18/20 (90%)
Aneuploid	Euploid	Discordant 34/218 (15.6%)	Discordant 18/104 (17.3%)	Discordant 2/20 (10%)
Euploid	Euploid	Concordant 17/19 (89.5%)	Concordant 12/16 (75%)	Concordant 1/1 (100%)
Euploid	Aneuploid	Discordant 3/19 (10.5%)	Discordant 4/16 (25%)	Discordant 0

Table 3. Impact of embryo grade at day of PGD/PGS analysis on level of concordance.

Data: re-analysis of FISH-based PGD (4)

PGD/PGS analysis	Embryo re-analysis	Result grade 1 embryos	Result grade 2 embryos	Result grade 3 embryos
Aneuploid	Aneuploid	Concordant 107/132 (81.1%)	Concordant 121/140 (86.4%)	Concordant 60/70 (85.7%)
Aneuploid	Euploid	Discordant 25/132 (18.9%)	Discordant 19/140 (13.6%)	Discordant 10/70 (14.3%)
Euploid	Euploid	Concordant 12/14 (85.7%)	Concordant 12/14 (85.7%)	Concordant 6/9 (66.7%)
Euploid	Aneuploid	Discordant 2/14 (14.3%)	Discordant 2.14 (14.3%)	Discordant 3/9 (33%)

Table 4. Impact of embryo grade at day of reanalysis on level of concordance.

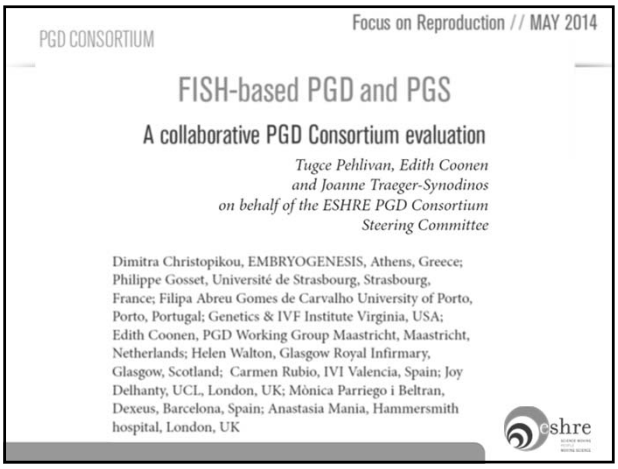
Data: re-analysis of FISH-based PGD (5)

PGD/PGS	Embryo re-analysis	Result day 4 re-analysis	Result day 5 re-analysis	Result day 6 re-anlysis	Result day 7 re-analysis
Aneuploid	Aneuploid	Concordant 89/103 (86.4%)	Concordant 187/224 (83.5%)	Concordant 10/13 (76.9%)	Concordant 2/2 (100%)
Aneuploid	Euploid	Discordant 14/103 (13.6%)	Discordant 37/224 (16.5%)	Discordant 3/13 (23.1%)	Discordant 0
Euploid	Euploid	Concordant 6/8 (75%)	Concordant 21/26 (80.8%)	Concordant 2/2 (100%)	Concordant 1/1 (100%)
Euploid	Aneuploid	Discordant 2/8 (25%)	Discordant 5/26 (19.2%)	Discordant 0	Discordant 0

Table 5. Impact of day of reanalysis on level of concordance.

Conclusions: re-analysis FISH-based PGD

- Due to the nature of the data (highly heterogeneous), the low numbers of comparable embryo-analyses preclude that the results reach statistical significance.
- However, as an observational study, it confirmed the presence of chromosomal mosaicism at different stages of human embryo development, which is something that should be taken into account when designing a PGD/PGS test in order to optimize clinical PGD/PGS results.



PGD CONSORTIUM

Focus on Reproduction // MAY 2014

FISH-based PGD and PGS

A collaborative PGD Consortium evaluation

Tugce Pehlivan, Edith Coonen and Joanne Traeger-Synodinos on behalf of the ESHRE PGD Consortium Steering Committee

Dimitra Christopikou, EMBRYOGENESIS, Athens, Greece; Philippe Gosset, Université de Strasbourg, Strasbourg, France; Filipa Abreu Gomes de Carvalho University of Porto, Porto, Portugal; Genetics & IVF Institute Virginia, USA; Edith Coonen, PGD Working Group Maastricht, Maastricht, Netherlands; Helen Walton, Glasgow Royal Infirmary, Glasgow, Scotland; Carmen Rubio, IVI Valencia, Spain; Joy Delhanty, UCL, London, UK; Mònica Parriego i Beltran, Dexeus, Barcelona, Spain; Anastasia Mania, Hammersmith hospital, London, UK

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SCIENCE MOVING PEOPLE MOVING SCIENCE

WG on monitoring new technologies in PGD

Martine de Rycke

Monitoring new technologies in PGD

- This study was set up to get a "snap-shot" of the introduction of new technologies in PGD/PGS, including:
- **New ART practices in PGD/PGS cycles**
- **The type of biopsy in PGD /PGS cycles**
- The **type of genetic tests in PGD /PGS cycles**

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Monitoring new technologies in PGD

New ART practices in PGD/PGS cycles, oocyte vitrification

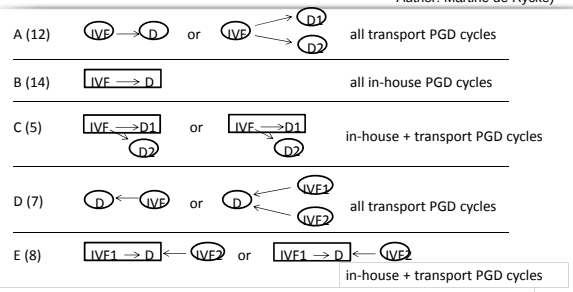
- oocyte *in vitro* maturation
- oocyte *in vitro* maturation and vitrification
- time-lapse imaging

The type of genetic tests in PGD /PGS cycles

- PCR-based
- FISH-based
- WGA + PCR-based
- WGA + array CGH based
- WGA + SNP array based
- WGA + NGS based

PGD centre set-up: 5 different possibilities were reported

Author: Martine de Rycke)



46 centres in total. Number of PGD centres with set-up indicated in brackets.
Rectangles indicate an IVF and diagnostic (D) centre at the **same** location,
Circles indicate IVF and diagnostic (D) centres at **different** locations.
The centre indicated in red is the centre submitting data to the PGD consortium.

Monitoring new technologies in PGD

Set-up A: 12/46 PGD centres encompass an IVF unit at one location which sends out samples to one or more diagnostic units at other locations. The IVF centre submits data to the PGD consortium: all PGD cycles are transport cycles.
Set-up B: 14/46 PGD centres consist of an IVF unit and a diagnostic unit in the same location. The diagnostic unit only receives samples from the IVF unit and all PGD cycles are in-house cycles. The IVF or the diagnostic unit submits data to the PGD consortium.
Set-up C: 5/46 PGD centres consist of an IVF unit and a diagnostic unit in the same location. The IVF unit also sends out samples to another diagnostic unit; PGD cycles comprise in-house cycles and transport cycles. Either the IVF/diagnostic unit submits all data to the PGD consortium or only data on in-house cycles. Data on transport cycles are then submitted by the other diagnostic unit.
Set-up D: 7/46 PGD centres involve a diagnostic unit in one location which receives samples from one or more IVF unit at other locations. All PGD cycles are transport cycles and the diagnostic unit submits data to the PGD consortium.
Set-up E: 8/46 PGD centres involve a diagnostic unit which receives samples from an IVF unit in the same location as well as samples from other IVF unit(s) in other location(s). PGD cycles include both in-house cycles and transport cycles. Either the IVF/diagnostic unit submits all data to the PGD consortium or only data on in-house cycles. Data on transport cycles are then submitted by the other IVF units.
One centre has a variant of set-up E as independent IVF units are replaced by IVF units of the same organization. PGD cycles include both in-house cycles and transport cycles. The main IVF/diagnostic unit submits data to the PGD consortium.

Planned working groups

Follow-up PGD cycles performed for HLA
(to be chaired by Jan Traeger-Synodinos),

Collaborative working practices between genetics & IVF teams in the context of a PGD service
(to be chaired by Sioban SenGupta)

Exchange of experience

Exchange of experience

Co-ordinated by Joyce Harper

Interactive webinars for exchange of experience on difficult/interesting cases, technical trouble-shooting etc

- May 2014: HLA PGD and clinical utility: A discussion,
- October 2014: FISH or CHIPs – how to diagnose chromosome abnormalities in embryos by PGD,

E-learning

In collaboration with SIG Reproductive Genetics

Four introduction webinars related to aspects of PGD are in preparation for open access through the ESHRE webpage for all consortium members:

a. Introduction to genetics; Joep Geraedts
b. Introduction to PGD; Joyce Harper, Jan Traeger-Synodinos
c. Embryo biopsy; Georgia Kokkali
d. Introduction to accreditation; Mike Morris, Sioban SenGupta

The PGD Consortium acknowledges

- All past and current members of the Steering Committee
- Veerle Goossens, the ESHRE Science officer
- All advisors and collaborators for data collections and other activities
- UK-NEQAS, CEQA and the SAG for support in EQA activities
- All centres who send in data and participate in PGD Consortium activities

Steering Committee 2012-2014

Joanne Traeger-Synodinos, GR, Chair, Edith Coonen, NL, Chair-elect, Martine De Rycke, BE, Céline Moutou, FR, Sioban SenGupta, UK, Joyce Harper, UK, Past Chair, Ursula Eichenlaub, SIG Chair, DE, Veerle Goossens, BE, ESHRE Scientific Officer

RCT results for PGS

Em. Professor Joep Geraedts
Maastricht University Medical Center

Disclosure

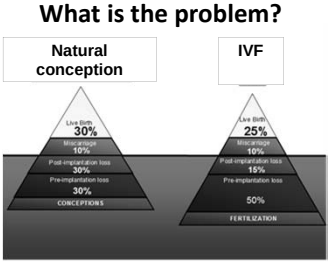
Joep Geraedts is co-ordinator of ESTEEM, the ESHRE polar body array CGH CRT, which is supported by a grant from BlueGnome®

Learning Objectives

At the conclusion of this presentation, participants should be able to:

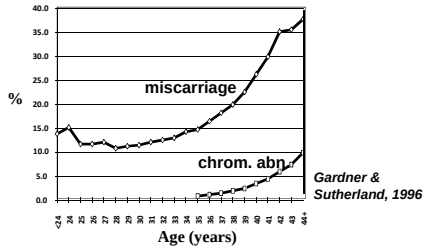
- Recapitulate the advantages and disadvantages of polar body biopsy, blastomere biopsy and trophectoderm biopsy;
- Summarise the methods available for analysis of all 24 chromosomes;
- Have an idea about the CRTs that have been published and that are underway.

What is the problem?



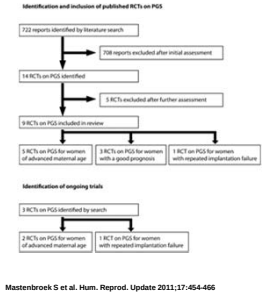
Macklon et al., 2002 & Boomsma et al., 2009

Maternal age specific risks



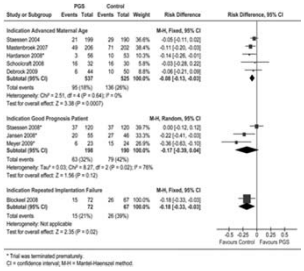
Gardner & Sutherland, 1996

Flow chart of search for RCTs on PGS



Mastenbroek S et al. Hum. Reprod. Update 2011;17:454-466

The effect of PGS on the live birth rate per patient



* Trial was terminated prematurely.
CI = confidence interval; M-H = Mantel-Haenszel method.

Mastenbroek S et al. Hum. Reprod. Update 2011;17:454-466

Explanations

- **The biopsied blastomere is not a true representation of the embryo at the 8-cell stage because of mosaicism**
- **The biopsy procedure might cause harm and negative influences on the developmental potential of the biopsied embryo**
- **Not all chromosomes were tested**

Mastenbroek S et al. Hum. Reprod. Update 2011;17:454-466

What is the problem?

Growth arrest

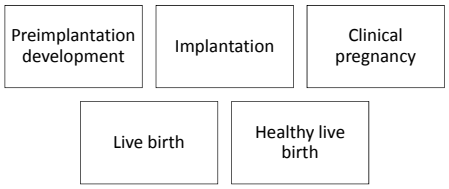
Implantation abnormal conceptus

Failed implantation
Early miscarriage
(Induced) late abortion
Delivery affected fetus

Comparison of PGS strategies: many differences

1) Patient selection / Indication groups
2) Aims
3) Biopsy strategies
4) Methods used for molecular analysis
5) Operator skills required for biopsy and molecular analysis
6) Definition of aneuploidy
7) Transfer policy
8) Definition of success
9) The estimation of the costs

What is success?



Patient selection / Indication groups

- Inclusion / Exclusion criteria

- Maternal age (advanced)
- Infertility or PGD patient
- Repeated implantation failure
- Recurrent miscarriage
- Male factor
- Selection of best embryo for SET
- etc

Different aims

The ESTEEM trial has for example two primary aims among women with advanced maternal age:

(1) to improve live birth rates

(2) To assess the prediction value of having no euploid oocytes in future ART cycles.

Biopsy strategies

- ***Polar body biopsy***
  - PB I, PB II or both
  - Simulaneous or sequential of both polar bodies
- ***Embryo biopsy***
  - Cleavage stage
  - Trophectoderm

Polar body biopsy

- Does not touch the future embryo
- More time for analysis
- No mosaicism
- Compatible with legal situation in some countries
- No paternal errors detected
- No diagnosis of postzygotic abnormalities

Cleavage stage biopsy

- Maternal and paternal errors detected
- Embryonic mosaicism (postzygotic errors)
- Detrimental to the embryo
- Incompatible with legal situation in some countries

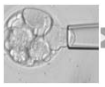
Trophectoderm biopsy

- Maternal and paternal errors detected
- Does not touch the future embryo
- Less embryos need to be analysed
- Multiple cells give more material for analysis
- Compatible with legal situation in some countries
- Less time for analysis
- Trophectoderm might not be representative for the inner cell mass (mosaicism)
- Longer in vitro culture: might give more epigenetic effects

Methods used for 24 chromosome analysis

- Karyotyping
- 24 chromosome FISH based detection (sequential hybridisation)
- Metaphase CGH
- Microarray CGH
- Genome wide SNP analysis
- Polymerase chain reaction-based detection
- Next generation sequencing

Amplification to obtain more material for the test
(screening or diagnostic)

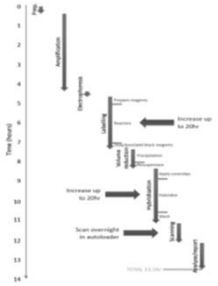


WHOLE GENOME AMPLIFICATION

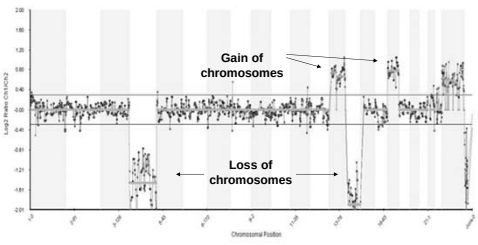
↓

Molecular analysis

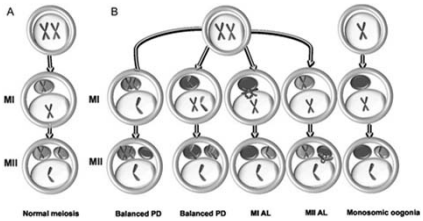
Protocol timings



Aneuploid PB1: +13, +17, -4, -14



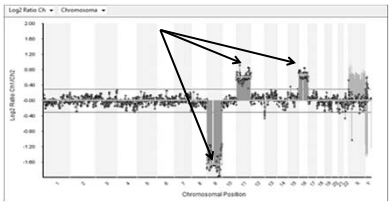
Normal female meiosis (A) and abnormal (B) meiotic segregation patterns resulting in misdiagnosis based on PB analysis when the genetic test is unable to discriminate between whole chromosome versus chromatid copy number gains or losses.



Capalbo A et al. Hum. Reprod. 2013;28:509-518

human reproduction

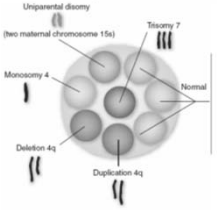
New accurate calling algorithm makes it possible to distinguish between 0, 1, 2, 3 and 4 chromatids



Detection of segmental aneuploidies?

Chromosome instability is common in human cleavage-stage embryos

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

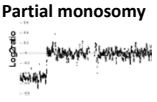


Now, new microarray-based technologies reveal that structural chromosome abnormalities also occur at a shockingly high rate in early embryos. In this issue of *Nature Medicine*, Vanneste *et al.*[7] show that only 9% of IVF-embryos have a normal karyotype in all blastomeres. The great majority show abnormalities of chromosome number or structure, such as large-scale duplications or deletions, or uniparental disomy, in which a chromosome pair is derived entirely from one parent. These abnormalities are often in mosaic form (Fig. 1). The normal diploid blastomeres, outnumbered, have to battle for survival to allow normal embryonic development and birth.

Nature Med 2009

Detection of segmental aneuploidies

Partial monosomy



Partial trisomy

If partial deletions and duplications are detected: which is the minimal size?

Embryo Transfer policy issues

- SET – DET – Multiple ET
- Fresh – Frozen – both
- Cleavage stage - Blastocyst
- Double blinded study required to have equal numbers in both arms
- What to do with undiagnosed embryos?

PGS#2 RCTs published

Data from: PubMed

1. **In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial**
2. **Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study**
3. **Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial.**
4. **Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial.**

1. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial
Forman et al. Fertil Steril. *2013 Jul;100(1):100-7*

CONCLUSION(S):

In women ≤ 42 years old, transferring a single euploid blastocyst results in ongoing pregnancy rates that are the same as transferring two untested blastocysts while dramatically reducing the risk of twins.

REMARK:

The original primary intent of the study was improvement of IVF pregnancy rates, which could not be demonstrated.

2. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study.
Yang Z. et al. Mol Cytogenet 2012 May 2;5(1):24.

CONCLUSION:

Although aCGH followed by frozen embryo transfer has been used to screen at risk embryos (e.g., known parental chromosomal translocation or history of recurrent pregnancy loss), this is the first description of aCGH fully integrated with a clinical IVF program to select single blastocysts for fresh SET in good prognosis patients. The observed aneuploidy rate (44.9%) among biopsied blastocysts highlights the inherent imprecision of SET when conventional morphology is used alone. Embryos randomized to the aCGH group implanted with greater efficiency, resulted in clinical pregnancy more often, and yielded a lower miscarriage rate than those selected without aCGH. Additional studies are needed to verify our pilot data and confirm a role for on-site, rapid aCGH for IVF patients contemplating fresh SET.

3. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial.
Scott RT et al. Fertil Steril. 2013 Sep;100(3):697-703

CONCLUSION(S):

Blastocyst biopsy with rapid qPCR-based comprehensive chromosomal screening results in statistically significantly improved IVF outcomes, as evidenced by meaningful increases in sustained implantation and delivery rates.

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

Scott RT et al. Fertil Steril. 2013 Sep;100(3):624-630

CONCLUSION(S):

Cleavage-stage biopsy markedly reduced embryonic reproductive potential.

In contrast, trophectoderm biopsy had no measurable impact and may be used safely when embryo biopsy is indicated.

PGS#2 RCTs open for participant recruitment: April 2014

Data from: *ClinicalTrials.gov*

1. **Preimplantation Genetic Screening (PGS) in Advanced Female Age and Male Severe Factor**
2. **The Eshre Study Into The Evaluation of Oocyte Euploidy by Microarray Analysis (ESTEEM)**
3. **Comparison of Standard ART Practice vs. Trophectoderm Biopsy and Whole Chromosome Analysis**

1. Preimplantation Genetic Screening (PGS) in Advanced Female Age and Male Severe Factor

Instituto Valenciano de Infertilidad, Spain

This prospective and randomized study seeks to study the results of chromosomal diagnosis using the new Comparative Genomic hybridization (CGH) arrays technique by practicing Preimplantation Genetic Screening (PGS) in day three biopsy on one arm of the study and not on the other arm in order to compare the results. The investigators will study the ongoing pregnancy rate of each oocyte retrieval and the ongoing implantation rate with Day 5 embryos (blastocysts) in IVF/ intracytoplasmic sperm injection (ICSI) treatments of embryos from two different groups of patients: Advanced Age Female Patients (38 - 41 years of age) and Male severe factor (≥2 million spermatozoids/ml.).

2. The Eshre Study Into The Evaluation of Oocyte Euploidy by Microarray Analysis (ESTEEM)

ESHRE

A pragmatic, multicentre, randomized double-blind controlled trial with an intention-to-treat analysis, of the use of preimplantation genetic screening (PGS) for aneuploidy by means of microarray comparative genomic hybridization (CGH) for the chromosomal analysis of the polar bodies (PB) of oocytes collected after ovarian stimulation for in vitro fertilization (IVF), and with the intention to assess the genetic competence of oocytes of advanced biological age, and the effect of this technique on reproductive outcome.

3. Comparison of Standard ART Practice vs. Trophectoderm Biopsy and Whole Chromosome Analysis

Reprogenetics

- We propose to perform a clinical randomized trial to evaluate the effect of blastocyst biopsy and whole chromosome analysis by Next Generation Sequencing (NGS) in comparison to standard Assisted Reproductive Technologies (ART) methods on on implantation rates, miscarriage rates, and pregnancy rates.
- This will be three studies into one: a) a comparison of treatment (NGS) and no treatment, b) a non-selection study based on the control group for which we will replace without knowing the ploidy of the embryos, but we will know it later, c) a retrospective study about the use of Mitochondrial DNA as a selection tool.

Literature (1)

Capalbo A et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. Hum Reprod. 2013 Feb;28(2):509-18.

Forman EJ et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013, 100:100-107.

Gleicher N et al. Preimplantation genetic screening (PGS) still in search of a clinical application: a systematic review. Reprod Biol Endocrinol. 2014 Mar 15;12:22.

Macklon NS et al. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update. 2002 Jul-Aug;8(4):333-43.

Literature (II)

Mastenbroek S et al. Preimplantation genetic screening: a systematic review and meta-analysis. Hum Reprod Update 2011, 17:454-546.

Scott RT et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013 Sep;100(3):624-630

Scott RT et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013 Sep;100(3):697-703

Vanneste E et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med. 2009 May;15(5):577-83.

Yang Z et al. Selection of single blastocyst for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results randomized pilot study. Mol Cytogenet 2012, 5:24.

Conclusions

- The reliability of 24 chromosome screening methods does not seem to be an issue anymore.
- No PGS results have been obtained yet after polar body analysis.
- Trohectoderm analysis looks promising since blastocyst embryos are less mosaic and larger number of cells are more representative.
- However, its applicability in different indication groups still needs to be shown.
- Although results of more randomised controlled trials are needed, only few are underway.
- Therefore it seems that PGS will be an experimental technique for several years to come.

Thank you!

I hope you enjoyed the presentation!

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g.en.e.r.a.

The biology of aneuploidy in pre-implantation embryos and implications in PGD/PGS

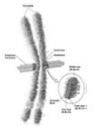
Dr Laura Rienzi
Senior Clinical Embryologist
Laboratory Director
GENERA Centres for Reproductive Medicine
Clinica Valle Giulia, Rome
Salus, Marostica
Studio Associato, Umbertide
Clinica RUESCH Napoli

I declare no conflit of interest related to my presentation

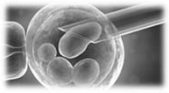
Learning objectives

1. Impact of chromosomes aneuploidies on human reproduction

2. Genesis of chromosomes aneuploidies:
   Meiotic aneuploidies
   Post-zygotic derived aneuploidies



3. Methods for aneuploidy screening in IVF



Impact of aneuploidies in human reproduction

Population	Methodology*	Timeframe of studies	Incidence of aneuploidy‡	Most common aneuploidies
Newborns	Karyotyping	1960s–1970s	0.3%	+13; +18; +21; XXX; XXY; XYY
Stillbirths	Karyotyping	1970s–1980s	4%	45,X; +13; +18; +21; XXX; XXY
Spontaneous abortions	Karyotyping	1970s–1980s	>35%	45,X; +15: +16; +21; +22
Preimplantation embryos	Karyotyping	1990s	20–40%	+16; +17; +18
	FISH	1990s–present	25–>70%	Various
	CGH, SNP array, CGH array	2000–present	30–60%	+15; +16; +21; +22
Eggs or polar bodies	Karyotyping	1990s	10–35%	+16; +17; +18; +21; +22
	FISH	1990s–present	20–>70%	Various
	CGH, SNP array, CGH array	2000–present	30–70%	+15; +16; +21; +22
Sperm	Karyotyping	1980s–1990s	1–4%	XY disomy; +21; +22
	FISH	1990s–present	1–3%	XY disomy; +13; +21; +22

Nagaoka et al., Nat.Gen.Rev. 2012

Learning objectives

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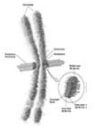

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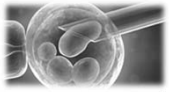
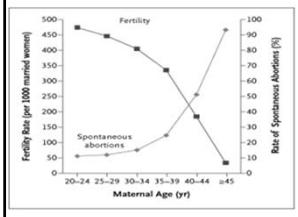
   Post-zygotic derived aneuploidies

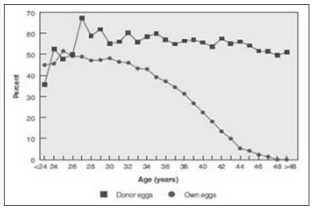


3. Methods for aneuploidy screening in IVF

Fertility and female reproductive aging



Heffner. N Engl J Med, 2004



CDC, 2009

Aneuploidies increase with female reproductive age

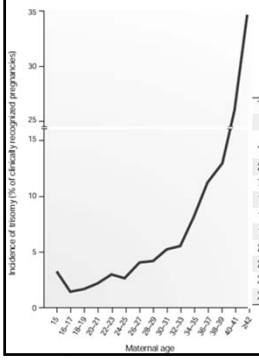
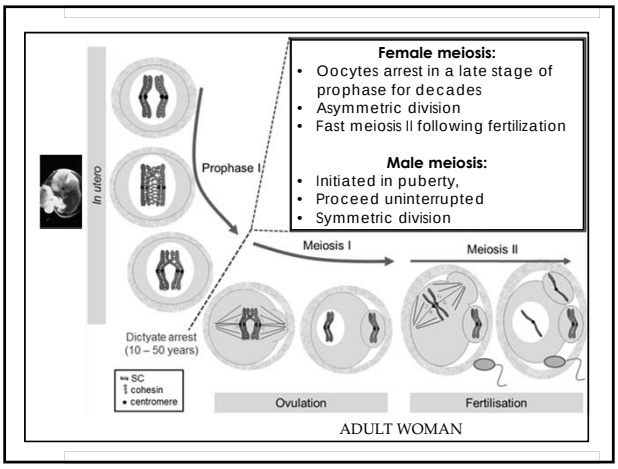
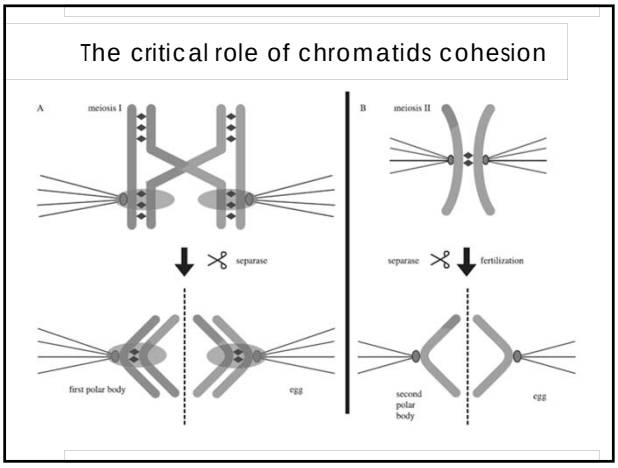


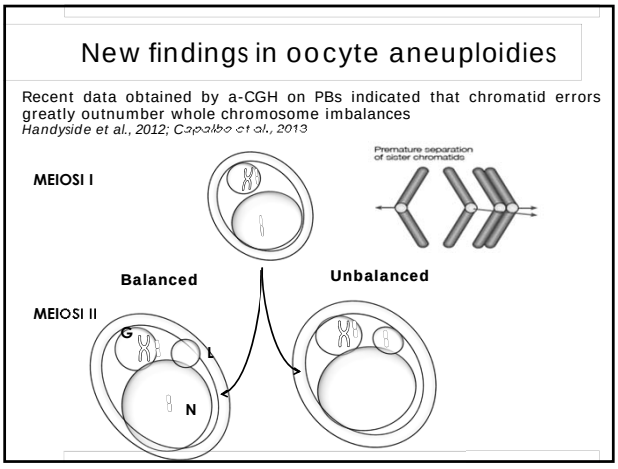
Table 2 | **The origin of human trisomy**

Trisomy	No. of cases	Paternal MI	Paternal MII	Origin (%) Maternal MI	Maternal MII	Post-zygotic mitosis
2	18	28	–	54	13	6
7	14	–	–	17	26	57
15	34	–	15	76	9	–
16	104	–	–	100	–	–
18	143	–	–	33	56	11
21	642	3	5	65	23	3
22	38	3	–	94	3	–
XXY	142	46	–	38	14	3
XXX	50	–	6	60	16	18

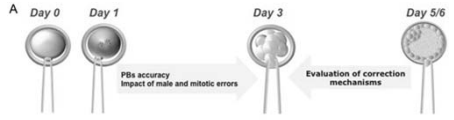
Hassold et al., Nature Genetics, 2001


Female meiosis:
• Oocytes arrest in a late stage of prophase for decades
• Asymmetric division
• Fast meiosis II following fertilization
Male meiosis:
• Initiated in puberty,
• Proceed uninterrupted
• Symmetric division
In utero
Prophase I
Meiosis I
Meiosis II
Dictyate arrest
(10 – 50 years)
SC
cohesin
centromere
Ovulation
Fertilisation
ADULT WOMAN


The critical role of chromatids cohesion
A
meiosis I
separase
first polar body
egg
B
meiosis II
separase
fertilization
second polar body
egg


New findings in oocyte aneuploidies
Recent data obtained by a-CGH on PBs indicated that chromatid errors greatly outnumber whole chromosome imbalances
Handyside et al., 2012; Capalbo et al., 2013
MEIOSI I
Premature separation of sister chromatids
Balanced
Unbalanced
MEIOSI II
G
L
N

Copy number segregation pattern analysis



B

	CELL TYPE				
	1PB	2PB	BLASTOMERE	TOPHOECTODERM	INTERPRETETION
SEGREGATION PATTERNS	G	L	L	L	*MI non-disjunction*
	L	G	G	G	*MI non-disjunction*
	G	L	N	N	*MI chromatid predivision balanced at MII*
	L	G	N	N	*MI chromatid predivision balanced at MII*
	G	N	L	L	*MI chromatid predivision unbalanced at MII*
	L	N	G	G	*MI chromatid predivision unbalanced at MII*
	N	N	G or L	N	*Mitotic error*
	N	N	N	G or L	*Mitotic error*
	G or L	G or L	G or L	N	*Aneuploid zygote rescue*

G= chromosome copy number gain; L, chromosome copy number loss;
N= normal chromosome copy number; PD, chromatids predivision

Capalbo A et al., Hum. Rep, 2013

Chromatids errors outnumber whole chromosome errors in Meiosis I

Table I Chromosomal segregation patterns of copy number gains (G) and loss (L) in the first (PB1), second polar bodies (PB2) and resulting embryos associated with errors in first and/or second meiosis.

Segregation pattern (PB1/PB2/blastomere)	*N* (% of total meiotic errors)	% MI errors	Interpretation
First meiosis errors			
G/L/L	1 (1.3)	4.3	MI non-disjunction
G/L/N	7 (9.0)	30.4	MI chromatid predivision balanced at MII
G/N/L	3 (3.8)	13.0	MI chromatid predivision unbalanced at MII
L/G/G	0	0	MI non-disjunction
L/G/N	5 (6.4)	21.7	MI chromatid predivision balanced at MII
L/G/L	1 (1.3)	4.3	MI chromatid predivision unbalanced at MII
L/N/G	5 (6.4)	21.7	MI chromatid predivision unbalanced at MII
L/N/N	1 (1.3)	4.3	MI anaphase lag or monosomic oogonia
Second meiosis errors			
N/L/N	3 (3.8)		MII anaphase lag
N/L/G	28 (35.9)		MII chromatids non-disjunction
N/G/L	24 (30.8)		MII chromatids non-disjunction
Total number of abnormal meiotic segregations	78		
Female meiotic errors in the embryos	62		
% of chromatid errors	97.4 (76/78)		

On the right column is reported the interpretation of meiotic mechanism causing error based on the chromosomal segregation pattern analysis. N, normal chromosome copy number.

Capalbo et al., Hum. Rep, 2013

Panel of possible sources of misdiagnosis when performing the analysis on PBs

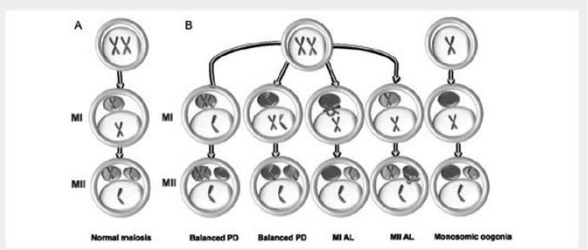


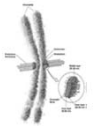
Figure 3 Normal female meiosis (**A**) and abnormal (**B**) meiotic segregation patterns resulting in misdiagnosis based on PB analysis when the genetic test is unable to discriminate between whole chromosome versus chromatid copy number gains or losses. Green coloured PBs indicate chromosome gains. Red coloured PBs indicate chromosome losses. AL, anaphase lag.

Capalbo et al., Hum. Rep, 2013

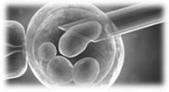
Learning objectives

1. Impact of chromosomes aneuploidies on human reproduction

2. Genesis of chromosomes aneuploidies:
   Meiotic aneuploidies
   Post-zygotic derived aneuploidies



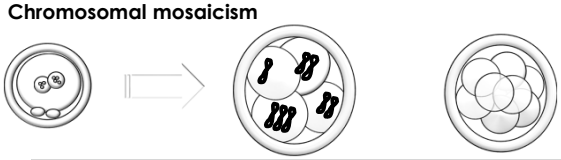
3. Methods for aneuploidy screening in IVF



Post-zygotic derived aneuploidies

Meiotic derived aneuploidies

Mitotic derived aneuploidies and Chromosomal mosaicism



Impact of mosaicism on Day 3 embryo aneuploidy screening

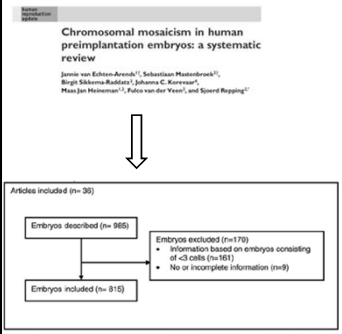


Table II Summary of the findings of 36 studies on the chromosomal makeup of human preimplantation embryos.

	All embryos (n = 815)	Developing, cleavage-stage embryos analysed for ≥8 chromosomes (n = 107)
Diploid	177 (22%)	15 (14%)
Mosaic	599 (73%)	77 (72%)
Diploid–aneuploid mosaic	480 (59%)	49 (46%)
% Diploid cells	*(10155/14116) (72%)*	*(151/324) (47%)*
Aneuploid mosaic	119 (15%)	28 (26%)
Other abnormalities	39 (5%)	15 (14%)
Haploid	3 (<1%)	1 (1%)
Polyploid	5 (<1%)	1 (1%)
Aneuploid	18 (2%)	4 (4%)
Monosomy	13 (2%)	3 (3%)
Trisomy	5 (<1%)	1 (1%)
Complex abnormal	13 (2%)	9 (8%)

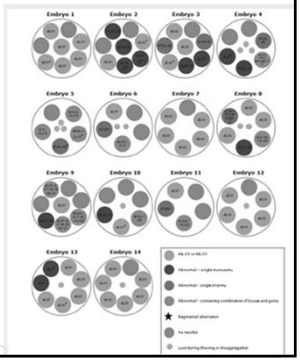
Impact of mosaicism on Day 3 embryo aneuploidy screening

Early studies suggested 25% of cleavage stage embryos are affected by mitotic segregation errors (Voullaiere et al., 2000; Wells and Delhanty 2000)

ORIGINAL ARTICLE Reproductive genetics

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

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

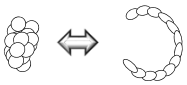


Mosaicism is a strong source of error when testing blastomeres sampled on day 3

Chromosomal constitution of embryos at blastocyst stage

1. Development of an efficient and reliable ICM isolation method with minimal TE cell contamination and without compromising the relative TE

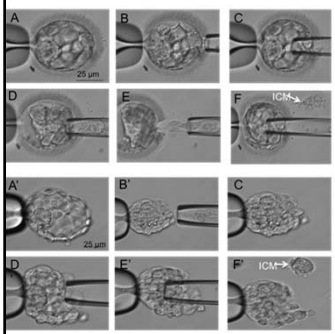
ICM isolation method

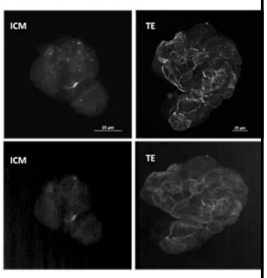


2. To provide further data concerning blastocyst cytogenetic constitution (i.e. impact of chromosomal mosaicism on diagnosis and allocation of aneuploid cells between ICM and TE)

Capalbo et al., *Hum Rep*, 2013

ICM biopsy: validation and application for the study of blastocyst mosaicism

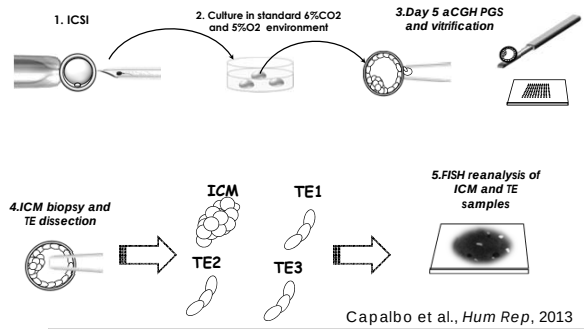




Capalbo et al., *Hum Rep*, 2013

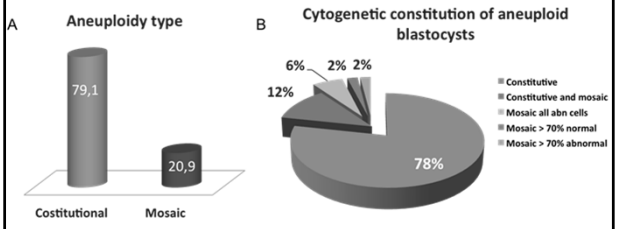
Chromosomal constitution of embryos at the blastocyst stage

Cross-sectional study



Capalbo et al., *Hum Rep*, 2013

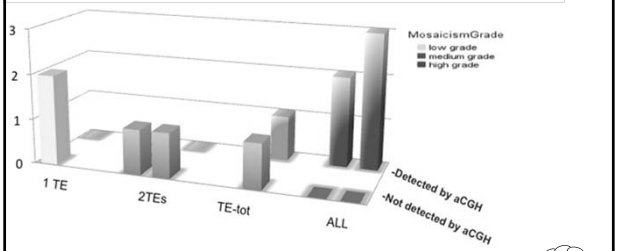
Low impact of mosaicism when testing at the blastocyst stage



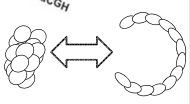
"Only" 4% of good quality blastocysts are mosaic diploid/aneuploid being at risk of misdiagnosis due to the biological issue of mosaicism

Capalbo et al., *Hum Rep*, 2013

Low impact of mosaicism when testing at the blastocyst stage



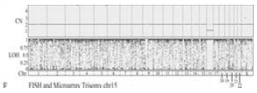
Excluding low grade mosaicism, concordance between ICM and TE was observed in 69/70 (98.6%; 95%CI 92.4-99.6)

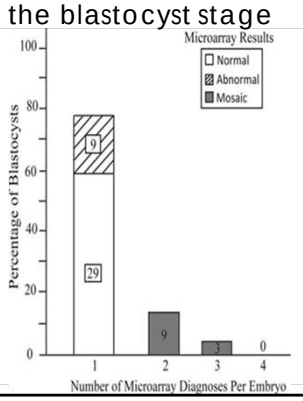


Capalbo et al., *Hum Rep*, 2013

Low impact of mosaicism when testing at the blastocyst stage

24-chromosome screening of 4 blastocyst sections by SNP array





Northrop et al., Mol Hum Repr, 2011

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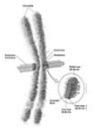
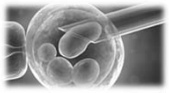
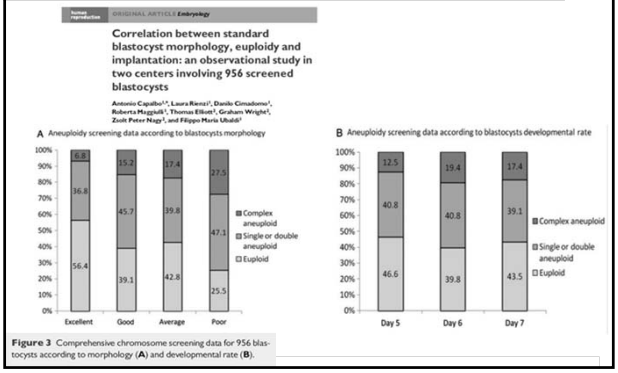


3. Methods for aneuploidy screening in IVF



Correlation between blastocysts morphology and aneuploidy screening



Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts

Antonio Capalbo[1,*], Laura Rienzi[2], Danilo Cimadomo[2], Roberta Maggiulli[2], Thomas Elluet[3], Graham Wright[3], Zsolt Peter Nagy[3], and Filippo Maria Ubaldi[2]

A Aneuploidy screening data according to blastocysts morphology

B Aneuploidy screening data according to blastocysts developmental rate

Figure 3 Comprehensive chromosome screening data for 956 blastocysts according to morphology (**A**) and developmental rate (**B**).

PGS potentiality

INDICATIONS

Advanced Maternal Age, **AMA**

Recurrent Pregnacy Loss, **RPL**

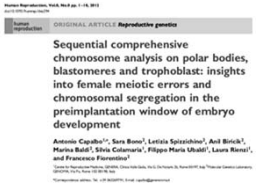
Recurrent implantation failure, **RIF**

ADVANTAGES

Decrease abnormal pregnancies	Decrease aborption rate	eSET	Increase pregnancy rate per ET	Decrease time-to-pregnancy	Increase treatment cost-effectiveness
					

When to perform the biopsy?

PBs approach limitations: false positives and false negatives



ORIGINAL ARTICLE *Reproductive genetics*

Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development

Antonio Capalbo[1,*], Sara Bono[1], Letizia Spizzichino[1], Anil Biricik[1], Marina Baldi[1], Silvia Colamaria[1], Filippo Maria Ubaldi[1], Laura Rienzi[1], and Francesco Fiorentino[1]

PBs FALSE POSITIVES RESULTS: 62 out of 78 (79.5%) of the abnormal meiotic segregations had errors in the either one or both PBs consistent with the aneuploidies observed in their resulting embryos.

PBs FALSE NEGATIVES: Ten of the 21 (47.6%) embryos had aneuploidies other than female meiotic-derived ones, confirmed at the blastocyst stage.

21.1% (48/227) of chromosome segregation errors detected as copy number changes in the polar bodies that did not result in the predicted outcome in the corresponding zygote were also reported by Handyside et al., 2012

Christopikou et al (2013) reported 17% (17/100) of false-positive PB results 7% of aneuploidies detected only in the embryo with normal segregation pattern in PB

Polar body biopsy is also associated with a lower embryo quality

Effects of laser polar-body biopsy on embryo quality

"... even though PBs are considered to be an innocuous procedure using material that has no rea importance for the developing embryos, it shown unfavorable effects on the standard measures of embryo quality, such as rate of fragmentation and number of blastomeres"

TABLE 1

Comparison between the study and control embryos on embryonic day 2, n (%).

	Study (n = 136)	Control (n = 264)	*P* value	Odds ratio (95% CI)
Noncleavage	5 (3.6)	2 (0.7)	.048	3.3 (1.2–9.6)
Inferior cleavage pattern	59 (43.3)	89 (33.7)	.06	1.5 (0.9–2.3)
High fragmentation rate*	13 (9.5)	8 (3.0)	.008	3.3 (1.2–9.5)
No. of blastomeres	1.0 ± 3.5	1.3 ± 3.3	.169	–
Embryos with ≥3 blastomeres	94 (69.1)	207 (78.4)	.04	1.6 (1.01–2.5)

* Level 3 (21%–40%) or 4 (>40%).

Levin. Laser polar-body biopsy effect on embryo quality. Fertil Steril 2012.

TABLE 2

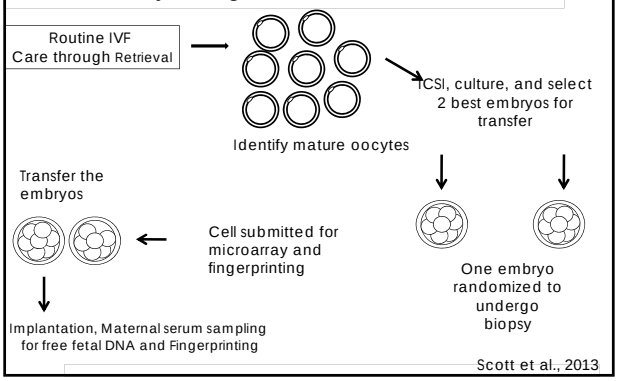
Comparison between the study and control embryos on embryonic day 3, n (%).

	Study (n = 136)	Control (n = 264)	*P* value	Odds ratio (95% CI)
Worse cleavage pattern	83 (57.2)	106 (38.4)	.0002	2.1 (1.3–3.3)
High fragmentation*	17 (11.7)	11 (3.9)	.003	3.1 (1.3–7.7)
No. of blastomeres	5.8 ± 2.1	6.6 ± 1.9	.001	–
Embryo with ≥6 blastomeres	82 (56.5)	206 (74.6)	.0002	2.2 (1.4–3.5)

* Level 3 (21%–40%) or 4 (>40%).

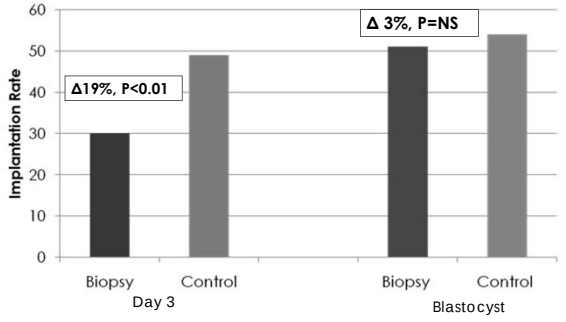
Levin. Laser polar-body biopsy effect on embryo quality. Fertil Steril 2012.

Impact of the biopsy at day 3 and at blastocyst stage



Scott et al., 2013

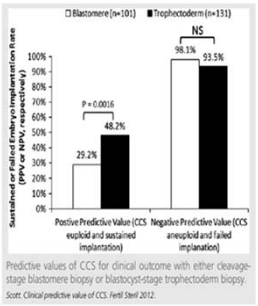
Blastomere but not trophectoderm biopsy affects implantation rate



Scott et al., 2013

High predictive value of blastocyst biopsy and screening

Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study



Predictive values of CCS for clinical outcome with either cleavage-stage blastomere biopsy or blastocyst-stage trophectoderm biopsy.

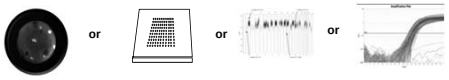
Scott. Clinical predictive value of CCS. Fertil Steril 2012.

Optimized PGD/PGS strategies

1. Stage of biopsy

+

2. Genetic testing method



9 chr. FISH | 24 chr. Array CGH | 24 chr. SNP array | 24 chr. qPCR

Advantages:

1) More DNA: More robust diagnosis
2) Low impact of mosaicism (Capalbo et al., HR 2014)
3) High clinical predictive value (Scott et al., FS 2012)
4) No impact of embryo biopsy (Scott et al., FS 2012)
5) Less embryos to process
6) More cost-effective

Platforms for 24-chromosomal screening

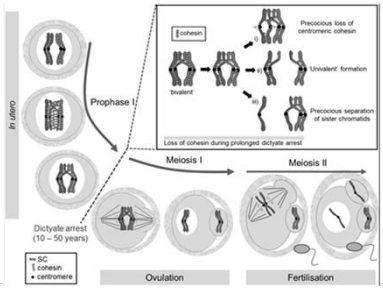
Comparison of methods for preimplantation CCS

Reported characteristics	CCS method		
	aCGH	SNP array	qPCR
Validation on cell lines samples	no	yes	yes
Accuracy	NR	94 – 99%	97-99%
Consistency between PB and oocyte	94%	NR	NR
Minimum turn-around time	12h	24 h	**4 h**
Blastocyst biopsy and fresh embryo transfer	Partial	NO	YES
Lab work-load	HIGH	HIGH	**LOW**
Number of probes	2-32 K	263-370 K	NR
Reported minimum detectable imbalance	2.5 Mb	1.7 Mb	NR
Direct monogenic disease screening	-	+	+
Contamination screening	-	+	+
Origin of aneuploidy screening	-	+[c]	NR

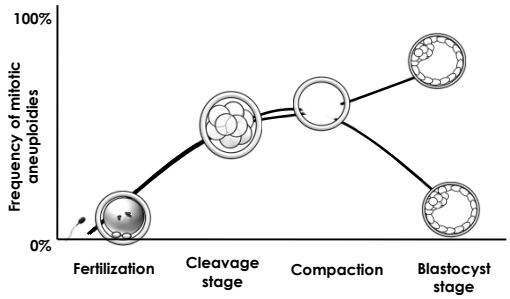
Adapted from Treff and Scott, JARG 2012

Conclusion

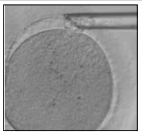
Maternal age effect is a spectrum of defects with multiple underlying mechanisms and with cohesion defects playing a central role



Mitotic errors in developing embryos are critical at cleavage stage but has no major impact for diagnosis at the blastocyst stage

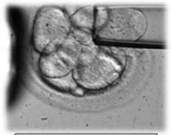


Blastocyst stage biopsy coupled with 24-chromosome analysis has the higher potentiality to be successful for PGS application in ART in an at risk population



PBs biopsy

- Paternal and post-zygotic errors not detected
- Need of 2°PB biopsy
- High false positive diagnostic rate
- Impact on embryo development
- Most expensive and time-consuming approach



Blastomere biopsy

- High worldwide experience
- Small reduction in embryo viability
- High impact of mosaicism
- Single cell analysis issue

TE biopsy

- More robust genetic analysis
- High clinical predictive value
- No impact of biopsy
- Low impact of mosaicism
- Reduced number of embryos/cycles
- Most cost-effective

References

Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, Nagy ZP, Ubaldi FM. *Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Capalbo A, Rienzi L, Hum Reprod. 2014 Mar 19. [Epub ahead of print]*

Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. *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.* Hum Reprod. 2013. 28(8):2298-307.

Capalbo A, Bono S, Spizzichino L, Biricik A, Baldi M, Colamaria S, Ubaldi FM, Rienzi L, Fiorentino F. *Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development.* Hum Reprod. 2013. 28(2):509-18.

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Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. *Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial.* Fertil Steril. 2013. 100(3):624-30.

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Paediatric follow-up of children born after PGD/PGS

Maryse Bonduelle

Conflict of interest

- Prof M Bonduelle's institution (UZBrussel) has received educational grants from
  - IBSA, Ferring, Organon, Shering-Plough, Merck, Merck Belgium…
- M. Bonduelle has received consultancy and speaker's fees from
  - Organon, Serono Symposia, Merck

Learning objectives

- Children born after PGD
  - Main outcome data after embryo biopsy are reassuring
  - More data are needed on outcome with other biopsy techniques
  - Limited data on psychological and development available

Content of presentation

- **Context and history**
- Definitions and procedure
- PGD in daily practice and results
- Babies born
- Future developments
- Conclusions

Who may benefit from PGD?

- Genetic counselling informs couples at high risk to transmit a genetic condition about the risks and the possible reproductive options
  - Take the risk
  - Refrain from children
  - Use donor gametes
  - Have prenatal diagnosis
  - Have preimplantation genetic diagnosis

Since when is PGD a possible option?

- Prenatal diagnosis was introduced in the 1970…
- Patients asked during counselling for an earlier form of prenatal diagnosis
- Scientists and physicians developed IVF, PCR…
- First preimplantation genetic diagnosis was offered in 1990
- (Handyside et al, 1990; Verlinsky et al, 1990) **…**

History

- Preimplantation genetic diagnosis can be considered as a very early form of prenatal diagnosis,
- However,

Differences between PGD and PND

PND	PGD

• Genetic diagnosis
• During pregnancy
• Termination of pregnancy
  • If foetus affected
	• Genetic diagnosis
	• Before pregnancy
	• Avoids termination of pregnancy
	  • If embryo affected: No transfer
	  • If embryo unaffected : Maybe pregnancy
	• Need for IVF

Content of presentation

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- **Definitions and procedure**
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Definitions

- Preimplantation Genetic Diagnosis (PGD)
  - Refers to a genetic diagnosis of an embryo in vitro
- Preconceptual Genetic Aneuploidy Screening (PGD-AS or PGS)
  - Aim: to improve the IVF outcome

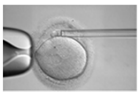
Indications PGD

PGD
- Monogenic diseases
- Chromosomal anomalies
  - Stuctural
  - Numerical
    - Klinefelter, Turner mozaic
    - Previous child with T21

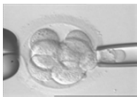
PGS
- Based on aneuploidy screening
- For low risk couples
- To improve outcome of ART
- Will decrease the risk of age related aneuploidies and miscarriages

PGD biopsy methods

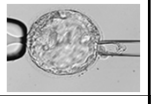
- **Polar body biopsy** / de la globule polaire
  Removal of 1st et 2nd polar body



- **Embryobiopsy** of 4-8 cell embryo
  Removal of 1 or 2 cells (blastomeres)



- **Trophectoderm biopsy**
  Removal of several cells at the blastocyste stage



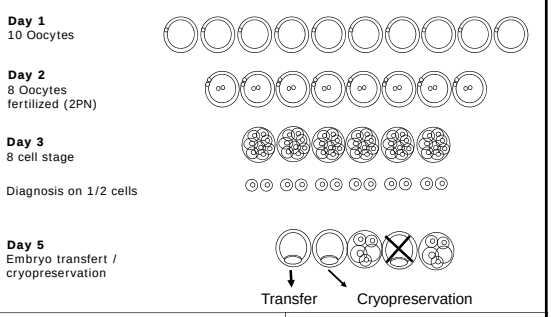
PGD biopsy methods

- **Early cleavage biopsy at 4-8 cell stage**
  - For monogenic conditions and chromosomal structural anomalies
- **Polar body biopsy** Information of maternal genome
  - For X-linked diseases or dominant in mother
  - Advantage if biopsy is not allowed (legally)oderm
- **Trophectectodermbiopsy of blastocyst**
  - Newer technique
  - Advantage for aneuploidy screening
  - Less embryo's to test

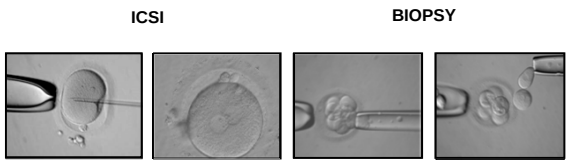
Clinical procedure pre PGD

- Aim is to make as many (good) embryos as possible in the lab (in vitro)
- Need for ovarian stimulation
- Oocyte retrieval
- Sperm collection
- IVF with ICSI

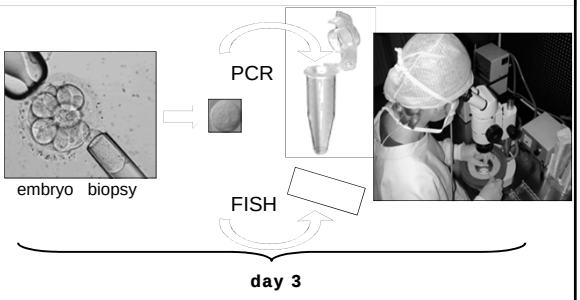
PGD procedure / biopsy on 8-cell E's



PGD procedure: embryo biopsy



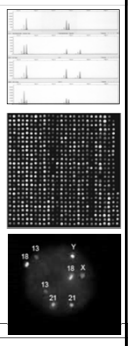
PGD procedure: biopsy and tubing



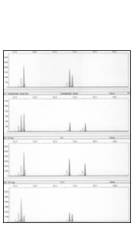
PGD procedure: day 4 diagnosis

Polymerase chain reaction (**PCR**)
- DNA analysis
  - monogenic conditions
  - Direct/linkage/HLA
- Array CGH
  - chromosomal anomalies
- SNP arrays
  - chromosomal/monogenic

Fluorescent in situ hybridisation (**FISH**)
- chromosomal aberrations or sexing



PGD procedure: day 5 tranfer



diagnosis

embryo transfer

dag 4 **dag 5**

Content of presentation

- Context and history
- Definitions and procedure
- **PGD in daily practice & results**
- Babies born
- Future developments
- Conclusions

PGD in daily practice

- Intake and evaluation of request
  - mail / consultation
- Combined appointment
- Development of diagnostic test
- Programming of the cycle
- ***Follow-up !!!***

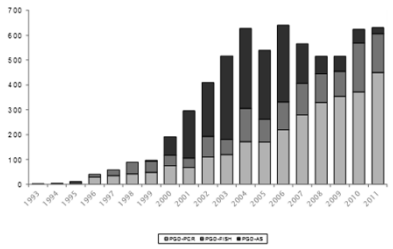
Intake and evaluation of request

- Is PGD possible ?
  - mutation known ?
- Is PGD acceptable ?
  - condition→ ethical committe
  - age of the female partner
  - medical evaluation of pregnancy risk (if woman affected)
  - psychological evaluation (HLA, late onset, limited life expectancy..)

Combined appointment

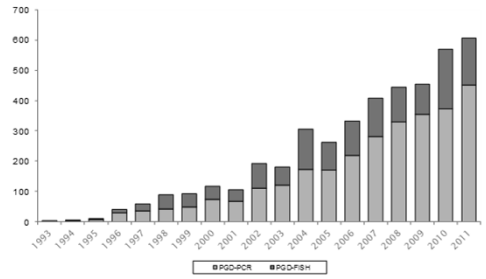
- Medical Genetics
  - diagnosis and pedigree
  - discussion on the reproductive options procedure and ***informed consent***
  - pre-PGD sampling of probands family
- Reproductive Medicine
  - pre-IVF examination and tests
  - pre-IVF/PGD counselling

PGD + PGS UZ Brussel 1993-2011



1500 children born
61% PGD DNA
39% PGD FISH

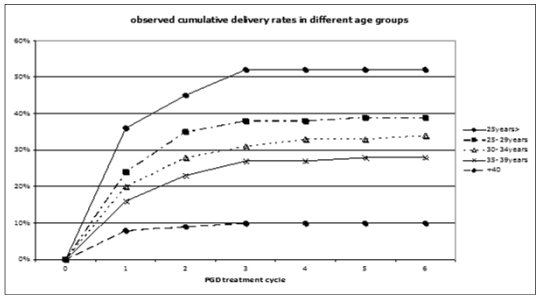
PGD UZ Brussel 1993-2011



Succes rates

- Overall pregnancy rate
  - + FHB/Oocyte Retrieval 25%
  - + FHB/ET 38%
- Cumulative delivery rates
  - Depending on maternal age
  - 40- 50% delivery rate after 3 cycles
    - if maternal age < 30years
  - <10% delivery rate after 3 cycles
    - if maternal age > 40 years

Observed cumulative delivery rates (1993-2005)



Verpoest et al. Hum Reprod 2009(11):2951-9

Misdiagnosis rate ?

- Very difficult to calculate accurately
- For monogenic conditions PGD PCR
  - 5 erroneous diagnoses in PGD-DNA
  - on 915 children born **0.5-1%**
- For FISH (PGD and PGS)
  - 2 errors (1 due to mosaicism)
  - on 600 children **0.3% -0.5%**

Content of presentation

- Context and history
- Definitions and procedure
- PGD in daily practice
- **Babies born**
- Future developments
- Conclusions

What are the concerns?

- PGD involves ICSI/IVF + embryo biopsy
  - Invasive procedure
  - Introduction with little data on human

➔ Data needed on outcome of the children
1997 ESHRE consortium was founded
1991 FU study at the UZ Brussel

ESHRE PGD Consortium: data I-X


Human Reproduction Update, Vol.18, No.3 pp. 234–247, 2012
Advanced Access publication on February 16, 2012 doi:10.1093/humupd/dmr052
human reproduction update

The ESHRE PGD Consortium: 10 years of data collection

J.C. Harper[1,2,*], L. Wilton[3], J. Traeger-Synodinos[4], V. Goossens[5], C. Moutou[6], S.B. SenGupta[1], T. Pehlivan Budak[7], P. Renwick[8], M. De Rycke[9], J.P.M. Geraedts[10], and G. Harton[11]

ESHRE PGD Consortium: data I-X

Aims

- 1997: foundation
  - availability
  - accuracy, reliability, effectiveness
  - follow-up studies
  - guidelines, protocols
  - consensus on use
  - education

ESHRE PGD Consortium: data I-X

- Data collection: 10 years: 1997-2007

Europe, North- and South-America, Africa, Asia, Australia, Russia

- > 27 000 cycles
  - 61% aneuploidy screening
  - 17% single gene disorders
  - 16% chromosomal abnormalities
  - 4% sexing for X - linked diseases
  - 2% social sexing
- 5 187 pregnancies → 4 140 children→
  - 62% singletons, 36% twins, 2% triplets

ESHRE PGD Consortium: data I-X data on children

- 5 135 newborns reported
  - Multiple pregnancies rate 23%
  - Pregnancy complications 14%
  - Birth weight
    - Singletons: 3219 g
    - Twins: 2386 g
  - Premature birth rate
    - Singletons: 15%
    - Twins: 64%
  - Major malformations 2%

ESHRE PGD Consortium: data I-X Critical remarks

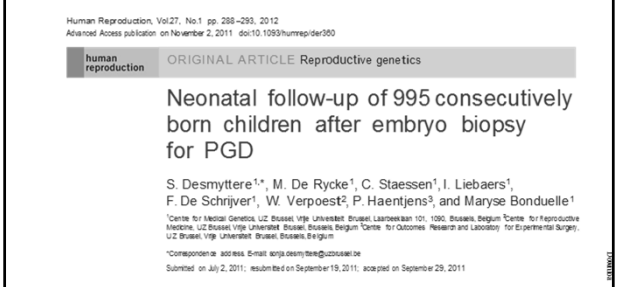
- Multiple pregnancies
  - seems comparable to literature data ART
- Pregnancy complications
  - definitions!
- Birth weight
  - seems comparable to literature data ART (ethnicity!)
- Preterm birth rate
  - seems comparable to literature data ART
- Major malformations
  - different approaches and definitions

ESHRE PGD Consortium: data I-X Conclusion

- Important effort to report on PGD activity in Europe and in the world
- No adverse outcome of the children, but…

- No valid study on the children's outcome
- Different evaluation method (letter, phone call's vs examination at the center) lack of definitions, incomplete data on children's, lost to FU rate?

Prospective controlled FU study UZ Brussel



Human Reproduction, Vol.27, No.1 pp. 288–293, 2012
Advanced Access publication on November 2, 2011 doi:10.1093/humrep/der360

human reproduction ORIGINAL ARTICLE Reproductive genetics

Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD

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Submitted on July 2, 2011; resubmitted on September 19, 2011; accepted on September 29, 2011

Prospective controlled FU study UZ Brussel

- Aim
  - health of children born after ART
  - ICSI since 1991, PGD since 1993
- Study design
  - Prospective controlled FU of PGD children
  - Control group of ICSI children
  - Both groups : day 5 embryo transfer

Prospective controlled FU study UZ Brussel

- Data collection
  - pregnancy and birth data obtained through written questionnaires
  - children examined at 2 months of age by trained pediatrician
  - developmental evaluation
    - Psycho-motor Bayley at age 2y
    - Socio-emotional and language at age 2y
  - Parents living abroad/refusals: questionnaire

Prospective controlled FU study UZ Brussel

- Outcome measurements
  - Parental characteristics

    cigarette smoking, alcohol use, medication, diseases (diabetes, hypertension, premature contractions), hospitalisations, weight gain and height mother
  - Neonatal parameters

    delivery, position baby, sex, weight, height, head circumference, Apgar score, complications, neonatal intensive care unit (NICU), breastfeeding, neonatal admission, perinatal death rate
  - Major Malformations

    examined at the centre of Medical Genetics

Children born

	PGD N=995	**ICSI N=1507**
Singletons	**670** 67%	**1059** 70%
Twins	**308** 31%	**433** 29%
Triplets	**17**	**15**

Parental parameters

	PGD(S)	**ICSI**	**P**
Maternal age (years)	33 ± 5	32 ± 4	< 0.001
Educational level mother H/M/L (%)	68/30/2	60/37/3	0.016
Educational level father	69/29/2	72/27/1	NS
Intake alcohol (%)	10	7	0.034
Cigarette smoking (%)	5	7	0.038
Parity =1 (%)	75	66	<0.001
Parity >1 (%)	25	34	<0.001
Complic pregnancy (%)	55	47	0.001
Prepreg BMI (kg/m^2)	22.7 ± 3.5	23.3 ± 4.3	0.002
Female subfertility (%)	10	54	<0.001
Male subfertility (%)	33	47	<0.001

Birthweight

	PGD N=995	ICSI N=1507	*P*
Singletons			
Mean birth weight (g)	**3263** ±544	**3237** ±583	NS
Birth weight SDS	-0.3 ±1.3	-0.4 ±1.4	NS
Twins			
Mean birth weight (g)	**2346** ±552	**2265** ±582	NS
Birth weight SDS	-2.7 ±1.6	-2.9 ±1.7	NS

No difference in mean birth weight (SDS) between PGD and ICSI

Gestational age

	PGD N=995	ICSI N=1507	*P*
Singletons			
Mean gestational age (w)	38.7 ±2.3	38.7 ±2.2	NS
Born < 37w (%)	11	11	NS
Born < 32w (%)	0.6	1.9	NS
Twins			
Mean gestational age (w)	35.3 ±1.6	35.0 ±3.1	NS
Born < 37w (%)	0.6	1.9	NS
Born < 32w (%)	9	14	NS

➔ No difference in mean gestational age and prematurity rate between PGD and ICSI

Major malformations

	PGD	ICSI	OR (95%CI)	*P*
Singletons	N= 670	N=1059		
Children with major Malformations	14 **2.1%**	25 **2.4%**	0.9 (0.4-1.8)	NS
Twins	N=308	N=433		
Children with major Malformations	7 2.3%	15 3.5%	0.7 (0.2-1.7)	NS
Total	N=995	N=1507		
Children with major Malformations	23 **2.3%**	40 **2.7%**	0.9 (0.5-1.5)	NS

Definition: Major malformations causing functional impairment and/or requiring surgical correction

MALFORMATION	PGD	ICSI
skin (ichthyosiform erythrodermia)	1	0
eye, ear, face and neck (cataract)	1	0
cardial, circulatory (VSD, ASD, pulmonary stenosis, Fallot)	3	9
respiratory (chylothorax, subglottis tracheal stenosis)	2	0
cleft lip and/or palate	0	2
digestive (duodenum atresia, oesophageal atresia)	2	1
genital organs (hypospadias, torsio testis, cryptorchism)	7	9
urinary (urethral valve, renal duplication, renal dysplasia)	1	3
musculoskeletal (syndactylia, club feet, polydactylia)	2	10
chromosomal (trisomy 21, 47 XXX)	1	2
neoplasms (lymphangioma, rhabdomyosarcoma)	2	0
other (myotonic dystrophy, S Beals, polymalformative S)	1	4

➔ No difference in overall major malformation rate and genital malformation rate between PGD and ICSI

Prospective controlled FU study at birth Conclusion neonatal outcome

- No difference in birthweight
- No difference in prematurity rate
  <37w , <32 weeks
- No difference in gestational age
- No difference in perinatal mortality
- No difference in neonatal hospitalisations
- No difference in major malformations

Prospective controlled FU study at birth Conclusion neonatal outcome

- Dependent variable: birth weight SDS
- Adjusting for maternal BMI, smoking, alcohol, parity, female and male infertility, pregnancy complications, parental educational level
- Results remained unaltered

Prospective FU studies at 2 years
UZ Brussel

- Prospective clinical and psychological follow-up on [1,2]
  - 70 singletons born after PGD/PGS
  - 70 singletons born after ICSI
  - 70 singletons born after SC
- Matching criteria
  - gender, mat. educational level, mother tongue, birth order
- Results
  - mental & psychomotor development
  - socio-emotional & language development

[1]S. De Smyttere et al Hum Reprod 2009; [2]J. Nekkebroeck et al. Hum Reprod 2008

Prospective FU studies at 2 years
Conclusion

- General health is comparable
- Growth and medical outcome in singletons born after PGD/PGS reveals reassuring findings as compared to ICSI and SC singletons[1]
- Cognitive and psycho-social development is similar[2]
- Socio-emotional and language development similar[3]

[1]Desmyttere et al. H Reprod, 2009 [2]Nekkebroek et al. H Reprod, 2008
[3]Nekkebroek et al H Reprod, 2008

Follow-up data in literature
Major malformations

Reference	Number	Major	% Major
1 Strom et al.	114	1	0.9
2 Horwitz et al.	413	8	1.9
3 Turk-Kapsa et al.	480	8	1.7
4 DeDie Smulders	48	0	0.0
5 ESHRE PGD consortium I –IX and consortium X	3929	68	2.0
	718	11	1.5
6 PGDIS meeting, Ginsberg et al, 2009	1230	23	1.9
7 Beukers et al. 2012	50	23	2.3

Follow-up data in literature
Major malformations

- Major congenital malformation rate
  - very few comparative studies
  - no difference with ICSI population
- In no study have anomalies been disproportionately clustered in any given organ system

Follow-up data in literature
FU data at older age

Reference	PGD	control	% Major
Banerjee et al 2008	49	66NC	Birthweight lower in PGD **Age 18 months** : Growth /Neuro-development / Parent-child relation similar
Middelburg et al 2011	54	77NC	**Age 2 years** : Mental/neurological/behavioral similar, but lower neurological scores

PGD compared to natural conception?

- PGD needs IVF/ICSI embryo's
- Therefore initial comparisons on risk of PGD with ICSI/IVF
- However meta analysis on ICSI shows an increased risk for
  - Major congenital malformation rate
  - Adverse neonatal outcome
  - Possible cardio-vascular risk at later age

Risk of ICSI and IVF

- Major congenital malformation[1]
  OR 1.29 (CI 1.19-1.39)
- Adverse neonatal outcome in SET embryo's[2]
  - LBW **OR 1.70** (CI 1.53 -1.89)
  - VLBW **OR 1.94** (CI 1.54-2.45)
  - Prematurity <32w
    OR 1.80 (CI 1.45-2.45)

[1] Hansen, 2005 Meta analysis
Adjusted for maternal age, parity, infant sex, not for plurality
[2] Panday, 2012, Meta analysis

Risk of ICSI and IVF

- Mainly related to subfertility of the parents
- Partially related to[1] ?
  - Hormonal stimulation
  - Suboptimal endometrium
  - Culture media
  - Vanishing twins
- ICSI not significantly different from IVF

[3]Pinborg et al, 2013 Review and meta analysis

Content of presentation

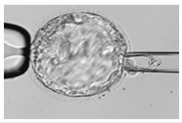
- Context and history
- Definitions and procedure
- PGD in daily practice
- Babies born
- **Future developments**
- Conclusions

Further developments

- New techniques have been introduced in the clinic
  - Array-comparative genomic hybridisation (for chromosomal aberration)
  - Single nucleotide polymorphism (SNP) arrays
- The use of **SNP arrays** brings ethical concerns as a large amount of genetic information will be available from each embryo
- Combined test for chromosomal and monogenic diseases will be possible on SNP arrays
- Possible advantages of blastocyst biopsy for monogenic conditions (combination with PGS?) should be evaluated

Trophectoderm biopsy

- Removal of several cells of blastocyst stage
  - Less embryo's to test → lowering cost of new technologies
  - Development of PGS
    - screening for chromosomal anomalies
    - avoidance of viable aneuploidies
    - Higher implantation chances
- No data on health of children after trophectodermbiopsy



Content of presentation

- Context and history
- Definitions and procedure
- PGD in daily practice
- Babies born
- Future developments
- **Conclusions**

Conclusion

- 1990: first PGD for sexing for X-linked conditions
- 2014: many centers offer PGD on 8-cell stage or trophectodermbiopsy
  - for monogenic and chromosomal diseases
  - as screening for aneuploidy

  few countries use Polar Body Biopsy
- Further evaluation needed on
  - succes rates / error rate
  - indications

Conclusions

- Ongoing ethical debate on indications for PGD for
  - late onset diseases / HLA typing / multifactorial diseases / sex selection without medical reasons
- Ongoing debate on screening
  - trophectoderm biopsy for screening for aneuploidy
- Ongoing evolution of technology
  - If whole genome analysis with SNP's → ethical problems, any normal embryo left?

Conclusions

- Medical outcome PGD newborns is reassuring
  - ESHRE PGD consortium and PGD UZ Brussel study
    → PGD similar to ICSI
- Medical and Psychological outcome 2-5 years
    → PGD similar to ICSI and NC on small numbers
  - Long-term studies are required to study
    - biometrical data
    - metabolic / cardiovascular /epigenetic risk
  - Outcome of PGD compared to NC needed

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CELEBRATING 20 YEARS
Brussels PGD
PGD kliniek | Clinique DPI | PGD clinic

Principles and Applications of Next-generation sequencing

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Professor
Li Ka Shing Institute of Health Sciences
Department of Chemical Pathology
The Chinese University of Hong Kong

Disclosures

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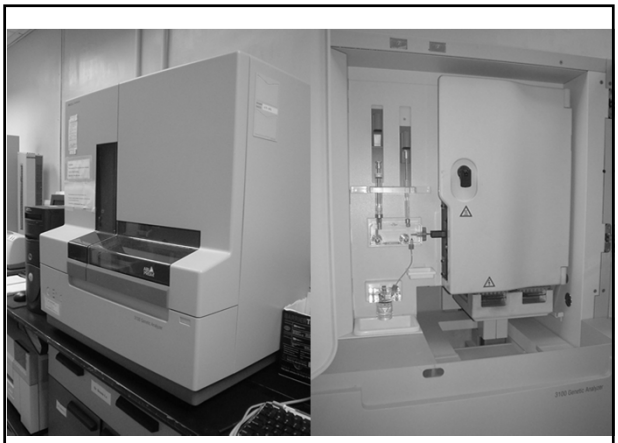
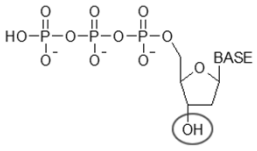
- Roche – research funding
- Illumina - Travel grants
- Life Technologies – Travel grants

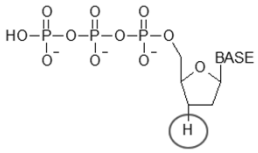
Learning objectives

- What is “next-generation” sequencing?
- Principles
- Applications related to prenatal diagnosis
- Pitfalls



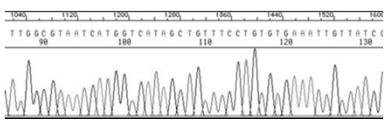


deoxynucleotide triphosphate



dideoxynucleotide triphosphate

Dideoxy sequencing
"Sanger" sequencing



Dideoxy sequencing
"Sanger" sequencing

- Up to ~ 800 basepairs
- 16 sequencing targets per run
- Target-specific primers (You control what gets sequenced)
- One target amplicon per sequencing reaction
- Sequence forward and reverse direction to get consensus sequence
- Alignment to confirm sequenced target
- Identify polymorphisms or mutations

Massively parallel sequencing

- "next-generation sequencing"
- Millions to billions of nucleic acid molecules sequenced in each run
- Enabled by the use of universal adaptors
- Clonal expansion of individual DNA / RNA template molecules
- Sequencing of each clone but many clones in parallel
- Short read sequencing
- Alignment is an approximation

Illumina sequencing

- DNA fragmentation
- Universal adaptors
- Clonal expansion by solid phase bridge amplification
- Sequencing initiated by universal sequencing primer
- Reversible dye terminators
- Optical monitoring

Amount of data output per run

- 150 x 10^6 reads per lane
- 2 x 100bp per read
- 8 lanes per flow cell

- = 2.4 x 10^{11} bp per run
- ~ 3.3 x 10^9 bp per haploid genome

- ~ 100 times coverage of the human genome

454 sequencing

- Emulsion PCR
- Pyrosequencing

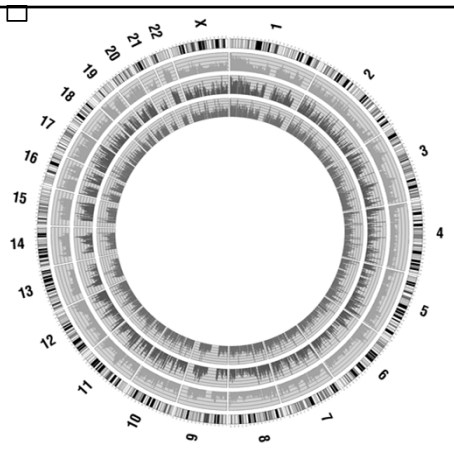
Semi-conductor sequencing

- Starts with emulsion PCR
- Detects H^+ released when deoxynucleotide is incorporated by DNA polymerase

After sequencing

Base calls (A, C, G, T)

Alignment

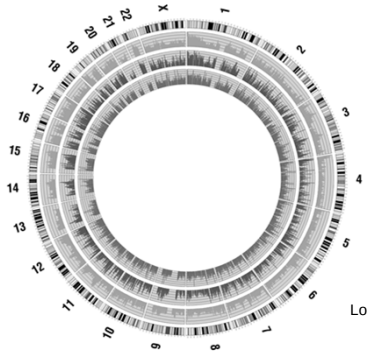


Clinical applications

de novo sequencing

New pathogen detection

Resequencing

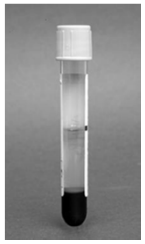


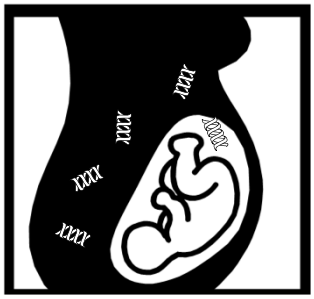
Fetal genome decoded noninvasively

Lo et al *Sci Transl Med* 2010

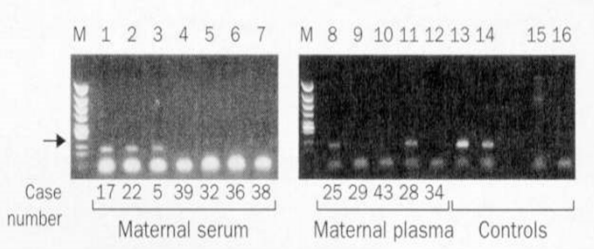
DNA-based prenatal diagnosis

Fetal DNA in maternal plasma



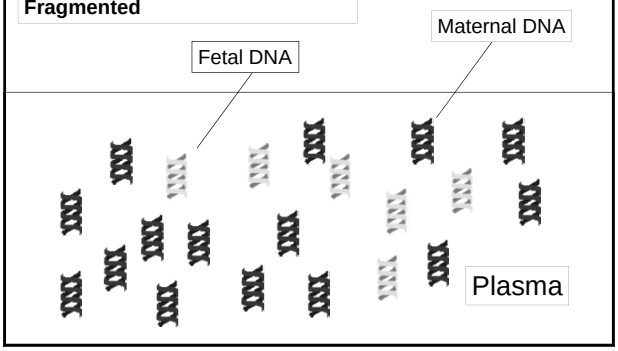


ChrY DNA in maternal plasma

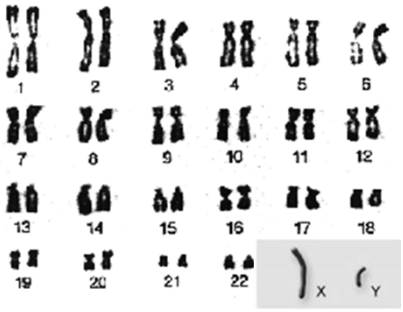


Lo et al *Lancet* 1997

Large maternal DNA background
Cell-free
Fragmented



Fetal sex

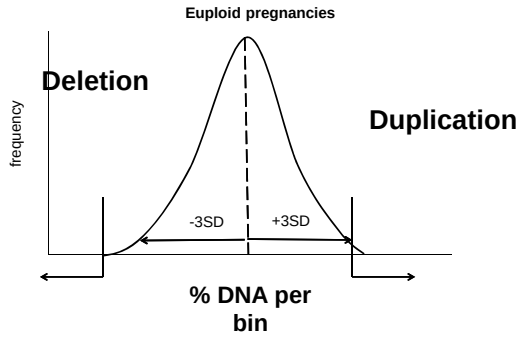


% DNA per bin

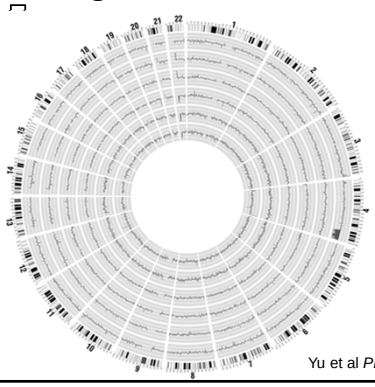
$$\frac{\text{\# DNA fragments per bin}}{\text{\# total DNA}}$$

- Test sample vs control samples

Cut-off



Structural genomic abnormalities



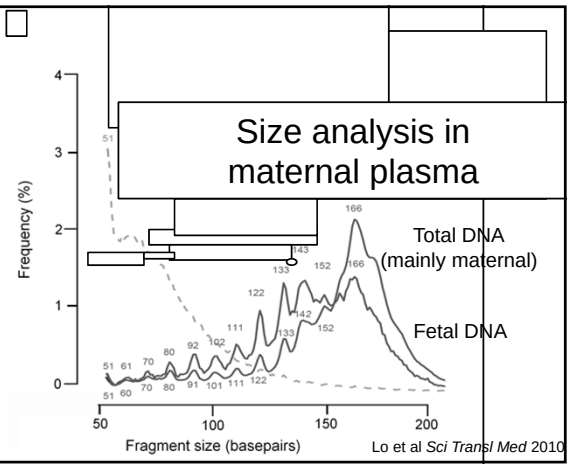
Yu et al *PLoS One* 2013

Paired end sequencing

Genome coordinates

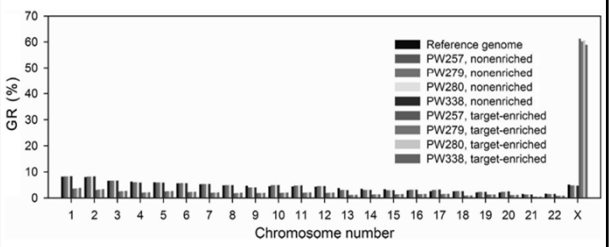
Size deduction

Size analysis in maternal plasma



Lo et al *Sci Transl Med* 2010

Targeted resequencing

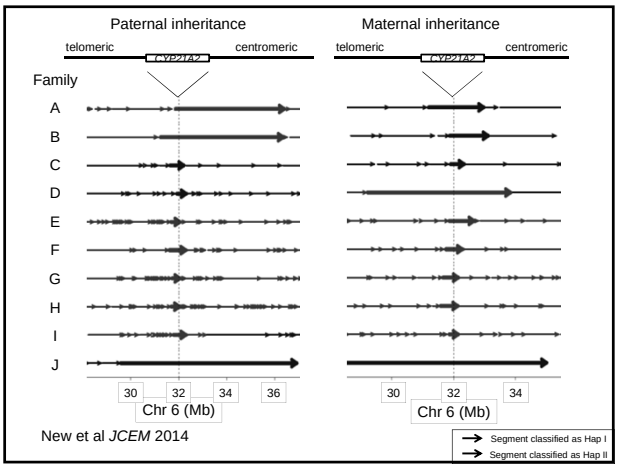


Target: exons on chr X

Liao et al *Clin Chem* 2010

Congenital adrenal hyperplasia

- Abnormalities in adrenal steroid synthesis
- > 95% cases 21-hydroxylase deficiency (*CYP21*)
- Autosomal recessive



Multiplex sequencing

- Mixing more than one sequencing libraries

Methylome

Unmethylated promoter

CGCGCGCGC Coding Sequence

Methylated promoter

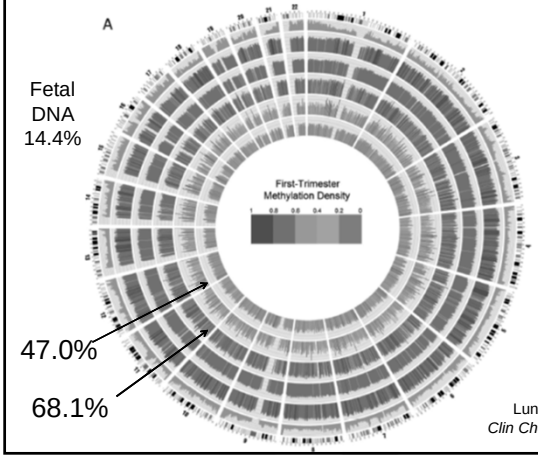
M M M M M

CG**C**G**C**G**C** Coding Sequence

X Gene Expression

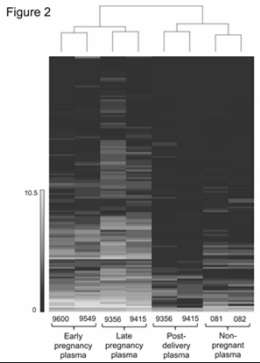
Placental epigenomics

- Growth and development
- Generally hypomethylated
- Tissue-specific DNA methylation
- Genomic imprinting
- Pregnancy-associated disorders



Lun et al *Clin Chem* 2013

Transcriptome profiling



Tsui et al *Clin Chem* 2014

Pitfalls

Sequencing error

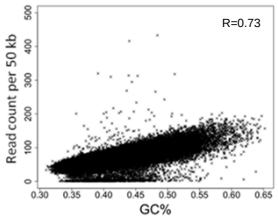
- 0.3%
- But 2.4 x 10^{11} bp per run
- = 7 x 10^{8} errors!

Alignment errors

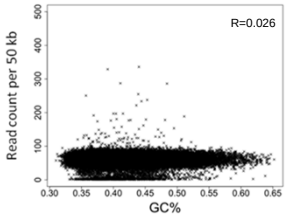
- Chr Y reads in females
- Alignment is an approximation

GC bias

Before GC correction



After GC correction



Chen et al *PLoS One* 2011

Single-cell sequencing

- Allele dropout

Other issues

- Amplicon sequencing
- Data storage costs

Once mastered the skill

- Very versatile
- Additive data
- Lots of data to interpret

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PGD in mitochondrial DNA disorders

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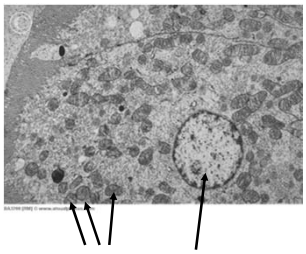
No conflict of interest to disclose

Learning objectives

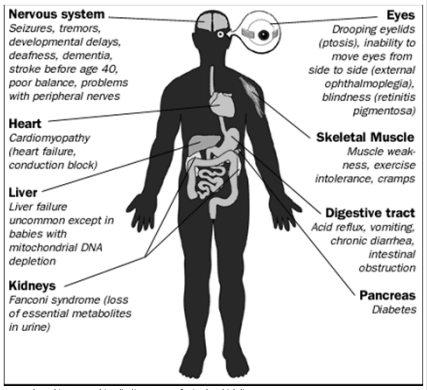
1. The heterogeneous clinical expression of mtDNA disorders
2. Pathogenic mutations in the mtDNA
3. Threshold of expression of mtDNA disorders
4. Unpredictable recurrence risk due to mtDNA transmission bottleneck
5. Current options to prevent the transmission of mtDNA disorders
   - Oocyte donation
   - Prenatal diagnosis (de novo mutations, some recurrent mutations)
   - Preimplantation Genetic Diagnosis (majority of heteroplasmic mutations)
6. Future options all mutations
   - Chromosome spindle-transfer
   - Pronuclear transfer
7. Ethical issues

Mitochondria: Power Plants of the Cell

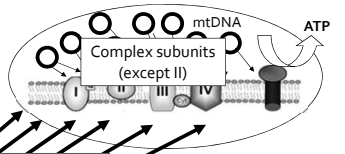


Mitochondrial Disease:

General or Local Power Failure



Maternal Inheritance
37 genes



MITOCHONDRION

Complex subunits
Assembly factors
Translation factors
mtDNA maintenance
Indirect OXPHOS proteins

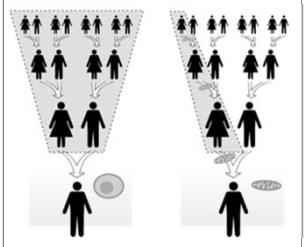
Mendelian Inheritance
>1500 genes

NUCLEUS

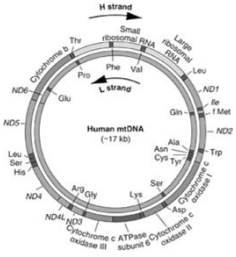
Mitochondrial Inheritance and mitochondrial DNA

Nuclear DNA is inherited from all ancestors

Mitochondrial DNA is inherited from a single lineage



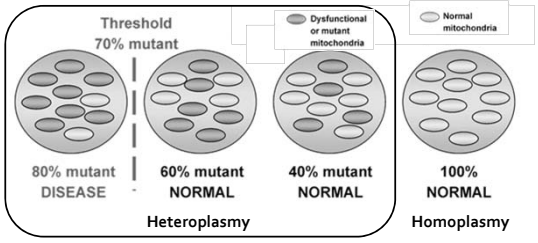
http://evolution.berkeley.edu



16.569 nucleotides

Deletions and >159 disease-causing point mutations in patients known

Threshold of Expression



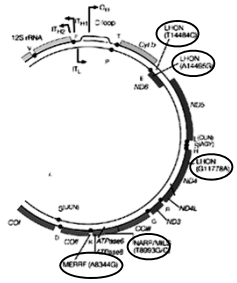
Threshold varies among tissues

Mutation percentage can change in time

Relation mutation percentage clinical symptoms often not straightforward

Most pathogenic mutation leading to severe disease are heteroplasmic
Homoplasmic pathogenic mutations exist (LHON mutations), but severe, life-threatening homoplasmic mutations are rare

Defects in the mtDNA

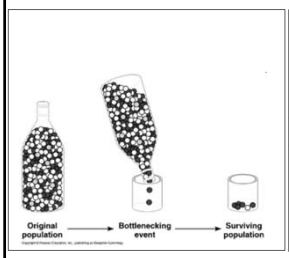


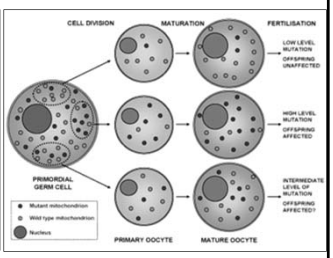
- Rearrangements
  - Deletions/duplications
  - Single (de novo/maternal-low RR)
  - Multiple (AR/AD)
  - Heteroplasmic
- Point mutations
  - tRNA genes
  - rRNA
  - Protein encoding genes
  - Heteroplasmic or homoplasmic
  - De novo/maternal (variable RR)
- mtDNA depletion
  - Reduced copy number
  - AR/AD/de novo

Genetic (mtDNA and nuclear genes) and environmental causes

Frequency mtDNA disease: 1 in 3,500-10,000
Frequency mtDNA mutations: 1 in 200-400

Mitochondrial Transmission Bottleneck





http://www.mitochondrialncg.nhs.uk/pa_genetics.html

Towards a Future without mitochondrial DNA Disease

1. Selecting the good guys (healthy oocyte/embryo)
   - Oocyte donation (homo/heteroplasmic mutations)
   - Prenatal diagnosis (some heteroplasmic/de novo mutations)
   - Preimplantation Genetic Diagnosis (all heteroplasmic mutations)
2. Kicking out the bad guys (exchange faulty mitochondria)
   - Spindle-chromosomal complex Transfer (homo/heteroplasmic mutations)
   - Pronuclear Transfer (homo/heteroplasmic mutations)

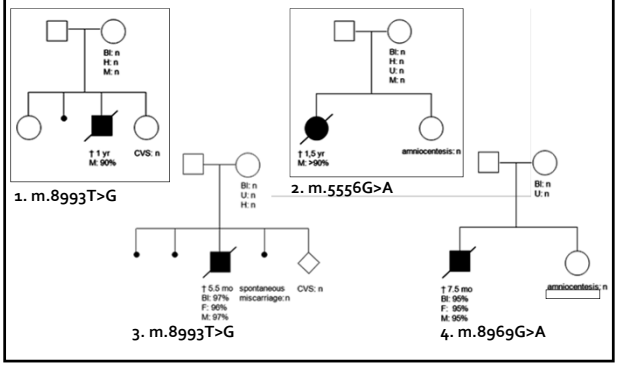
Prenatal Diagnosis for mtDNA Mutations

Criteria mutations
- Close correlation mutation load - disease severity
- Uniform distribution in all tissues
- No change mutation load in time

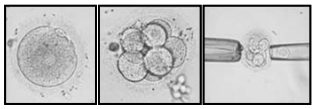
(ENMC International Workshops)

- **For most carriers of mtDNA mutations PND no option**
  - Technically feasible/accurate, but interpretation is the problem
  - Only few specific mtDNA mutations match criteria
  - Many private mutations
- **PND is an option for *de novo* mutations**
  - *De novo* mtDNA mutations frequent (based on absence mutation in different tissues of the mother of an mtDNA patient)
  - Chances of having another child without the mutation very high
  - PND for confirmation or reassurance
  - Requires appropriate counselling

Prenatal Diagnosis for *de novo* mtDNA mutations



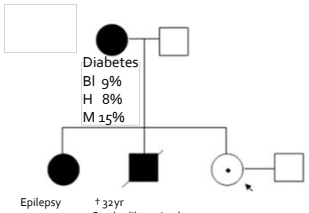
Is Preimplantation Genetic Diagnosis (PGD) a better option for recurrent mtDNA mutations?



Selection embryos with mutation load below threshold expression, but:

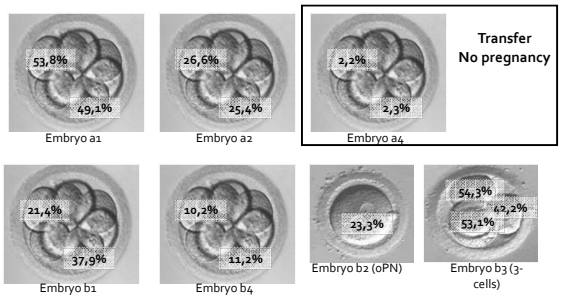
- Only heteroplasmic mutations (main group of severe mutations)
- Is it reliable? (mutation load blastomere representative?)
- What is the threshold? (many private mutations)
- Do such embryos exist?
- Additional advantage no dilemma of termination pregnancy

PGD in Family with m.3243A>G Mutation

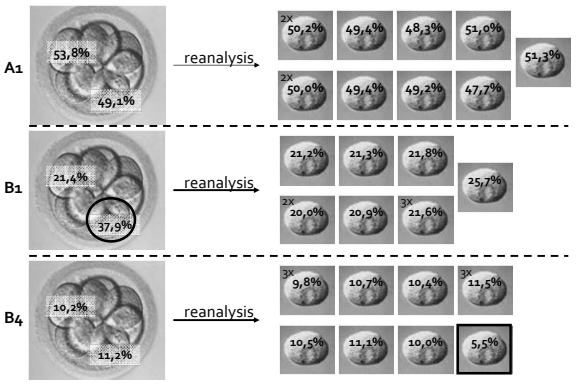


Bl 23%	Bl 35%	Bl 25%	Bl= blood
H 9%	H 40%	H 10%	H= hair
M 28%	F 63%	M 28%	M= muscle
			F= fibroblasts

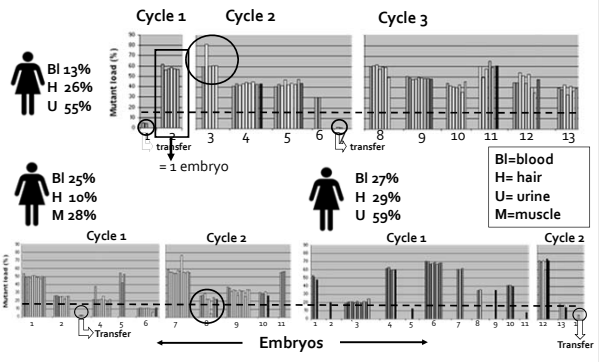
PGD in Family with m.3243A>G Mutation



Reanalysis PGD m.3243A>G

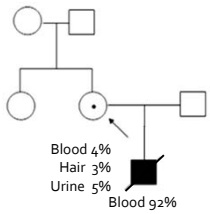


Interblastomere Differences m.3243A>G



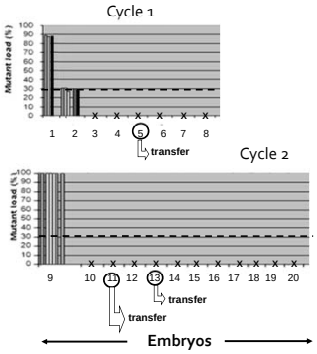
Interblastomere Differences m.8993T>G

Leigh syndrome

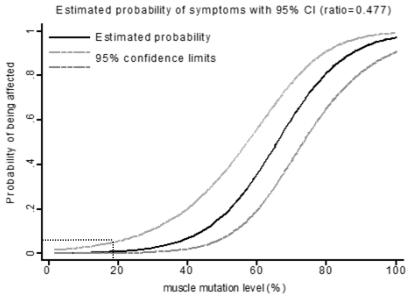


A healthy son was born

Sallevelt et al. J Med Genet 50:125-132



The Likelihood of being affected as a Function of mtDNA Mutation Load

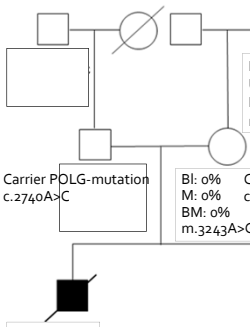


Systematic review on 159 different heteroplasmic mtDNA mutations (327 pedigrees)

Combined data of muscle mutant % from affected individuals and unaffected maternal relatives

Hellebrekers et al. Hum Reprod Update. (2012) 18:341-9.

At mutant level ≤ 18% → P(unaffected) ≥ 95% **irrespective of mutation**



More complex Preimplantation Genetic Diagnosis

- Patient: severe, infantile-onset, clinical presentation with feeding problems, hypotonia, psychomotor retardation and epilepsy
- Low percentage m.3243A>G
- Can not explain phenotype
- Additional POLG mutation
- PGD for both POLG mutations and m.3243A>G on 2 separate blastomeres

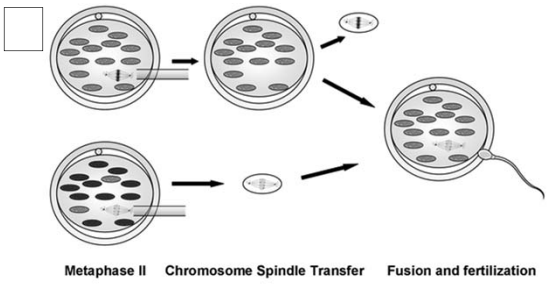
How far will Preimplantation Genetic Diagnosis in mtDNA Disease bring us?

- Carriers of **all** heteroplasmic mtDNA mutations have a fair chance of having healthy offspring by applying PGD
- PGD of heteroplasmic mtDNA mutations is **technically safe and reliable** (preferably on 2 blastomeres, polar bodies not reliable)
- Estimating a **"safe" cut-off mutation percentage** at which the risk of being affected is acceptably low (risk reduction strategy)
- Based on limited PGD cycles for specific mutations we expect that **most mtDNA mutation carriers will have oocytes below this threshold** (depends on mutation and mutation load, issue of stimulation)
- Exact cut-off mutation percentage determined by **case-by-case counselling**, considering uncertainties, disease severity, family circumstances, risk perceptions, availability of embryos below the threshold
- Selection of male embryos (sex analysis) could definitely eliminate mtDNA disease in future generations (ethical issue), but consecutive cycles of female embryos in subsequent generations might have the same effect

Towards a Future without mitochondrial DNA disease

1. Selecting the good guys (healthy oocyte/embryo)
   - Oocyte donation (homo/heteroplasmic mutations)
   - Prenatal diagnosis (heteroplasmic/de novo mutations)
   - Preimplantation Genetic Diagnosis (heteroplasmic mutations)
2. Kicking out the bad guys (exchange faulty mitochondria)
   - Spindle-chromosomal complex Transfer (homo/heteroplasmic mutations)
   - Pronuclear Transfer (homo/heteroplasmic mutations)

Chromosome Spindle Transfer



Smeets (2013) Reprod Biomed Online. 27:599-610.

Current Status of Spindle Transfer

Proof of concept demonstrated in non-human primates

- Spindle-chromosomal complex is devoid of surrounding mitochondria
- Carry-over nuclear-donor mtDNA is less than 3% (below detection limit)
- Fertilization was successful – primates were born

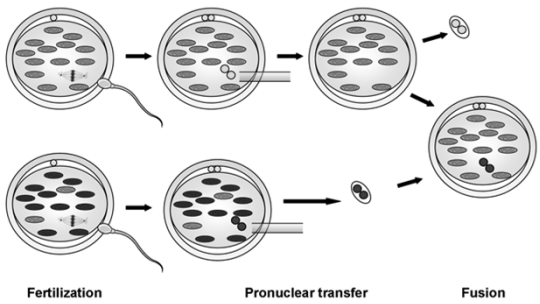
Tachibana et al. (2009) Nature 461: 367-372

Proof of concept in human oocytes

- Spindle transfer in 65 oocytes
- Fertilization rate similar to controls (73%, 75%)
- 52% spindle transfer zygotes: abnormal fertilization (irregular number pronuclei) – different from monkeys (premature activation?)
- Normal fertilized spindle transfer zygotes: blastocyst development, embryonic stem cell isolation, proliferation, karyotypes, copy number variants and OXPHOS activity comparable to controls
- Spindles from vitrified oocytes in fresh cytoplasts results similar to controls (prevents premature activation oocytes)

Tachibana et al. (2013) Nature 493:627-631/Paull et al. (2013) Nature 493:632-637

Pronuclear Transfer



Smeets (2013) Reprod Biomed Online. 27:599-610.

Status Pronuclear Transfer

- Abnormally fertilized human zygotes used (approved test model)
- Transfer 1 or 2 pronuclei with a minimal volume of cytoplasm
- Reconstituted zygotes cultured 6-8 days to monitor development *in vitro*
- Onward development comparable to controls (abnormal fertilized zygotes)
- 8.3% developed to blastocyst stage after transfer 2 pronuclei (50% of controls)
- Average carry-over was less than 2%

Craven et al. Nature (2010) 465:82-5

Ethical Issues concerning Spindle or Pronuclear Transfer Technologies

Ethical considerations:

- Implications for identity
- Germline therapy
- Introduction of novel techniques and follow-up
- Parentage of the child (genetic contribution third party)
- Status of the mitochondrial donor
- Implications for wider society and future generations (creating boys)

Conclusions and issues for future consideration:

- Treatment as part of a research trial (safety issues - specialized centres)
- Regulation: follow-up (central register)
- Parentage of the child (no 'third parent' or 'second mother')
- Regulation: status of the mitochondrial donor (identity not required)
- Further issues for discussion (germline therapy)

Bredenoord et al. J Med Ethics (2011) 37:97-100
Report Nuffield Council on Bioethics 2012

How far will nuclear Transfer in mtDNA Disease bring us?

- Spindle Tranfer and Pronuclear Transfer are capable of generating (almost) mtDNA mutation-free embryos
- The minimal amount of mtDNA carry-over is unlikely to cause disease
- In primates and (abnormally) fertilized oocytes the methods seem safe, but issues remain (long term effects, epigenetic issues)
- Both methods can be used for heteroplasmic and homoplasmic mutations
- The clinical safety of the methods needs to be further demonstrated but it may not be possible to demonstrate the safety before the first clinical trial
- Ethical issues need to be settled
- Require sufficient donor oocytes or zygotes (vitrification possible)

De novo mtDNA disease: a remaining issue

- De novo mtDNA disease is frequent (1 in 10,000)
- Oocytes contain *de novo* heteroplasmic point mutations
- Different oocyte of the same woman have different heteroplasmic mutations
- Random de novo mutations at high percentage occur in 1% oocytes (Jacobs et al. (2007) Mol Hum Reprod 13:149-154)
- De novo deletion are frequent (50%) in oocyte, usually in extremely low heteroplasmy levels

De novo mutations can only be identified by Preimplantation Genetic Screening and maybe in future by Non-Invasive Prenatal Testing (NIPT-technical challenge)

Need for development of new therapeutics to treat patients

Towards a Future without mitochondrial DNA Disease

1. **The transmission of mtDNA disease can be effectively stopped** by:
   - **Prenatal Diagnosis**: mother of a child with a *de novo* mutation, some recurrent mutations
   - **Preimplantation Genetic Diagnosis**: most or all heteroplasmic mutations
   - Both methods are safe with a small residual risk based on heteroplasmy level of embryo/foetus
2. **Future options are nuclear transfer technologies**:
   - **Spindle Transfer**: homoplasmic and heteroplasmic mutations
   - **Pronuclear Transfer**: homoplasmic and heteroplasmic mutations
   - Residual risk based on carry-over seems low
   - Safety of the methods needs to be further demonstrated
   - Ethical issues need to be settled
3. **Therapy development is fundamental** as mtDNA disease occurs *de novo in* 1 in 10.000

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MITOCIRCLE

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Ethical dilemmas in preimplantation genetic testing

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- Commercial relationships: none
- Conflict of interest: none

Learning objectives

- to understand the dynamics of preimplantation genetic testing and its moral implications
- to contribute to an adequate ethical framework for preimplantation genetic diagnosis (PGD) and screening (PGS)
- to understand the ethical complexities and pitfalls of 'comprehensive' PGS
- to stimulate further reflection on the ethics of reproductive genetic testing

Outline

- Types of preimplantation genetic testing
- PGD: a strong consensus, some general questions
- PGS: tema con variazioni
- Comprehensive PGS (WESA/WGSA): rationale, problems and pitfalls
- Alternative approaches, incl. preconception carrier screening (PCS) → targeted PGD: advantages and questions
- Conclusions
- Literature

Two types of preimplantation genetic testing

- PGD: testing (IVF/ICSI-)embryos 'on indication', mostly because of a high risk of having an affected child
- PGS: the routine testing of (IVF/ICSI)embryos. Its primary aim so far is to increase the 'take home baby rate' (THBR) of IVF.

PGD

- Strong consensus: PGD is ethically sound if there is a 'high risk of serious disease'
- Normative debates concentrate on specific new possible indications, incl.: reduced penetrance alleles (RPA) for Huntington disease (HD); mitochondrial disorders; cardiogenetic disorders, etc.
- Ethical agenda-setting: some more general issues (incl.)

  A. Fertile or sub-/infertile applicants: does it matter?

  B. 'Never transfer an affected embryo'?

PGD (cont.)

A. Proportionality: does sub-/infertility matter?

The moral acceptability of PGD depends on its proportionality, taking account of a.o. the efforts, burdens and risks of IVF/ICSI for women, the possible risks of IVF/ICSI and the biopsy for future children thus conceived, the inherent embryo loss, and the costs of the procedure.

This, then, seems to imply that the criteria for PGD in *sub-/infertile* couples who will have IVF/ICSI *anyway* may be somewhat more permissive.*

* De Wert & Dondorp, 2014

PGD (cont.)

B. 'Never transfer an affected embryo?'

Background: the aim of PGD and the doctor's responsibility to avoid a 'high risk of serious harm' to future children*

But what if all embryos tested prove to be affected? Some exceptions to the rule seem to be be justified, taking account of (a.o.)**

- a flexible use of the proportionality criterion reg. the indications for PGD
- possible less serious, incidental findings
- the burdens and costs of an additional IVF/ICSI-cycle
- the dynamics of parental motives.

Obviously, adequate counseling is to be provided.

* ESHRE Task Force E&L, Hum Reprod 2007; ** id. Hum Reprod 2014 (in press)

PGS: tema con variazioni

Different methods, different aims, different normative frameworks:

I. PGS for tripronuclear zygotes (non-viable)

II. PGS for aneuploidy (mostly non-viable)

III. Comprehensive PGS (WESA/WGSA): the 'best' embryo → the 'healthiest'/'best' child

PGS I: Triploidy/PN screening (PGS-PN)

Aims (incl.)

- >THBR
- prevention of dreadful disease/suffering

Ethics

- acceptable even for pro-life ethicists …?
- locus of decision-making: patients' or professional autonomy?
- the status of 3PN zygotes (nb hESC research): truly embryos?

PGS II: Aneuploidy screening (PGS-A)

Primary aim: > THBR

State of the art: cf. former presentations*

Ethical issues include**:

- the imperative of evidence based reproductive medicine
- ethical prerequisites reg. experimental PGS-A (if such screening is not a misguided effort in view of the data)
- the just distribution of scarce resources/opportunity costs
- the status of non-viable embryos
- the locus of decision-making: what about e.g. XYY embryos?

Again: the 'high risk of serious harm'-standard

* Braude, 2013; ** De Wert, 2009

PGS III: Comprehensive PGS (WESA/WGSA): rationale

Both

- increasing the THBR of IVF/ICSI and
- avoiding genetic risks for future children, in order to guarantee, as far is this is possible, a healthy baby.

Isn't this the Holy Grail of medically assisted reproduction and the dream of prospective parents?

Conceiving 'the best possible child' might even include selecting for non-medical characteristics.

I

Comprehensive PGS: problems and dilemmas

1. A suitable screening test?

A. What about the analytical validity?

The wider the scope of PGS,
the more genetic defects/variants are screened for,
the more *false positives* →
the more embryos wrongly excluded from transfer →
lower THBR →
lower proportionality

Comprehensive PGS: problems and dilemmas (cont.)

1. A suitable screening test? (cont.)

B. What about the clinical validity?

The more complex the disorders screened for,
the *lower the predictive value* (clinical validity) of a positive test result, the lower the proportionality of the screening

Both sufficient analytical and clinical validity are a *necessary* (but not sufficient) condition for sound screening →
Comprehensive PGS does not (at least: not now) meet this primary, 'technical' (but morally relevant) criterion*****

* Winand et al., 2014

Comprehensive PGS: problems and dilemmas (cont.)

2. Does comprehensive PGD really facilitate reproductive autonomy?*****

A. Informed consent - a prerequisite

- would *informed* consent, taking account of the complexity of WESA/WGSA, be feasible?

- is *presumed* consent morally acceptable?

- what about *generic* consent (not as an alternative for, but as a variant of informed consent)?

* De Wert, 2009; Hens et al., 2013

Comprehensive PGS: problems and dilemmas (cont.)

2. Does comprehensive PGD really facilitate reproductive autonomy? (cont.)

B. Complex trade-offs. Just a simple case, please, make your choice for single embryo transfer:

- Embryo 2: probably infertile, slightly increased risk of late-onset AD
- Embryo 5: slightly increased risk of stomach cancer and type 2 diabetes
- Embryo 6: somewhat higher risk of kidney failure and Parkinson disease

Comprehensive PGS: problems and dilemmas (cont.)

3. Possible moral limits to reproductive autonomy/choice

A. The doctor's responsibility for the welfare of the child

First, *avoid a high risk of serious harm*

If various embryos are available, the choice which embryo to transfer may not be morally neutral →

What, then, about the *maximisation principle*: 'choose the embryo with the best prospect of the highest quality of life'. But what is best …?

Conflicting views. Burdens and costs of additional cycles

Comprehensive PGS: problems and dilemmas (cont.)

3. Possible moral limits to reproductive autonomy/choice (cont.)

B. The future child's right not to know ('open future'')

What about the transfer of embryos at (higher) risk of later-onset diseases?

The relevance of the ethical framework regarding predictive testing in (actual) children for sound comprehensive PGS →

Violating or respecting the future child's right to an open future - that's the question. How to make this respect operational?

Comprehensive PGS: problems and dilemmas (cont.)

3. Possible moral limits to reproductive autonomy/choice (cont.)

C. Non-medical embryo selection?

The issues: not really new, but still troubling

- select for sex, for 'talent' and/or to avoid social harm?
- Isn't such selection at odds with the interests ('open future') of the ('designer') child? Dissent: Habermas (dignity) vs Glover (flourishing/'all purpose means')*
- what about possible adverse social effects?

Habermas, 2003; Glover, 2006

Some possible alternatives for comprehensive (WESA/WGSA) PGS

1. PGS using WES/WGS, but → *targeted* analysis

This might have some of the possible advantages of comprehensive PGS, while avoiding some of its disadvantages.

But what to include in/exclude from the analysis? What are the in-/exclusion criteria – and who decides?

Some possible alternatives for comprehensive (WGSA/WESA) PGS

2. Preconception carrier screening (PCS)→ targeted PGD

Some advantages:

- More time for reflection
- More reproductive options, incl.:
  - refrain from having children,
  - use of donor gametes,
  - (targeted) PGD
- Avoid some of the problems of comprehensive PGS

But, obviously, this scenario raises some questions

PCS → targeted PGD: some questions

1. aim(s) of the offer? Facilitate reproductive autonomy? Improve public health? Taking professional responsibility seriously?

2. for whom/which target group? Just IVF-patients? All prospective parents? Selective or universal PCS?

3. for which disorders/what scope of PCS? All recessive conditions? More? Less?*

* De Wert et al., 2012

Conclusions

1. The ethical debate on PGD concentrates on acceptable indications. The relevance of the distinction between fertile and sub-/infertile applicants is, however, wrongly disregarded.

2. The adagium 'never transfer an affected embryo' needs revision.

3. PGS (like other types of screening) can only be ethically sound if it meets the proportionality criterion; its advantages shoud clearly (based on strong evidence) outweigh its disadvantages and costs.

Conclusions (cont.)

4. Comprehensive (WESA/WGSA) PGS seems to be driven by a technological imperative, does not (yet) meet widely accepted 'technical' criteria (analytical and clinical validity) for genetic screening, and raises puzzling ethical issues. Its implementation is, therefore, premature at best.

5. Preconception carrier screening for a wider set of (recessive) conditions plus targeted PGD may be a sound strategy – but needs further ethical scrutiny.

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