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"Latest developments in preimplantation genetic diagnosis"

SPECIAL INTEREST GROUP
REPRODUCTIVE GENETICS

6

28 June 2009
Amsterdam
The Netherlands

PRE-CONGRESS COURSE 6

Organised by the Special Interest Group Reproductive Genetics

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PRE-CONGRESS COURSE 6 - PROGRAM

Latest developments in preimplantation genetic diagnosis

Organised by the Special Interest Group Reproductive Genetics

Course co-ordinators: Karen Sermon (Belgium), Stephane Viville (France), Sjoerd Repping (The Netherlands), Filipa Carvalho (Portugal)

Course description: This course aims to bring the latest developments in preimplantation genetic diagnosis primarily in the technical field, but also in the field of patient follow-up and ethics. For the technical aspects, an update will be given on the latest efforts to improve efficiency and accuracy for PGD in monogenic disease and chromosomal abnormalities. The technology behind and the first clinical results on microarray-CGH will be presented and discussed. The organisation of a PGD centre, as well as the long term follow-up of children born after PGD will also be discussed

Target audience: Scientists and clinicians with an interest in PGD: those who already provide PGD to their patients and wish to expand their experience as well as those who have followed the developments in PGD through literature
Participants are expected to have a minimal background on basic techniques used in PGD (ICSI, embryo culture, biopsy, FISH, PCR)

09:00 - 09:30	Set-up a PGD lab and clinic: guidelines, QA and accreditation - ***Alan Thornhill (United Kingdom)***
09:30 - 09:45	Discussion
09:45 - 10:15	One vs two cell biopsy - the same answer for monogenic and chromosomal abnormalities? - ***Catherine Combelles (USA)***
10:15 - 10:30	Discussion
10:30 - 11:00	**Coffee break**
11:00 - 11:30	Optimizing PGD for monogenic diseases: minimal requirements in

	multiplex PCR and MDA - ***Céline Moutou (France)***
11:30 - 11:45	Discussion
11:45 - 12:15	Chromosomal abnormalities: development of generic tests – ***Catherine Staessen (Belgium)***
12:15 - 12:30	Discussion
12:30 - 13:30	**Lunch**
13:30 - 14:00	Studying all chromosomes: is more better? - ***Evelien Vanneste (Belgium)***
14:00 - 14:15	Discussion
14:15 - 14:45	PGS: the final settlement - ***Sebastiaan Mastenbroek (The Netherlands)***
14:45 - 15:00	Discussion
15:00 - 15:30	**Coffee break**
15:30 - 16:00	Long-term children follow-up - ***Alison Lashwood (United Kingdom)***
16:00 - 16:15	Discussion
16:15 - 16:45	What have we learned from 10 years of the PGD Consortium? – ***Peter Braude (United Kingdom)***
16:45 - 17:00	Discussion

Setting up a PGD lab and clinic: Guidelines, QA and accreditation

Latest developments in preimplantation genetic diagnosis

ESHRE SIG: Reproductive Genetics, 2009

Alan R. Thornhill Ph.D.
Scientific Director, The London Bridge Fertility, Gynaecology and Genetics Centre
Director, Bridge Genoma

Disclosure: I have no commercial relationships, nor am I engaged in other activities that might be perceived as a potential conflict of interest

Learning Objectives

- Understand what is required to provide PGD treatment
- Describe activities required to perform PGD according to specific guidelines and general laboratory accreditation standards
- Identify the differences between transport, satellite and in-house PGD
- Discriminate between satisfactory and excellent quality PGD treatment
- Identify future improvements in PGD services

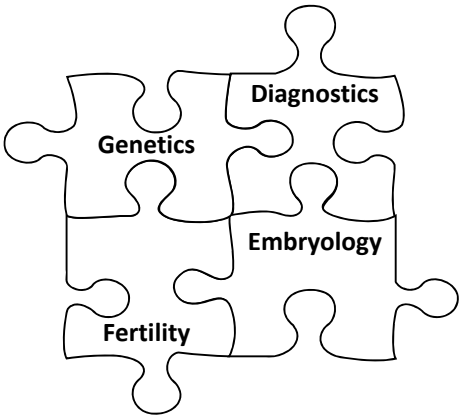
Organisation of a PGD centre

- What is required to perform PGD?
- Building the PGD puzzle
- Patient vs centre experience of PGD
- Satellite/transport PGD - Pros and Cons
- What makes an excellent PGD centre?
- Impact of future developments on PGD
- Bibliography

What is required to perform PGD?

- *Building the PGD puzzle*

- Appropriate testing (genetic counselling/testing)
- In Vitro Fertilization
- Embryo biopsy
- Diagnostic test on biopsied blastomere
- Reporting and explaining results
- Transfer of selected embryos to the uterus
- Follow-up of pregnancy and resulting child

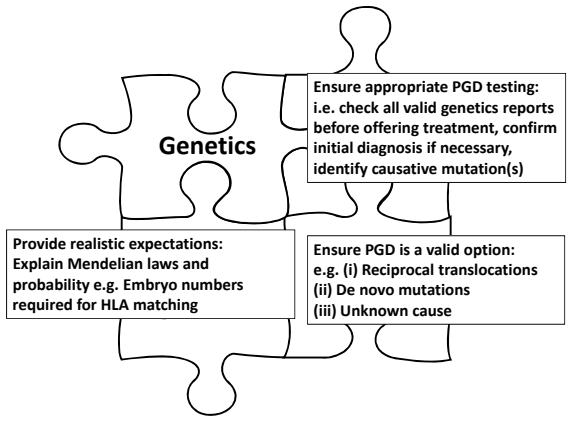


Genetics

- Full family and medical history
- Assess severity of condition
- Estimate genetic risk
- Provide realistic expectations
- Explain PGD process, disorder and tests
- Ensure appropriate tests offered
- Discuss options (risk/benefit)
- Obtain informed consent

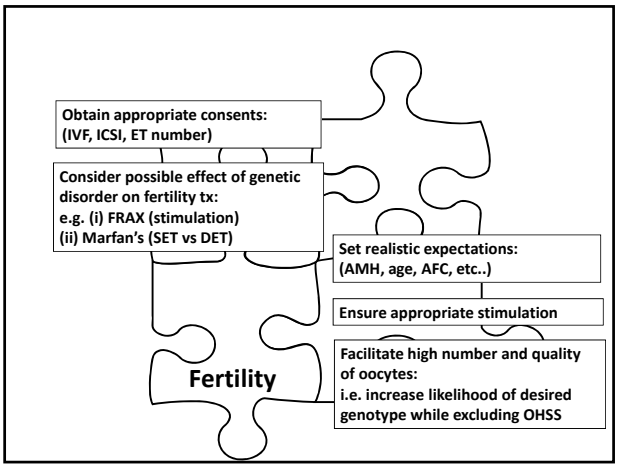
Options for potential 'PGD' patients

- Contraception
- Childlessness
- Prenatal testing (± pregnancy termination)
- Donation (egg, sperm, embryo)
- Adoption
- Reproductive roulette (emotional, physicial & financial cost of affected child?)



Fertility

- Full reproductive and medical history
- Provide realistic expectations
- Explain IVF process and tests
- Discuss options (risk/benefit)
- Obtain informed consent
- Prescribe IVF medication
- Perform IVF procedures (EC and ET)

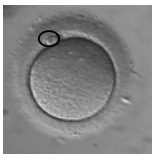
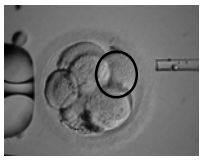
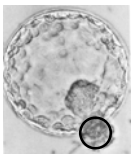


Embryology

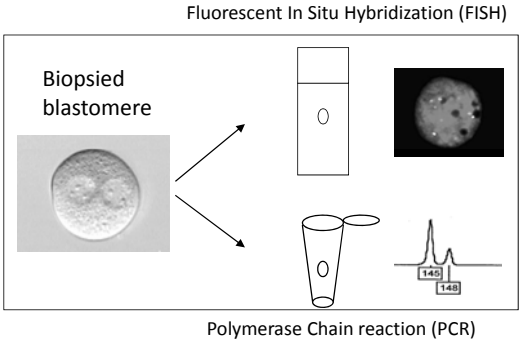
- Prepare and introduce gametes in vitro
- Culture embryos
- Biopsy (method, equipment, competency)
- Prepare diagnostic sample (single cells)
- Culture biopsied embryos (label, d3-d5)
- Select embryo(s) for transfer (based on genetic result and morphology)
- Surplus embryos (freeze, QC/QA, research)

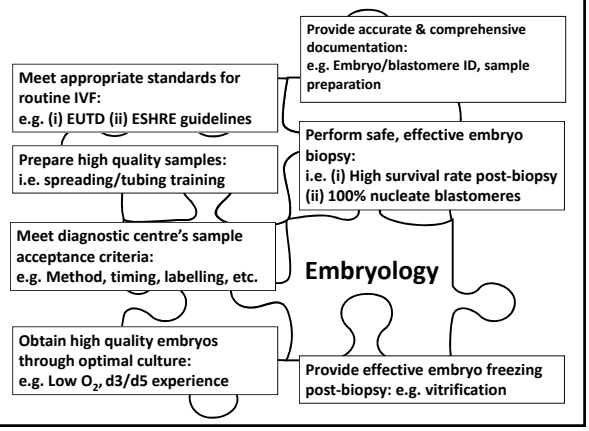
Embryo Biopsy - Stages

Polar Body | Cleavage stage | Blastocyst

Day 1 | Day 3 | Day 5/6





Embryology - EUTD/Guidelines

General examples (standards)

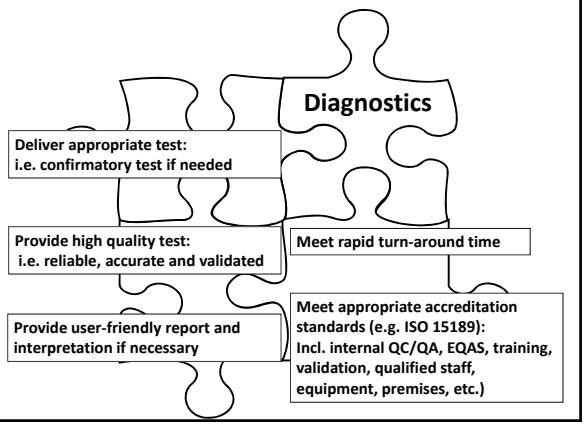
- Presence of QMS
- Air Quality
- Trained, competent staff
- Labelling and traceability

Specific examples (guidelines)

- Biopsy efficiency >95%
- ICSI for PCR based cases
- Biopsy embryos with >6 cells?
- 1 cell biopsy wherever possible

Diagnostics

- Develop and validate single cell test
- Receive and accession sample
- Perform test
- Analyse and Report results
- Provide interpretation



Diagnostics – ISO 15189/Guidelines

Examples

- Internal QC/QA (controls, int. Standards, confirmation of diagnosis, TAT, failure rates)
- EQAS (pilots for FISH and PCR)
- Validation (new specific test vs generic test, pre-cycle polymorphism check)
- Qualified staff (for testing and sign-off, training plan, ongoing competency assessments)
- Labelling (from pencil to barcodes)
- Premises and equipment (single cell diagnostics, 'clean room', FISH microscope)

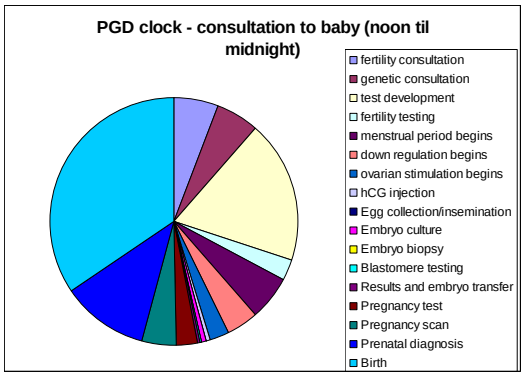
Patient experience of PGD

- Comprehensive information
- Calm
- Communication
- Control
- Consent
- Clear instructions

IVF centre experience of PGD

- Confusing
- Complex
- Control (lack of)
- Clear instructions (Ts and Cs)
- Communication
  - Smart NOT necessarily more!

Where does all the time go?



Satellite/transport PGD - Pros and Cons

- Pros
  - Improved patient access and convenience
  - Lower costs
  - Experienced reference diagnostics lab
  - Centres of excellence model
- Cons
  - Quality of sample preparation
  - Transportation risks and timings
  - Inadequate counselling/pre-cycle screening
  - Negligible follow-up /responsibilities

Impact of future developments on PGD

More….

- Satellite and transport PGD
- Quality control/quality assurance (reliability/accuracy)
- PGD laboratories accredited
- Test methods (whole genome amplification/microarray – cost?)
- Types of screening test (mutation analysis, linkage, haplotyping, aneuploidy, gene expression, protein)
- Patient access (funding for PGD, shorter wait times)
- Information for patients (report/interpretation complexity)
- Time for diagnosis (vitrification of biopsied embryos)

What makes an excellent PGD centre?

- Genetic Evaluation and Counselling
  - Best performed by genetics professionals, support throughout
- IVF Platform
  - Routine IVF results must be good
- Diagnostics Laboratory
  - Accreditation, experience, reputation
- Patient experience
  - Manage expectations (wait time, cost, success, misdiagnosis)
- Integration of Services
  - No blame culture, smart communication, follow rules
- Quality Control/Quality Assurance
  - Req. in accredited labs, Best test = best chance, follow-up
- Comprehensive Ethical Review

Relevant bibliography

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One *vs.* two cell biopsy - the same answer for monogenic and chromosomal abnormalities?

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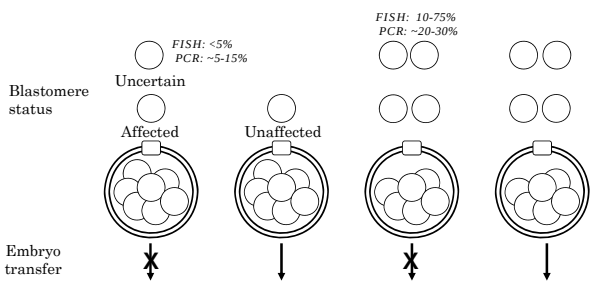
Learning objectives

- To appreciate the differences in clinical practices with respect to one *vs.* two cell biopsy
- To evaluate the current data on the efficiency, accuracy, outcomes of one *vs.* two cell biopsy
- To compare and contrast diagnostic parameters based on the type of analysis
- To relate clinical findings to our current understanding of early human embryogenesis

The biopsy of one *vs.* two cells



Potential trade-offs in the biopsy of one *vs.* two cells

Reasons to biopsy two cells:

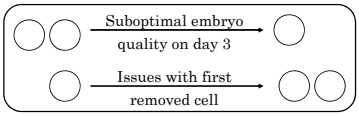
- ↑ diagnostic efficiency: human, embryo errors; technical limitations
- ↑ diagnostic accuracy
- Detection of discordant genetic make-ups: mosaicism
- Performed during an already invasive procedure

Reasons NOT to biopsy two cells:

- Developmental handicap(s): ↑ cell loss
- ↑ workload

The biopsy of one *vs.* two cells: who does what?

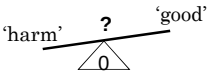
- No comprehensive data on how PGD is performed
- In the U.S., 1/4 of PGD clinics report on the biopsy of two cells (Baruch *et al.*, 2006)
- PGD labs with a chosen biopsy protocol except:



- PGD/PGS studies are based on one or two cells, or both.
- Practice guidelines from PGDIS, ESHRE, and ASRM

The biopsy of one *vs.* two cells: why under scrutiny?

Different lab procedures
↕ ?
Argued benefits of PGS



Arguments based on modeling and assumptions
Cryopreservation, error rates

Direct comparisons
Embryo quality bias
Sample sizes
Retrospective designs

Some of the key diagnostic measures based on number of cells and type of analysis

Diagnostic measures (%)	1-cell		1- and 2-cell	
	PCR	**FISH**	**PCR**	**FISH**
Efficiency	79[5]-89[6]	95-98[1]	85[5]-88[3]	95[2]-96[3]
Accuracy (false-positive)	14[4]-16[5]	7-15[1]	11[5]-12[4]	8[2]

[1]Li *et al.* (2005); [2]Staessen *et al.* (2004); [3]ESHRE PGD data collection VIII for 2005; [4]Dreesen *et al.* (2008); [5]Ray *et al.* (1998); [6]Goossens *et al.* (2008)

Direct comparisons of one *vs.* two cell biopsy

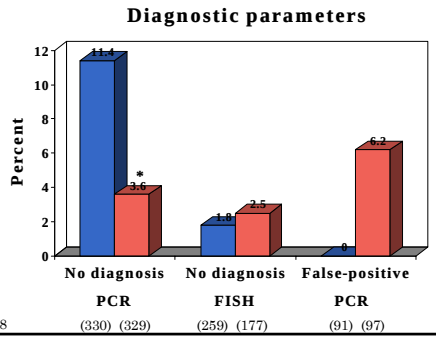
	1-cell	2-cell	Analysis	Study design	Ref.
Diagnostic efficiency (embryos)	70% (23)	78% (41)	FISH	retrospective 3 probes	Emiliani *et al.* '04
	95.9% (413) p = 0.04	98.2% (1366)	FISH	not randomized 2-c if ≥6 c	Michiels *et al.* '06
False-positives (embryos)	12.6%	6%	FISH	modeling assumptions	Los *et al.* '04
	25.6% (39)	13.6% (66)	FISH	not randomized 2-c if ≥ 6 c	Michiels *et al.* '06
	42% (29)	43% (54) 18% if concordant (11)	FISH	not randomized levels of mosaicism	Baart *et al.* '06

Some further insights into one *vs.* two cell biopsy

	1-cell	2-cell	Analysis	Study design	Ref.
Unaffected embryos transferred	69% 47%	88% 85%	PCR- rec. - dom.	modeling assumptions	Lewis *et al.* '01
False-positives (embryos)	14.3% (98)	9.1% (99)	PCR	retrospective 2-c if ≥8 c	Dreesen *et al.* '08
Cycle outcomes:					
Preg.	21.4%	29.1%	PCR + FISH	retrospective 2-c if ≥7 c	van de Velde *et al.* '01
Impl.	20.0%	18.6%			
Birth (transfers)	20.0% (14)	17.0% (117)			

A first RCT comparing the biopsy of one *vs.* two cells

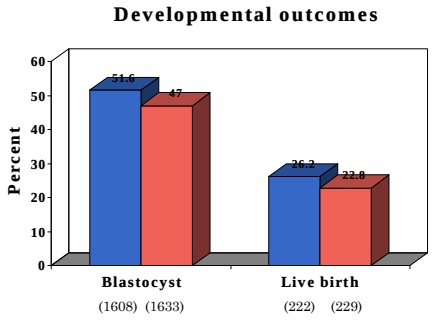
Goossens *et al.* (2008)



*, p=0.008

A first RCT comparing the biopsy of one *vs.* two cells

Goossens *et al.* (2008)



Rates of unacceptable misdiagnosis: false-negatives

- Anecdotal cases in published studies
- Reported rates from ESHRE PGD Consortium data collection: 0.16% misdiagnosis (FISH + PCR; 1-cell + 2-cell)
- Limitations in data collection
- Preliminary results from one *vs.* two cell re-analyses:

1-cell	2-cell	Analysis	Ref.
1/12	0/24	FISH	Emiliani *et al.* '04
0/114	0/267	FISH	Michiels *et al.* '06
2/78	5/147	PCR	Dreesen *et al.* '08
0/54	0/57	PCR	Goossens *et al.* '08

So how many cells to biopsy?

It depends on:

- the methodology for genetic analysis
- the disease screened

A further understanding of human embryogenesis awaits…

Are cleavage-stage blastomeres totipotent?
How much pre-patterning is in place?

↕

Which cell or two cells to biopsy?

Is early embryogenesis regulative in human?

↕

How much & how well can an embryo adapt post-biopsy?

Additional questions that remain unanswered

- ❑ What to do with the current evidence on mosaic embryos?
- ❑ Can, and how much can, the embryo 'self-correct'?
- ❑ How 'normal' do embryos have to be?
- ❑ Are any detrimental effects due to the loss of cell(s) *per se* or the biopsy itself?
- ❑ How may changes in lab practices and techniques influence the relative risks and benefits of 1 or 2 cell biopsy?

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ESHRE Annual Meeting 2009
AMSTERSDAM

Pre-congress course 6
Latest developments in preimplantation genetic diagnosis

Optimizing PGD for monogenic diseases : minimal requirements in multiplex PCR and MDA

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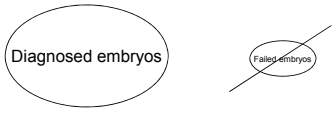
28.06.2009

Objectives

- describe the different approaches in PCR PGD
- explain why multiplex PCR is the standard for PGD for monogenic diseases
- describe the minimum requirements to set up a multiplex protocole
- explain the principle of multiplex PCR and MDA
- give tools to optimize protocols
- describe the advantages and disavantages of both methods
- help to choose the best approach for a specific indication

C.MOUTOU
28.06.2009

Requirements for PGD protocols

- Fast (transfer day 3-5) 
- Sensitive : single cell PCR 
- Powerful : 

- Distinguish affected / unaffected embryos

⇒ Never transfer an affected embryo

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28.06.2009

Once upon a time...

PGD for monogenic diseases Original methods

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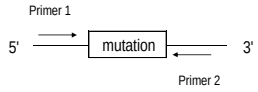
28.06.2009

Once upon a time...

PGD for monogenic diseases Original methods

Simplex PCR for direct diagnosis

1. Flanking PCR (fluorescent PCR = golden standard)



2. Mutation detection

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Once upon a time...

PGD for monogenic diseases Original methods

Simplex PCR for indirect diagnosis

Indications :

- Direct mutation testing not possible
  - Unknown mutation, large insertion/deletion
  - No efficient single cell PCR available : CG-rich regions, uninformative couples for triplet diseases, pseudogenes
- Genes with a wide spectrum of mutations (1 test for several couples)
- Exclusion testing (ie : Huntington's disease)

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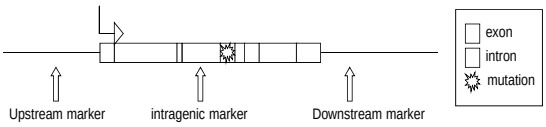
Principle of Indirect diagnosis



- Use of markers close to the mutation
  - intronic or flanking the disease gene
  - Polymorphic : **STRs (microsatellites)** or VNTR (minisatellites) or SNPs (single nucleotide polymorphisms)
- Linkage analysis (2 affected members needed)
- No mutation detection
- Detection of the marker allele linked to the mutation

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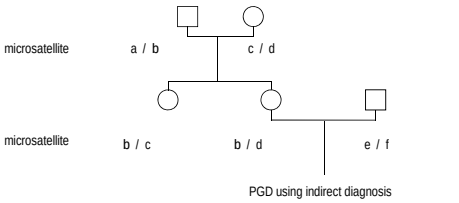
Principle of Indirect diagnosis

Example : autosomal dominant disease



Possible genotypes in embryos	d / e	d / f	b / e	b / f
Embryo status Transferable	Unaffected YES	Unaffected YES	Affected NO	Affected NO

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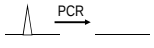
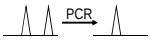
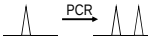
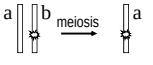
BUT… Pitfalls in single cell PCR

Simplex PCR may lead to misdiagnosis

PCR problem	Observation	Adverse misdiagnosis
Amplification failure (AOF)		autosomal dominant diseases
Allele drop-out (ADO)		autosomal dominant diseases
Contamination		recessive diseases
Recombination during meiosis (indirect diagnosis)		Dominant and recessive diseases

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Conclusion : simplex PCR

- Not safe enough for PGD of monogenic disease
- Misdiagnosis risk :
  - « benign misdiagnosis » (no transfer of unaffected embryos or transfer of carrier embryos thought to be free of the mutation)
  - « adverse misdiagnosis » : transfer of affected embryos thought to be unaffected (TOP or birth of an affected child)

- **Golden standard : Multiplex PCR**
  - **mutation detection and linkage**
  - **or linkage with several markers**

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Multiplex PCR for linkage analysis in PGD

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Multiplex PCR for PDG

Advantages :

- Several tests in the same cell : increased reliability
- 1 universal test for 1 disease (for familial cases)
- Valid for combined indications
- Possible detection of problems :
  - PCR level : contamination, ADO, AOF
  - Embryo/blastomere level : recombination event, blastomere quality

➢ Avoidance of misdiagnosis leading to affected embryo transfer

Disavantages :

- PCR setup more difficult
- Family study needed

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Type of Marker

- SNP : Single nucleotide polymorphisms
- VNTR : variable number of tandem repeats : minisatellites
- STR : Short tandem repeats : microsatellites
  - High heterozygousity rate
  - Abundant and spanning the whole genome
  - Polymorphic in the general population
  - Stable during transmission
  - Short fragments compatible with single cell PCR and multiplexing

⇒ STR : Marker of choice for PGD

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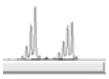
How to choose a Microsatellite

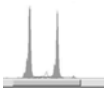
Number of Repetitions : from 2 to 5 nucleotides

⇒ Dinucleotide repeats : (CA)n, (GT)n, (TA)n.
- the most common
- characteristic stutters (training needed for interpretation)



Trinucleotide repeats : (CAG)n (few stutters)

⇒ Tetranucleotide repeats : (GATA)n (no stutters)



Pentanucleotide repeats : (AAAAT)n (rare)

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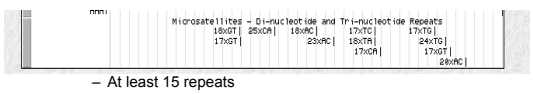
How to choose a Microsatellite

Human genome database :
www.genome.ucsc.edu

Known markers (AFM or others)

- Intragenic ; flanking (proximal AND distal) to reduce the risk of recombination events
- max Het >70% (better if >80%)
- If flanking : close to the gene (<1MB)

– + search repeat :



– At least 15 repeats

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How many markers ?

Depending of the test

Test	Minimum N° of markers (full informative)	Information
Mutation + contamination detection	1, linked or not	detection of contamination only
Mutation + linkage	1 linked (intragenic) 2 flanking if semi-informative	Confirmation of embryo status + Detection of contamination, ADO, recombination event
Linkage	2 flanking the mutation (intragenic or not)	
HLA typing	4-5 within HLA region	

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Family study

Familial case : at least 2 affected (mutation carrier) needed in 2 generations.

De novo mutation :

- Mutation + markers : phase can be deduced during PGD or using single sperm or polar body

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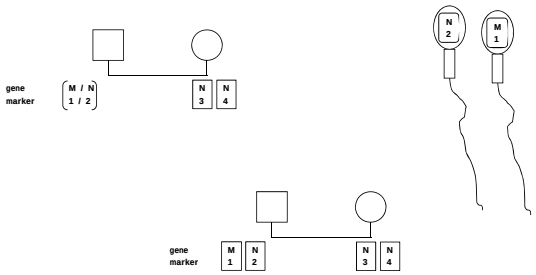
Single sperm amplification



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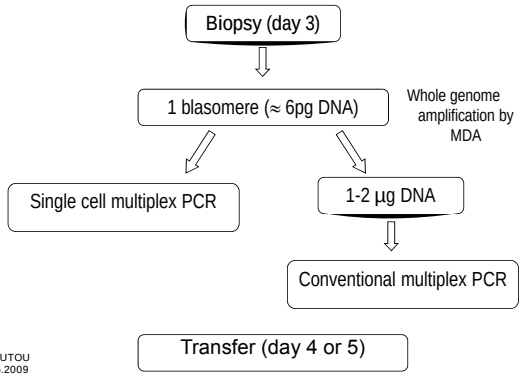
PGD by Multiplex PCR : 2 strategies



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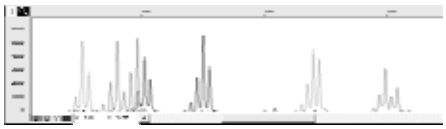
Principle of single cell multiplex PCR

- All primer sets in the PCR mastermix
- Amplification :
  - single round PCR
  - or split after a few cycles to run each part with opimized conditions



HD : D4S1614 + D4S127 + Intron1 + D4S3034 + D4S3038 + D4S412

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Single cell multiplex PCR

Optimization

Preliminary work :
- map the region and find STRs (5-6)
- primer design : similarTm, no stable dimer, same expected annealing T° for PCR
- (informativity testing for the couple)
- heterozygousity estimation for new markers
- marker choice
- PCR set up and validation

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Single cell multiplex PCR

Optimization

– primer : test different ratios for primer set concentration

– mastermix : [MgCl2], modified dNTPs (CG-rich regions), polymerases, activators (glycerol, DMSO)

– try different amplification conditions (step duration, annealing T°)

– or use commercial kits dedicated to multiplex PCR

Validation on heterozygous cells (≥50)

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Single cell multiplex PCR

Warning …

N° of loci ↗ ⇨

ADO rate per locus ↗
Complete genotyping per cell ↘
Contamination by carry-over ↗

- Validation should be done per locus but also per cell.
- Evaluation : haplotypes rather than genotypes
- Lower contamination rate when small series during validation.

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MDA: Multiple displacement Amplification

- For limited DNA sample size : forensic, paleonthology, precious samples, single cell
- Idea :
  - whole genome amplification ⇒ sufficient amount of DNA.
  - conventional molecular testing
- Requirement for PGD
  - high fidelity (not sufficient using DOP-PCR or PEP-PCR)
  - compatible with the timing between biopsy and transfer

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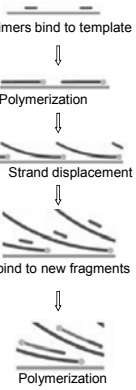
Principle of MDA

Polymérase : Φ29 :
displacemement propriety

Random exonuclease -resistant hexamer
: 5'-NpNpNpNpsNpsN-3'

dNTPs

DNA template (denaturation needed)



Isotheral amplification (No cycling)
Incubation at 30°C (6-18h)
Product : 70kb fragments
uniform amplification
few µg DNA
available for STR, RFLP,
SNP, sequencing, CGH arrays…

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MDA: Multiple displacement Amplification

Optimization

- Single cell optimization :
  - MDA : YES
  - post MDA steps : NO
- cell storing before MDA (freezing / fresh cells)
- lysis buffer / time / temperature
- denaturation
- amplification time : initial protocols 16 hours, PGD protocols 1.5 to 2 hours

- Validation on single cells (>50 cells)

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Single cell multiplex PCR

Warning …Major drawback :

MDA increases ADO and preferential amplification (PA)
!

- ≈ 30% ADO (versus ≈ 10% for multiplex single cell PCR)

⇒ More loci needed for linkage analysis
Evaluation : haplotypes rather than genotypes

⇒ PGH : preimplantation genetic haplotyping

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Single cell Multiplex / MDA

	Single cell Multiplex	MDA
single cell setup	Foreach mutliplex	For MDA only
Time for setup	A few weeks to months	-
Nb of loci	Limited	Unlimited but Higher number needed (ADO)
ADO rate	≈ 10% (5-6 loci)	≈ 30%
PGD Application	Linkage (± mutation) Haplotyping (few loci)	Linkage Haplotyping (HLA)
De novo mutation	Yes (single sperm, polar body)	No

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How to choose a strategy ?

Type of lab

Genetic unit performing DNA test, PND, PGD :
- MDA : avoid to develop new tests

•PGD lab only : if no test for the disease, setup needed
- balance time for setup and number of loci needed

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How to choose a strategy ?

Indication

- Common disease, 1 major mutation
  - multiplex PCR (mutation + linkage)
- Common disease, no major mutation
  - multiplex PCR (linkage) or MDA
- Rare disease : « private disease/mutation »
  - multiplex PCR (mutation + linkage or linkage) or MDA
- •HLA
  - •MDA
- Combined indications
  - MDA
- GC-rich region + linkage
  - MDA ?

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How to choose a strategy ?

Family history

- affected relatives available
  - multiplex PCR (mutation + linkage or linkage) or MDA
- de novo mutation (or no relative available)
  - multiplex PCR (mutation + linkage)

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Conclusion

- Single cell multiplex PCR and MDA followed by multiplex can both be used in PGD.
- Single cell multiplex PCR : mutation detection + linkage
- MDA : more complex indications (HLA, combined indications) + other possibilities than monogenic (aneuploidy, chromosome rearrangements

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- Sermon et al. Preimplantation genetic diagnosis. Lancet. 2004 May 15;363(9421):1633-41.
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Chromosomal abnormalities: development of generic tests

Staessen Catherine

CENTRE FOR MEDICAL GENETICS
CENTRE FOR REPRODUCTIVE MEDICINE

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Learning objectives

- List the basic categories of chromosomal abnormalities
- Describe the various strategies used clinically in PGD for chromosomal abnormalities
- Results obtained with clinical application of PGD for structural chromosomal abnormalities
- Describe research for implementation of micro-array as a generic test for PGD for chromosomal abnormalities

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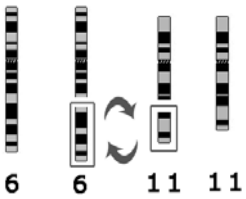
Types of chromosomal abnormalities

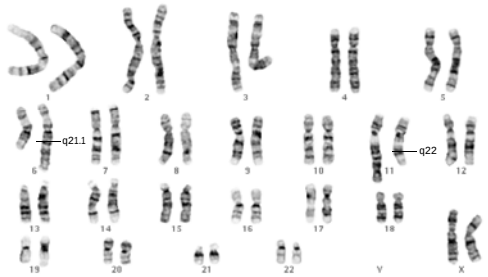
- Numerical abnormalities: aneuploidy
  - One chromosome too many or too few: monosomy - trisomy – tetrasomy
  - One or more sets of chromosomes too many: triploidy - tetraploidy – polyploidy
- Structural abnormalities: balanced or unbalanced
  - Translocations (reciprocal; Robertsonian)
  - Inversions (paracentric; pericentric)
  - Deleties (interstitial; terminal)

Reciprocal translocation

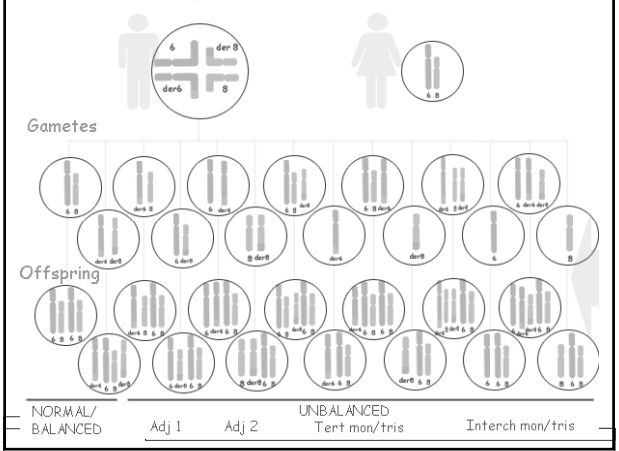
Exchange of chromosomal fragments (= usually an exchange of 2 terminal segments) between 2 non-homologous chromosomes



Reciprocal translocation
46,XX,t(6;11)(q21.1;q22)

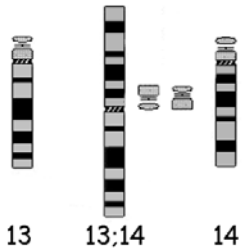


46,XX,t(6;11)(q21.1;q22)

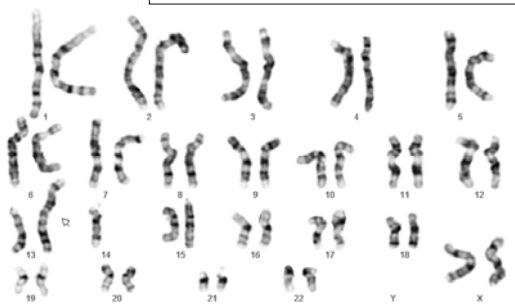


Robertsonian translocation

Centric fusion of 2 acrocentric chromosomes (chromosomes 13, 14, 15, 21, 22)

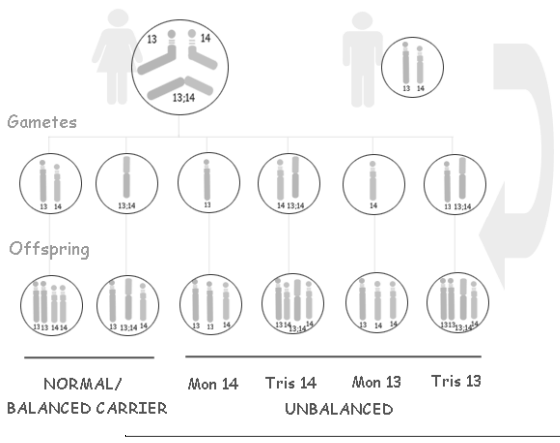


Robertsonian translocation



45,XX,der(13;14)(q10;q10)

Medical genetics AZ-VUB



Indications for prenatal diagnosis for chromosomal abnormalities

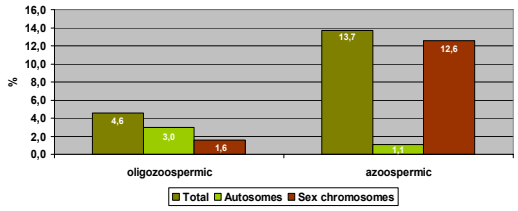
- Presence of balanced structural chromosome abnormality in one of the parents
- Previous child with de novo chromosomal aneuploidy
- Advanced maternal age

For carriers of balanced translocations

PGD

- As an alternative for prenatal diagnosis
- Associated infertility and in need of ART:
  - high incidence of unbalanced embryos
  - limited number of embryos transferred
  - selection of normal/balanced embryos for transfer: a necessity to obtain the highest chance for the delivery of a healthy child

Chromosome abnormalities in oligozoospermic and NOA males



Total of 1701 oligospermic males investigated (5 studies)
Total of 1151 non-obstructive azoospermic males investigated (6 studies)

Van Assche et al., Hum Reprod, Vol 11, suppl 4, 1996

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Approaches to PGD of aneuploidy

- polar bodies
- cleavage stage embryos

Detection of aneuploidy by FISH

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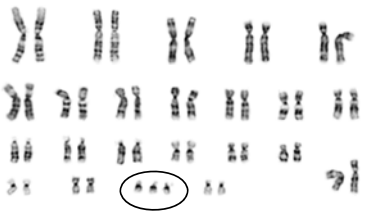
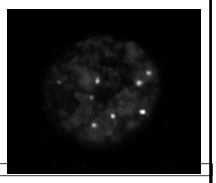
Trisomy 21: 47, XX, +21



Karyotype :47,XX,+21 met G-bandering

Xp11.1-q11.1 Blue
Yp11.1-q11.1 Gold
13q14 Red
18p11.1-q11.1 Aqua
21q22.13-q22.2 Green



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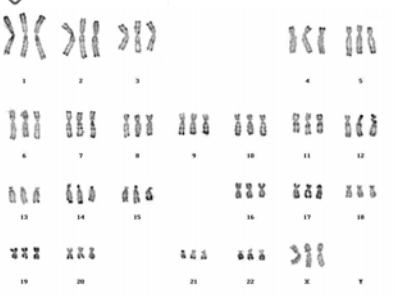
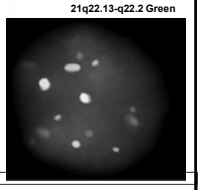


Xp11.1-q11.1 Blue
Yp11.1-q11.1 Gold
13q14 Red
18p11.1-q11.1 Aqua
21q22.13-q22.2 Green

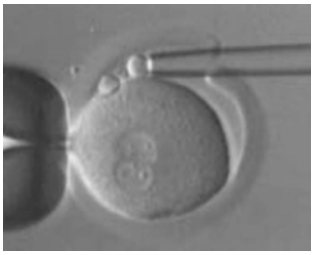


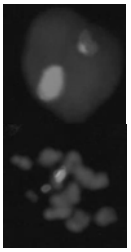
Different approaches to PGD of translocations

- Metaphase analysis from
  - first polar bodies

Metaphase analysis from first polar bodies

Munne S et al., *J Assist Reprod Genet,* 15(5):290-296, 1998





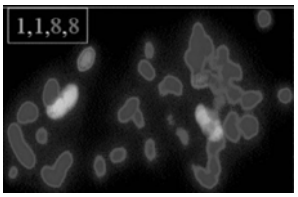
Different approaches to PGD of translocations

- Metaphase analysis from
  - first polar bodies
  - single blastomeres or second polar bodies by oocyte fusion

Metaphase analysis from single blastomeres by oocyte fusion

Day-3 blastomere nuclear conversion and metaphase FISH (fusion with murine or bovine zygotes)

Verlinsky Y et al., *Reprod Biomed Online,* 5(3):300-305, 2002



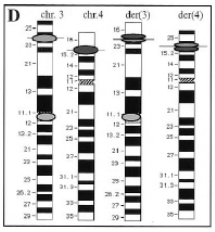
Different approaches to PGD of translocations

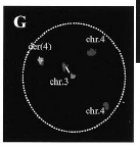
- Metaphase analysis from
  - first polar bodies
  - single blastomeres or second polar bodies by oocyte fusion
- Interphase FISH on blastomeres
  - for reciprocal translocations: breakpoint spanning probes

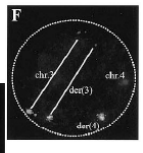
probes which spanned the breakpoints of a translocation

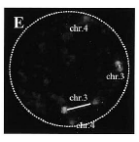
Munné et al., Hum Genet, 102: 663-674, 1998

46,XY,t(3;4)(p24;p15)





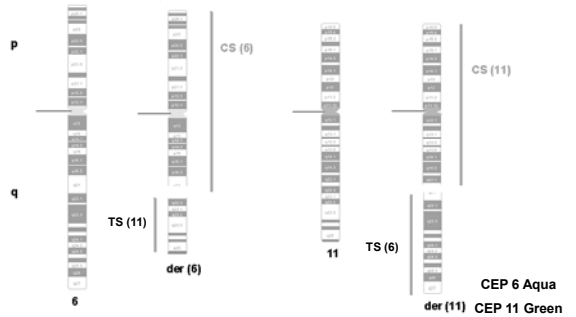


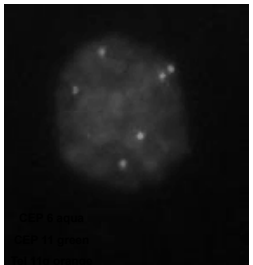


Different approaches to PGD of translocations

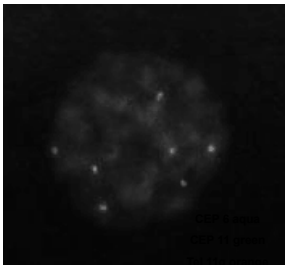
- **Metaphase analysis from**
  - **first polar bodies**
  - **single blastomeres or second polar bodies by oocyte fusion**
- **Interphase FISH on blastomeres**
  - **for reciprocal translocations: breakpoint spanning probes**
  - **for reciprocal translocations combination of centromeric and telomere probes or probes distal to the breakpoints (*Scriven et al., 1998*)**

Reciprocal translocation
46,XX,t(6;11)(q21.1;q22)





Normal/balanced embryo



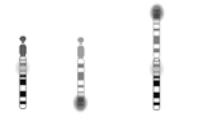
unbalanced embryo

Different approaches to PGD of translocations

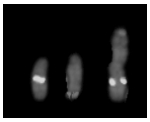
- Metaphase analysis from
  - first polar bodies
  - single blastomeres or second polar bodies by oocyte fusion
- Interphase FISH on blastomeres
  - for reciprocal translocations: breakpoint spanning probes
  - for reciprocal translocations: combination of centromeric and telomere probes or probes distal to the breakpoints
  - for Robertsonian translocations: combination of alpha satellite/locus specific probes

FISH for Robertsonian translocation

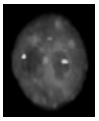
Robertsonian translocation der(13;14)(q10;q10)



normal 13 normal 14 der 13-14

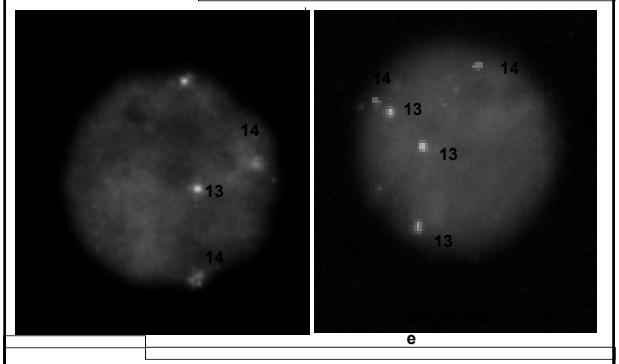


Metaphase chromosomes



blastomere

2-color FISH for der(13;14)



e

UZBrussel experience: PGD for structural abnormalities

- From 2001 → 2007: 558 cycles (with biopsy) performed (285 patients)

  167: Robertsonian translocations (103 patients)
  337: Reciprocal translocations (162 patients)
  14: Pericentric inversion (6 patients)
  3: Paracentric inversion (1 patient)
  18: Deletion: 22q11; del (X); ... (6 patients)
  19: Various: + marker, both parents rec translocation, (7 patients)

UZ-Brussel experience

	Robertsonian	Reciprocal
No. of OPU (patients)	181 (103)	366 (162)
Mean age	33.0 ± 4.8	33.2 ± 4.3
No. of oocytes	13.6 ± 6.9	12.9 ± 5.5
Fertilization (%)	73.7%	65.6 %
Cycles to biopsy	167 (92.3%)	337 (92.1%)

UZ-Brussel experience

	Robertsonian	Reciprocal
Mean n° biopsied	5.5 ± 4.1	5.5 ± 3.7
Number of embryos for biopsy	926	1844
Diagnosed embryos	92.1%	94.3%
% normal embryos	50.4	19.2
Cycles to ET (%)	113 (67.7)	159 (43.4)

Male versus female carriers

	rob ♂	rob ♀	rcp ♂	rcp ♀
Mean N° biopsied	5.3 ± 3.9	6.1 ± 4.6	5.7 ± 3.8	5.3 ± 3.7
% normal embryos	49.3	52.4	19.8	18.6
Cycles to ET	75 (66.4%)	38 (70.4%)	77 (43.1%)	82 (44.2%)
Mean N° transferred	1.7 ± 0.7	1.6 ± 0.6	1.5 ± 0.7	1.3 ± 0.5

UZ-Brussel experience

	Robertsonian	Reciprocal
Mean N° transferred	1.6 ± 0.7	1.4 ± 0.6
No. of transfers	113	159
N° +HCG	57	63
%/OR	34.1%	17.2%
%/ET	50.4%	39.6%
N° +FHB	45 (+1 no info)	44 (+2 no info)
%/OR	26.9%	13.1%
%/ET	38.7%	27.7%

Outcome pregnancies

	Robertsonian	Reciprocal
No. of pos. HCG	57	63
Lost of follow up	1 (1.8%)	2 (3.2%)
Preclin misc	11 (19.3%)	17 (27.0%)
Clinical misc	3 (5.3%)	2 (3.2%)
singleton	1	1
twin	2	1
triplet	0	0
Ongoing/delivered	42 (73.7%)	42 (66.6%)
singleton	25	31
twin	16	11
triplet	1	0

ESHRE PGD consortium data I-VIII: Chromosome abnormalities

- 2712 to oocyte retrieval; 2514 to biopsy
- % infertile (63%)
- Majority cleavage stage aspiration (93%)

- 1788 cycles to embryo transfer (71%)
- 332 clinical pregnancies (16% per OR, 24% per ET)

- No male/female differences.
- Robertsonian higher pregnancy rate than reciprocal

Goossens et al., Hum. Reprod., 2008

ESHRE consortium data Misdiagnoses in PGD with FISH

3 misdiagnosis for translocations on 2514 cycles = 0.12%

Indication	Diagnosis	Outcome
Translocations		
T13 after 45,XY,der(13;14)(q10;q10)	Miscarried	Miscarried
47,XX,+der(22)t(11;22)(q23.3;q11.2)mat	PND	TOP
46,XY,der(15)t(13;15)(q25.1;q26.3)pat	PND	TOP

Wilton et al., 2009

Single-cell Comparative Genomic Hybridization

- Whole genome amplification:
  - degenerate Oligonucleotide Primed Polymerase Chain Reaction (DOP-PCR)
  - multiple displacement amplification (MDA)

- Comparative Genomic Hybridization (CGH)
  - metaphase CGH
  - array CGH

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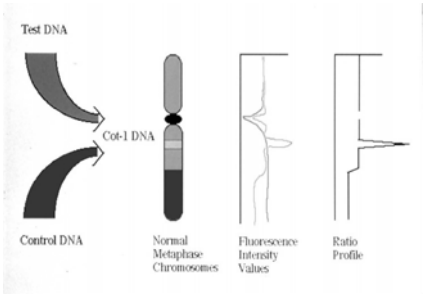
Metaphase-CGH: principle



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Wells et al., Mol. Hum. Reprod., 6, 1055-1062, 2000

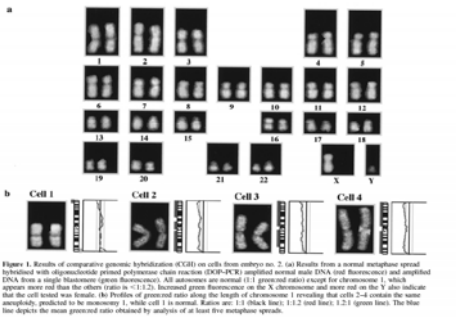

Figure 1. Results of comparative genomic hybridization (CGH) on cells from embryo no. 2. (a) Results from a normal metaphase spread hybridized with oligonucleotide primed polymerase chain reaction (DOP-PCR) amplified normal male DNA (red fluorescence) and amplified DNA from a single blastomere (green fluorescence). All autosomes are normal (1:1 green:red ratio) except for chromosome 1, which appears more red than the others (ratio is <1:1.2). Increased green fluorescence on the X chromosome and more red on the Y also indicate that the cell tested was female. (b) Profiles of green:red ratio along the length of chromosome 1 revealing that cells 2–4 contain the same aneuploidy, predicted to be monosomy 1, while cell 1 is normal. Ratios are: 1:1 (black line); 1:1.2 (red line); 1.2:1 (green line). The blue line depicts the mean green:red ratio obtained by analysis of at least five metaphase spreads.

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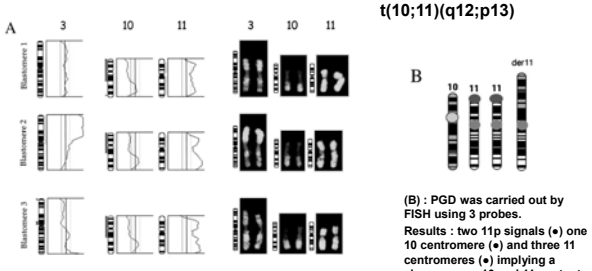
Malmgren et al., Mol Hum Reprod, 2002

Individual blastomeres from translocation carriers: analysed by CGH

- **single cell CGH analysis reveals a high degree of mosaicism in human embryos from translocation carriers**
- **found a resolution limit of 10-20Mb for CGH**
- **small deletions or amplifications of the telomeric regions were difficult to interpret**
- **all protocols have a time requirement that is impossible to fit into the PGD situation with day 4-5 transfer**

Malmgren et al., Mol Hum Reprod, 2002

t(10;11)(q12;p13)

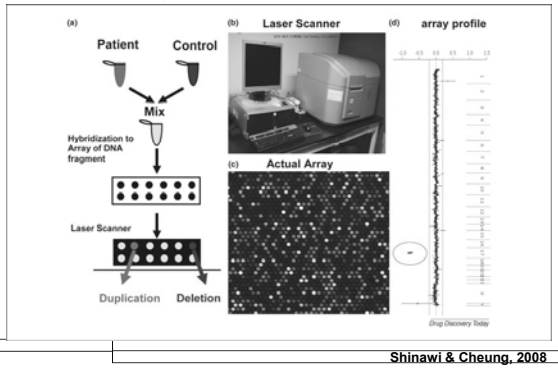


(A) : Single cell CGH and revealed an excess of almost one entire chromosome11 complement and lack of 10p-10q12 in all 3 blastomeres. In addition extra chromosome 3p material was detected in blastomere 2

(B) : PGD was carried out by FISH using 3 probes.

Results : two 11p signals (•) one 10 centromere (•) and three 11 centromeres (•) implying a chromosome 10 and 11 content in agreement with the CGH results

array-CGH: principle



Shinawi & Cheung, 2008

Different array-CGH platforms

- array CGH using chromosome libraries
- array CGH using bacterial artificial chromosome (BAC) probes
- array CGH using oligonucleotide probes
- array CGH using single nucleotide polymorphism (SNP)

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array CGH using chromosome libraries

- Arrays utilizing DOP-PCR products from chromosome-specific DNA libraries, depleted of repetitive sequences, as probes
- Hu DG et al. (2004): detecting of chromosomal copy-number variation from single lymphoblasts and fibroblasts
- No robust results were obtained since incorrect ratios were sometimes observed for chromosomes 2, 4, 9, 11, 17, 22, X and Y.
- Disadvantage: inability to detect deletions and duplications
- Advantage: it takes only 30 h to perform

Hu et al., Mol. Hum. Reprod., 2004

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array CGH using BAC probes

- Arrays consisting of a thousand of individual probes (BAC clones)
- Le Caignec et al., 2006:
  Method accurately detects chromosomal imbalances from a single lymphoblast, fibroblast and blastomere within a single day following MDA
- Demonstrate the accurate detection of the del(4q) and the unbalanced translocation t(X;14) with deletion sizes of 34 and 58 Mb, respectively and a duplication of 47Mb in the fibroblasts.

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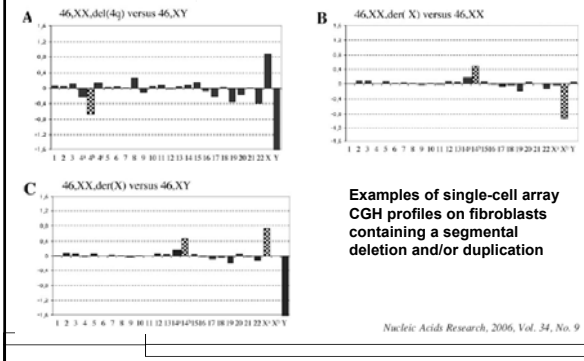
Nucleic Acids Research, 2006, Vol. 34, No. 9 e68
doi:10.1093/nar/gkl336

Single-cell chromosomal imbalances detection by array CGH

Cedric Le Caignec[1,2], Claudia Spits[2], Karen Sermon[2], Martine De Rycke[2], Bernard Thienpont[1], Sophie Debrock[4], Catherine Staessen[2], Yves Moreau[3], Jean-Pierre Fryns[1], Andre Van Steirteghem[2], Inge Liebaers[2] and Joris R. Vermeesch[1,*]

[1]Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium, [2]Research Centre Reproduction and Genetics, University Hospital and Medical School, Vrije Universiteit Brussel, Brussels, Belgium, [3]ESAT-SISTA, K.U. Leuven, Leuven, Belgium and [4]Leuven University Fertility Center, University Hospital Gasthuisberg, Leuven, Belgium

Le caignec et al., 2006

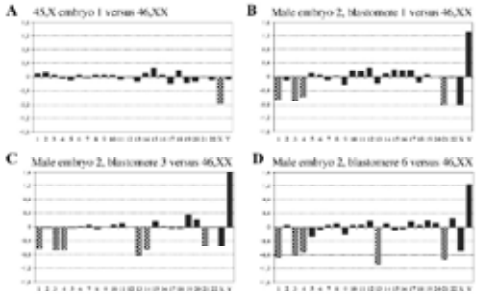


Examples of single-cell array CGH profiles on fibroblasts containing a segmental deletion and/or duplication

Nucleic Acids Research, 2006, Vol. 34, No. 9 **e68**

Le caignec et al., 2006

Examples of single-cell array CGH profiles on blastomeres



Nucleic Acids Research, 2006, Vol. 34, No. 9 **e68**

array CGH using oligonucleotide probes

- **Microarray platform utilizing oligonucleotides, which are synthesized in situ.**
- **The probes typically vary from 25-85 nucleotides in length (Agilent 60 nucleotides in length**
- **Agilent has been used clinically, for the purpose of PGS (Hellani et al., 2008)**
- **Agilent platform is compatible with single-cell array-CGH used in conjunction with different WGA techniques**

Hellani et al., RBM online, 2008

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RBMOnline
Vol 17 No 6. 2008 841-847

- **Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening**
  Dr Ali Hellani **- Laboratory, Saad Specialist Hospital - Kingdom of Saudi Arabia**
- **Preimplantation genetic screening (PGS) using multiple displacement amplifications (MDA) and array comparative genomic hybridization (aCGH) was successfully performed on eight patients with a minimum of seven recurrent IVF failures with the aim of detecting aneuploidy and ameliorating pregnancy rate.**

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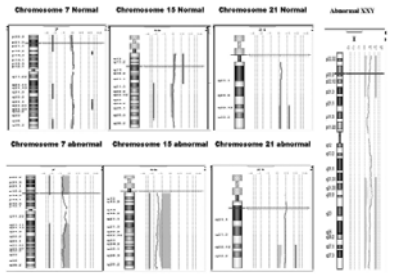

Figure 1. Result of a set of representative chromosomes with normal and abnormal aCGH status. Abnormality in the sex chromosomes is also shown where the embryo was diagnosed as male by amelogenin PCR and the microarray showed XX profile when hybridized with XY DNA.

Hellani et al., RBM online, 2008

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Array CGH using single nucleotide polymorphism

- **An oligonucleotide array based upon the analysis of single nucleotide polymorphisms (SNPs): common polymorphic DNA sequences throughout the genome.**
- **The amplified material from the test sample is hybridized separately, with reference DNA samples assessed in parallel.**
- **A significant advantage of SNP micro-arrays is that the probes used provide genotype data in addition to chromosome copy number information. The simultaneous analysis of thousands of polymorphisms scattered throughout the genome produces a unique DNA fingerprint for each embryo tested.**

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Conclusions

- **Array CGH on single blastomeres:**
  - determination of the copy number of all chromosomes
  - enabled the detection of imbalance of chromosomal segments, providing a universal platform, whereas different FISH probes have to be optimized for each specific translocation
- **Array CGH requires further development and investigation before general clinical application can be considered: still clinical research**
- **Array CGH: still too expensive for clinical application**

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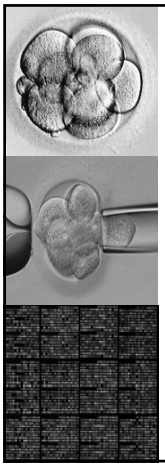

Studying all chromosomes: is more better?

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Belgium

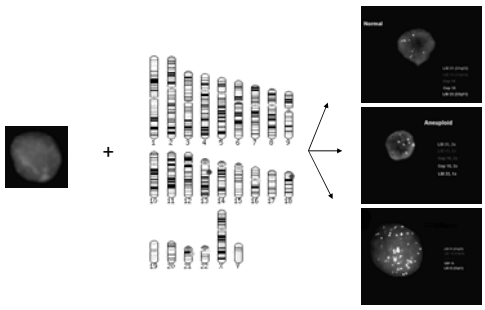
PCC ESHRE, Amsterdam, 28-6-2009

Overview

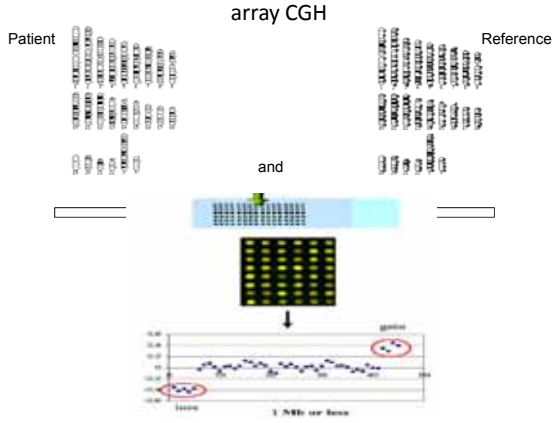
Studying all chromosomes: is more better ?

- Introduction
  - From FISH to arrays
  - Amplifications of single cells
  - Basic analysis of single cell array results
- Copy number variation detection in single cells
  - Advanced analysis of single cell array results
    - BAC array
    - SNP array
- Clinical implementation
  - Array screening results of GQE from young fertile patients
- Biological implications
  - Consequences of array technique for PGD and PGS

Fluorescent *In Situ* Hybridisation (FISH)



array CGH



Sanlaville *et al.*, 2005

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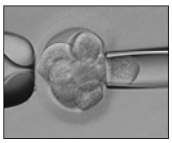
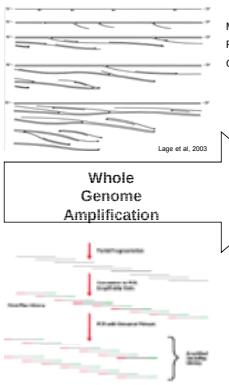
Whole Genome Amplification (WGA): current methods



Single blastomere

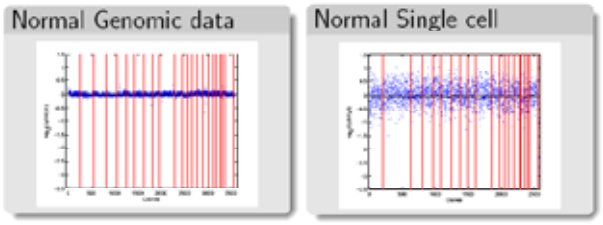
7 pg DNA



Spits *et al.*, 2006

Fiegler *et al.*, 2007

Micro array data of a single cell



Stochastic and systematic scatter

Overview

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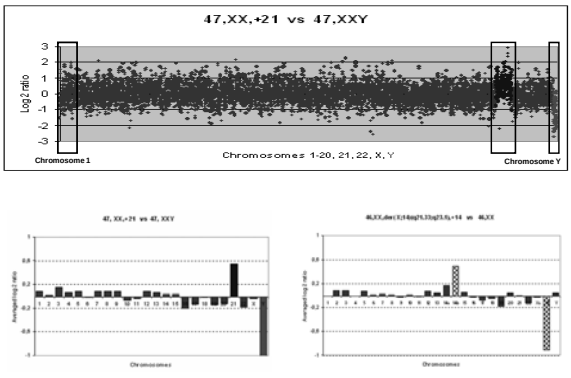
Single cell aneuploidy detection by array CGH



Le Caignec *et al.*, 2006 and Fiegler *et al.*, 2007

Conclusion introduction

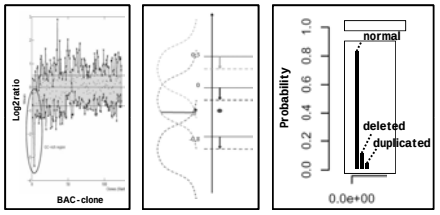
- Array > FISH
  - Accuracy
- Array of single cells : amplification needed
- Advantages single cell array :
  - Screen complete genome at once
  - Detection of whole chromosome aberrations
  - Detection of *a priori* known partial chromosome imbalances
- Disadvantages single cell array :
  - Threshold ?
  - Non *a priori* known aberrations ?
- *Replace FISH by array CGH for PGD and PGS ??*

Overview

Studying all chromosomes: is more better ?

- Introduction
  - From FISH to arrays
  - Amplifications of single cells
  - Basic analysis of single cell array results
- Copy number variation detection in single cells
  - Advanced analysis of single cell array results
    - BAC array
    - SNP array
- Clinical implementation
  - Array screening results of GQE from young fertile patients
- Biological implications
  - Consequences of array technique for PGD and PGS

A novel statistical method that calculates likelyhood estimates on imbalances detected with SC BAC-arrayCGH

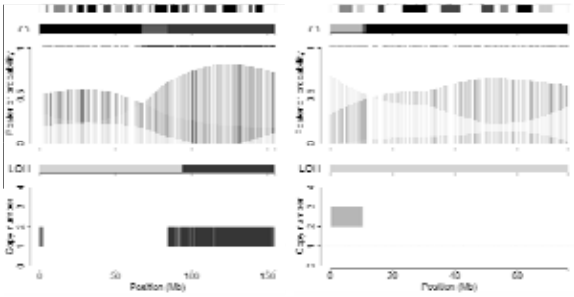


Clone specific variability | Clone specific correction | Clone specific likelyhood estimate

Ampe *et al.*, 2009

Validation using single-cells with known imbalances

Xq terminal deletion (58 Mb) | 18p terminal duplication (9.3 Mb)

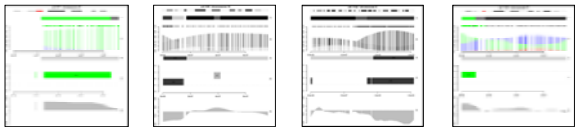


Vanneste *et al.*, 2009

Validation using single-cells with known imbalances

- 4 single cells with known imbalances

Trisomy 21 | 14 Mb 18p deletion | Unbalanced translocation 58 Mb Xq deletion | Unbalanced transloaction 9.3 Mb 18p duplication

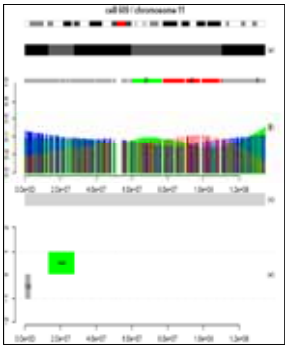


Conclusion : Reliable detection if combination of both techniques

(no combination SNP : 7 extra duplications, 1 extra deletion; BAC 1 extra deletion)

Vanneste *et al.*, 2009

Genome-wide equal probabilities for all copy numbers states



- Origin :High SD between intensity ratios of consecutive BAC-clones ?
- Technical or biological ?
- Hypothesis : relation with cell cycle
  - S-phase : more scatter
  - M-phase : chromosomes highly condensed
- Consequence:

  cell type specific reference set

Vanneste *et al.*, 2009

Conclusion CNV detection in single cells

- Combination of BAC and SNP : reliable detection of aberrations
  - Advantages of BAC-model
    - Correction for systematic biases
    - Posterior probabilities for deletions, duplications and diploidy
    - Quality of SC array data (equal probabilities = not informative)
  - Advantages of SNP-model
    - CN + LOH
    - Detection of nullisomy and amplifications
    - Detection of uniparental disomy
- High 'drop out' due to high SD
- Cell type specific

Overview

Studying all chromosomes: is more better ?

- Introduction
  - From FISH to arrays
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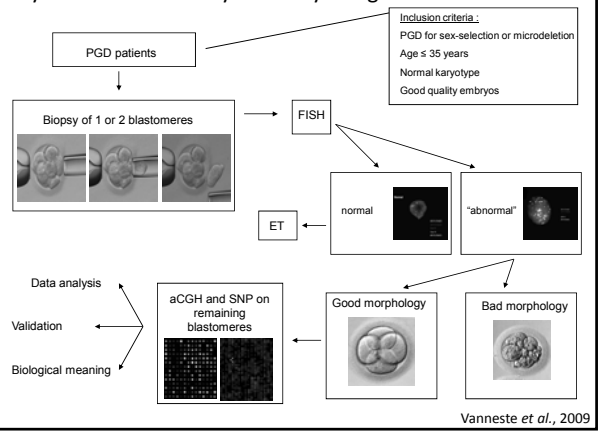
  - Consequences of array technique for PGD and PGS

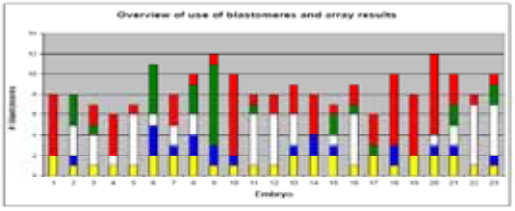
Analysis of human embryos : study design



Vanneste *et al.*, 2009

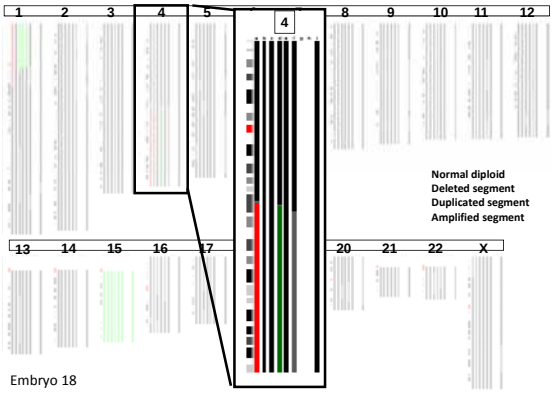
The majority of human cleavage stage embryos contain chromsomally imbalanced blastomeres

- 2/23 (9%) : normal diploid in all cells
- 1/23 (4%) : diploid, but UPID
- 8/23 (35%) : mosaic diploid/aneuploid (5 embryos : ratio diploid/aneuploid > 1)
- 12/23 (52%) : mosaic aneuploid (3 embryos : meiotic (same aberration in all cells)



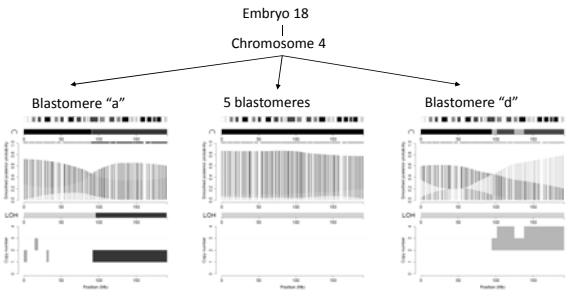
Vanneste *et al.*, 2009

Overview of the chromosomal status of all blastomeres of embryo 18



Embryo 18

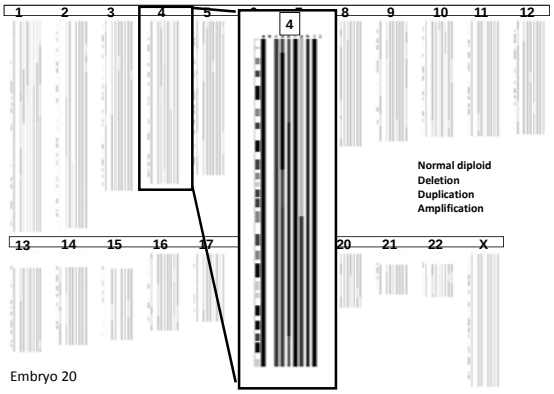
Simple terminal imbalances are terminal deletions, duplications or amplifications



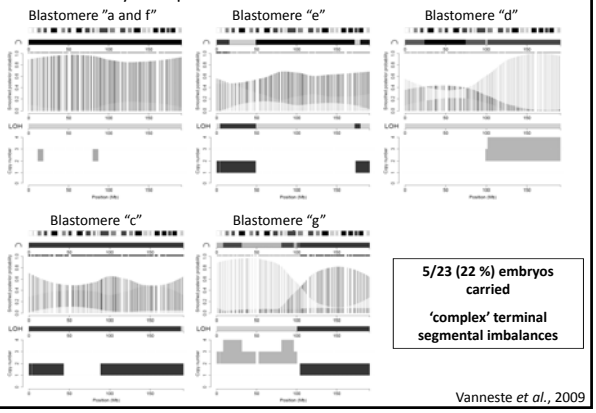
12/23 (52%) carried 'simple' terminal imbalances

Vanneste *et al.*, 2009

Overview of the chromosomal status of all blastomeres of embryo 20



Complex terminal imbalances are terminal imbalances accompanied by aneuploidies for the same chromosome



5/23 (22 %) embryos carried

'complex' terminal segmental imbalances

Vanneste *et al.*, 2009

Conclusion clinical implementation

Chromosomal aberrations (> 9 Mb) can be accurately detected in single cells by microarrays

Questions to solve before clinical implementation :

Combination of array results in practice ('no golden standard')

Duration of protocols

Resolution

Quality criteria

Overview

Studying all chromosomes: is more better ?

- Introduction
  - From FISH to arrays
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Studying all chromosomes: is more better?

CHROMOSOME INSTABILITY = common to human IVF embryogenesis

91% of early human IVF embryos are chromosomally abnormal

- FISH not appropriate to detect segmental aberrations
- Not confined to aneuploidy risk couples
- IVF/ICSI success rate > normal diploidy rate
- Self correction and/or self selection mechanisms

↓

Explanation for low human fecundity

↓

Biological basis for failure of preimplantation genetic screening (PGS) in improving baby take home rates

↙ ↘

NO, it isn't !!!
Not in preimplantation context

YES, is it
Better insight in early embryogenesis

Vanneste *et al.*, 2009

Near Future

- Arrays for PGD
  - Translocations
    - Which chromosomes?
    - De novo events (Kasakyan *et al.*, 2008)
- More studies using high resolution arrays : confirmation of results
  - Percentage of normal diploid embryos
  - Segmental aberrations
  - UPD
- Segmental aberrations
  - Origin

Acknowlegdements…

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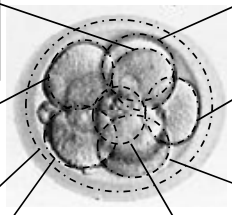
All participating patients

De Duve Institute
UCL
Miikka Vikkula
Mustapha Amyere

Leuven University Fertility Center
K.U.Leuven
Thomas D'Hooghe
Sophie Debrock



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Preimplantation Genetic Screening
the final settlement

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Academic Medical Center
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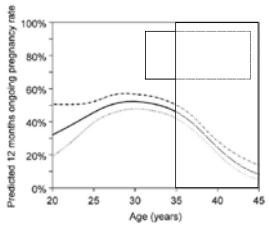
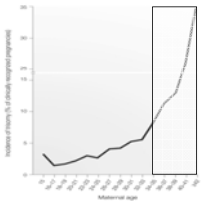
Amsterdam, The Netherlands

Universiteit van Amsterdam

am center for reproductive medicine

Rationale PGS





Lintsen, et al., Hum.Rep. 2007, Hassold & Hunt, Nat.Rev.Gen. 2001

am center for reproductive medicine

Promising results

Preimplantation genetic diagnosis increases the implantation rate in human in vitro fertilization by avoiding the transfer of chromosomally abnormal embryos

Luca Gianaroli, M.D. Agnese Fiorentino, B.Sc.
M. Cristina Magli, M.Sc. John Garrisi, Ph.D.
Anna Pia Ferraretti, Ph.D. Santiago Munné, Ph.D.

Preimplantation genetic diagnosis significantly reduces pregnancy loss in infertile couples: a multicenter study

Santiago Munné, Ph.D.,* Jill Fischer, M.Sc.,* Alison Warner, M.Sc.,* Serena Chen, M.D.,* Christo Zouves, M.D.,* Jacques Cohen, Ph.D.,* and the Referring Centers PGD Group

Fertility and Sterility® Vol. 85, No. 2, February 2006

Improved implantation after preimplantation genetic diagnosis of aneuploidy

Authors: Santiago Munné[1]; Mireia Sandalinas[2]; Tomas Escudero[2]; Esther Velilla[2]; Renee Walmsley[2]; Sasha Sadowy[1]; Jacques Cohen[1]; David Sable[1]
Source: Reproductive BioMedicine Online, Volume 7, Number 1, July 2003, pp. 91-97(7)

Human Reproduction vol.14 no.8 pp.2191–2199, 1999

OUTSTANDING CONTRIBUTION

Positive outcome after preimplantation diagnosis of aneuploidy in human embryos*

Santiago Munné[1,4], Cristina Magli[1], Jacques Cohen[1], Paula Morton[1], Sasha Sadowy[1], Luca Gianaroli[2], Michael Tucker[3], Carmen Márquez[1], David Sable[1], Anna Pia Ferraretti[2], Joe B.Massey[3] and Richard Scott[1]

Human Reproduction vol.15 no.9 pp.2003–2007, 2000

Healthy births and ongoing pregnancies obtained by preimplantation genetic diagnosis in patients with advanced maternal age and recurrent implantation failure

S.Kahraman[1,4], M.Bahçe[2], H. Samli[2], N.İmirzalioğlu[2], K.Yakın[1], G.Cengiz[2] and E.Dönmez[2]

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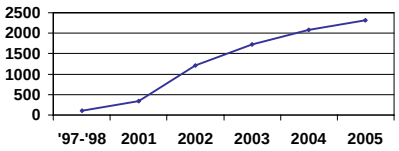
Use of PGS

PGS - all indications[1]



USA survey[2]: 127/186 respondents (68%) reported to have performed a total of 2197 IVF cycles with PGS in 2005

1 – Harper, et al., Hum.Rep. 2008
2 – Baruch, et al., Fertil.Steril. 2008

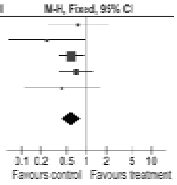
What is the evidence?

PGS RCTs – Advanced Maternal Age

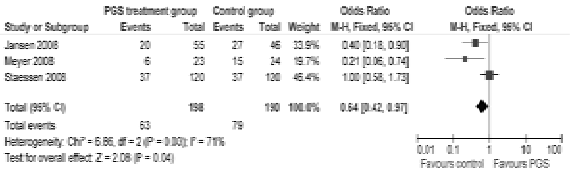
ongoing pregnancy

Study or Subgroup	PGS Events	PGS Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Debrock 2007	7	46	10	51	7.5%	0.74 [0.25, 2.13]
Henderson 2008	3	56	10	53	9.1%	0.24 [0.06, 0.94]
Mastenbroek 2007	52	206	74	202	52.4%	0.58 [0.36, 0.89]
Staessen 2004	22	199	29	150	24.7%	0.59 [0.36, 1.25]
Stevens 2004	11	21	13	18	6.2%	0.42 [0.11, 1.62]
Total (95% CI)		528		514	100.0%	0.58 [0.43, 0.79]
Total events	95		136			

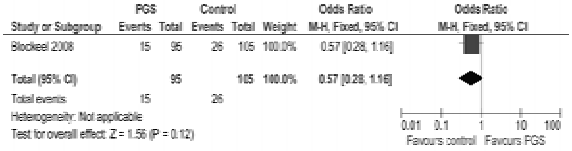
Heterogeneity: Chi² = 2.32, df = 4 (P = 0.68); I² = 0%
Test for overall effect: Z = 3.45 (P = 0.0006)



PGS RCTs – Good Prognosis Patients
live birth



PGS RCTs – Repeated Implantation Failure
ongoing pregnancy



PGS – Unexplained Recurrent Miscarriage

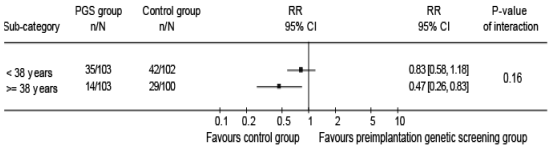
Systematic review:

- No RCTs
- No comparative studies
- Comparison of cohorts:

Outcome	PGS N = 181 couples 223 ET cycles	Sp conception N = 165 couples 6-12 months
Livebirth rate per couple % (range)	35% (19 – 46)	44% (11 – 63)
Miscarriage rate per couple % (range)	9% (0-10)	24% (22 – 32)

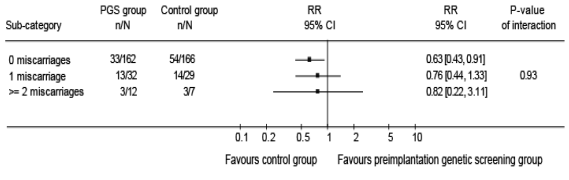
Musters et al - submitted

Subgroup female age



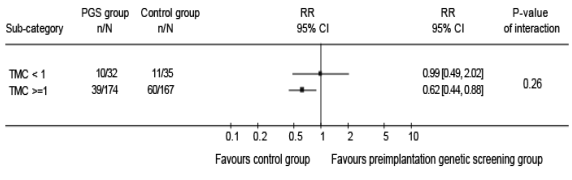
Twisk et al Hum Rep 2008 – PGS for advanced maternal age

Subgroup miscarriage



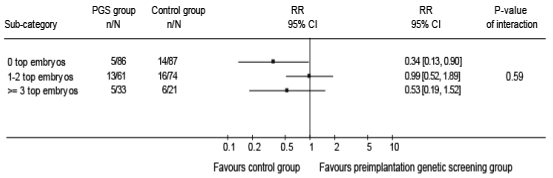
Twisk et al Hum Rep 2008 – PGS for advanced maternal age

Subgroup sperm quality



Twisk et al Hum Rep 2008 – PGS for advanced maternal age

Subgroup embryo quality



Twisk et al Hum Rep 2008 – PGS for advanced maternal age

Recommendations

Preimplantation genetic testing: a Practice Committee opinion

The Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

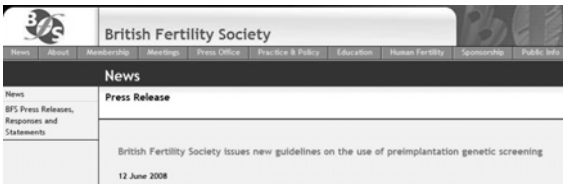
RECOMMENDATIONS: PGS

- Before PGS is performed, thorough education and counseling must be provided to ensure that patients fully understand the limitations of the technique, the risk of error, and the lack of evidence that PGS improves live-birth rates.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with advanced maternal age.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with previous implantation failure.
- Because the prevalence of aneuploidy is high in the embryos of patients with recurrent implantation failure, decisions concerning future treatment should not be based on the results of PGS in one or more cycles.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with recurrent pregnancy loss.
- Available evidence does not support the use of PGS as currently performed to reduce miscarriage rates in patients with recurrent pregnancy loss related to aneuploidy.

"Available evidence does not support the use of PGS as currently performed…"

SART ASRM Fertil Steril 2007

Recommendations



• Current evidence indicates that PGS does not improve pregnancy rates or decrease miscarriage rates for women over the age of 35. Clinical trials carried out to date show that live birth rates may be significantly reduced following PGS treatment.

• The BFS advises that PGS should preferably only be offered to patients within the context of a robustly designed clinical trial

www.fertility.org.uk

Recommendations

ACOG COMMITTEE OPINION

Number 430 • March 2009

Preimplantation Genetic Screening for Aneuploidy

Committee on Genetics

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

ABSTRACT: Preimplantation genetic screening differs from preimplantation genetic diagnosis for single gene disorders and was introduced for the detection of chromosomal aneuploidy. Current data does not support a recommendation for preimplantation genetic screening for aneuploidy using fluorescence in situ hybridization solely because of maternal age. Also, preimplantation genetic screening for aneuploidy does not improve in vitro fertilization success rates and may be detrimental. At this time there are no data to support preimplantation genetic screening for recurrent unexplained miscarriage and recurrent implantation failures; its use for these indications should be restricted to research studies with appropriate informed consent.

Why doesn't it work?

Technical aspects
failure rate

	AMC/UMCG[1]	VUB[2]	ESHRE PGD consortium[3]	Saint Barnabas[4]
biopsy ***(embryos)***	**2.8%**	**5.8%**	**1.3%**	
fixation ***(blastomeres)***	**10.9%**	**6.4%**	**12.5%**	
FISH ***(blastomeres)***	**6.7%**	**7.1%**		
Total unknown ***(embryos)***	**20.1%**	**2.4%***	**13.7%**	**4.4%****

* 2-cell biopsy
** Transferpolicy: ET of abnormal embryos allowed, but excluded from analysis. No definition of abnormal.

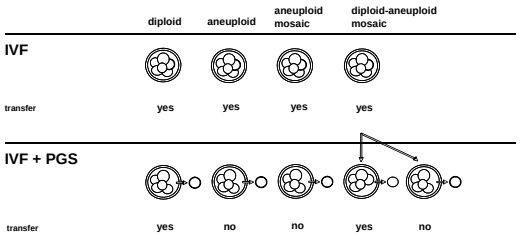
1 – Mastenbroek, et al., NEJM (2007), 2 – Michiels, et al., Hum.Rep. (2006)
3 – Sermon, et al., Hum.Rep. (2007), 4 – Munne, et al., RBMO (2003)

Technical aspects

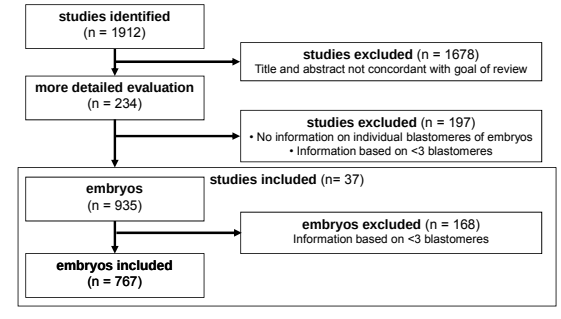
- Biopsy possibly more harmful than previously thought
  - the effect of biopsy alone on pregnancy rates has never been studied[1]
- FISH is not 100% accurate
  - Estimated accuracy in lymphocytes 92-99%
  - Not determined for PGS probe set on human blastomeres
  - Estimated FISH error rate
    - 0.92^8 → 49% error
    - 0.94^8 → 39% error
    - 0.96^8 → 28% error
    - 0.98^8 → 15% error

1 – De vos and Van Steirteghem, 2001

Mosaicism
theoretical implications for PGS



Mosaicism - prevalence



Van Echten, et al., submitted

Mosaicism - prevalence

Chromosomal constitution	Van Echten (n=767) *all*		Van Echten (n=260) *cleavage stage >5 chromosomes*		Mastenbroek (n=360)	
Diploid	168	22%	39	15%	29	8%
Aneuploid	36	5%	36	5%	11	3%
haploid	2	0%	0	0%	1	0%
polyploid	4	0%	1	0%	0	0%
aneuploid	17	2%	10	4%	6	2%
complex abnormal	13	2%	11	4%	4	1%
Mosaic	563	73%	199	76%	320	89%
aneuploid-mosaic	108	14%	47	18%	122	34%
diploid-aneuploid mosaic *diploid blastomeres*	455	59% *72%*	152	58% *61%*		55% *46%*

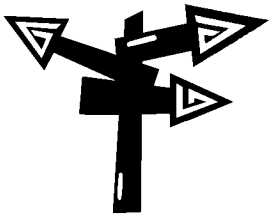
Van Echten, et al., submitted
Mastenbroek, et al., submitted

Mosaicism
theoretical implications for PGS

	diploid	aneuploid	aneuploid mosaic	diploid-aneuploid mosaic		total cohort
IVF						
percentage of all embryos	8	3	34	55		
percentage of diploid blastomeres	100	0	0	46		
transfer	yes	yes	yes	yes		
theoretical implantation potential	800	0	0	2530		3330
IVF + PGS						
percentage of all embryos	8	3	34	25	30	
percentage of diploid blastomeres	100	0	0	35	55	
transfer	yes	no	no	yes	no	
theoretical implantation potential	800	-	-	875	-	1675

- 49%

Alternatives



New or improved forms of PGS

- Developmental stage at which biopsy is performed
  - polar body
  - blastocyst
- Methods of analysis[1]
  - CGH arrays
  - SNP arrays
- First experiences
- Development stage or pilot-study stage
  - too premature for routine clinical application

1 - Wells Mol Hum Reprod 2008

New or improved forms of PGS

ESHRE PGS Task Force

- No need for further RCT using cleavage stage biopsy and FISH
- Polar-body biopsy and 24-chromosome analysis in theory best alternative
- Stepwise approach
  1. Preclinical study / method assessment
  2. Pilot study
  3. Randomised clinical trial

  → currently phase1

Geraedts Focus on Reproduction January 2009

Non-invasive selection

- ≠ Preimplantation Genetic Screening
- Examples[1]
  - metabolomic profiling
    - spectrophotometric analysis of metabolomic changes in culture medium
  - amino acid profiling
    - assessment of amino acid depletion and production by embryo in culture medium
  - respiration-rate measurement
    - respiration-rate of embryos is assessed
  - birefringence imaging
    - polarization light microscopy is used to assess meiotic spindle or zona pellucida
- Development stage or pilot-study stage
  - too premature for routine clinical application

1 -Reprod Biomed Online 2008; 17(4):451-507

Summary

- All clinical evidence shows that PGS does not work
  - Harmful
- Reason for inefficacy
  - Technical aspects
    - Failure of biopsy / fixation / FISH
    - Biopsy harmful
    - FISH not 100% accurate
  - Mosaicism
    - Half of all embryos are diploid-aneuploid mosaic
      - Half of all blastomeres are diploid
    - Discarding of potentially viable embryos

Future

- Further development and evaluation of new or improved forms of PGS
  - Polar body biopsy, blastocyst biopsy
  - CGH arrays, SNP arrays
- Alternative selection methods (non-invasive)
- Preimplantation embryology
  - Origin of diploid-aneuploid mosaic embryos
    - Physiological? Pathological?
    - Induced through hyperstimulation and/or in vitro culture?
  - Fate of diploid-aneuploid mosaic embryos
    - Rescue mechanisms?

PGS: the final settlement?

- PGS with the use of current techniques
  - cleavage stage biopsy and FISH
- Routine clinical application not justified

PGS: the final settlement?

- New or improved forms of PGS
  - Polar body biopsy, blastocyst biopsy
  - CGH arrays, SNP arrays, etc
- Method assessment, pilot studies and subsequent RCTs needed
- We should learn from the past
  - No routine clinical application before efficacy is proven
  - Harm of biopsy, accuracy of analysis, mosaicism

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ESHRE Pre course congress
Latest developments in preimplantation genetic diagnosis
Long-term children follow up

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Objectives

- To be aware of the data available to date and convey this to couples requesting treatment for PGD.
- To be aware of on going studies and the importance of these.
- To understand the difficulties inherent in long term follow up

Recommendations for paediatric follow up

- PGDIS- guidelines for good practice in PGD *(PGDIS- 2008)*
- ESHRE- best practice guidelines for clinical PGD *(Thornhill 2005)*
- Human Genetics Commission- UK (*Making babies 2006)*
- Huge variation in practise across the EU- impact on follow up *(Lawford Davies 2007)*
- In 1996 Simpson and Leibaers recommended a standardized approach to collating data at birth on PGD babies

Paediatric outcome after PGD

Reports on outcome at birth:

- Case controlled study 2007 (Brussels) 563 babies- increased NND and SB rate, but major abnormality 2.9% less than ICSI *(personal communication 2008)*
- ESHRE *(Goossens 2009)*-indicated abnormality rate at birth 3.99%
- 109 babies from polar body biopsy- no increase in abnormality rate *(Strom 2000)*
- 24 babies over 5 years PGD for CF- no increase in major abnormalities *(Keymolen 2007)*

Paediatric outcome after PGD

Long term follow up:

- Little long term data on PGD babies
  - 3 papers have reported longer term case controlled outcomes
- Mental & psychomotor development at 2 yrs- 70 children *(Nekkebroeck 2008)*
- Auxological & medical follow up at 2 yrs- 70 children (Desmyttere 2009)
- General health at 2 yrs- 49 children *(Banerjee 2008)*

All report developmental parameters same as control groups

What are the abnormalities?

Malformations in context- back ground risks

Major malformation

"major structural anomalies have medical and social consequences"

(Stevenson & Hall- Human malformations & related anomalies)

- 2-3% at birth
- 5% at 5 years

Minor malformations

"minor anomalies are relatively frequent structural alterations that pose no significant health or social burden"

(occur in <4% of population)

- 15% at birth

Range of major abnormalities

(ESHRE 2009)

- Aorta coarction
- Absence of corpus callosum
- Absence of corpus callosum, haemivertebra
- Absence of corpus callosum, kidney dilatation, growth
- Retardation
- Absence of ductus venosus
- Bilateral clubfoot
- Cataract
- Choanal artresia
- Cleft lip unilateral
- Cleft lip and palate
- Congenital hip luxation
- Congenital ichtyosiform erythrodermia
- Fryns syndrome, neonatal death
- Hemangioma
- Hydrocephaly
- Large cavernous haemangioma
- Laryngomalacia, receding chin, strawberry naevus
- Left ventricular hypoplasy syndrome, Aorta stenosis
- Pes equinovarus
- Phocomelia and pulmonary deficiency
- Right ear- external meatus obstruction
- Sacrale dimple without intestinal connections
- Pulmonary stenosis
- Tachycardia of Courmel
- Tetralogy of Fallot
- Unilateral cryptorchidia
- Unilateral intrauterine torsio testis
- Ventricular septum defect
- Ventricular septum defect, retrognatia

Range of minor abnormalities

(ESHRE 2009)

- Bilateral hydrocoele
- Capillary haemangioma
- Cardiac septum defect
- Cerebral calcifications + limb malformation
- Congenital hip luxation
- Cryptorchidy
- Heart murmur
- Heart problems + 1 testicle + mental retardation
- Hypospadias
- Kidney and bladder problems, mental
- Microcephaly
- Mongolean spot
- Positional talipes
- Pre-auricular tags
- Pyelourethral junctional stenosis
- Pyelo-caliectasy bilateral
- Sacral dimple
- Syndactyly digit iv-v
- Syndrome of Rubinstein-Taybe
- Uniumbilical artery

What can we learn from ART (1)

- In developed countries ART babies represent 1-4% of babies born
- There is a need to compare singleton pregnancies to control for confounding multiple birth factors
- It is difficult to assess whether the underlying cause of sub-fertility or ART is responsible

- 3 meta-analyses *(Hansen 2005, Lie 2005 & Rimm 2004)* and a controlled study of nearly 3000 infants *(Bonduelle 2002)* confirmed relative risk of major abnormality of 1.24 in ART babies
- Longer term studies on ICSI/IVF babies show a relative increase risk of abnormality- ICSI (2.77) and IVF (1.8) *(2005 Bonduelle)*
- Possible increase risk of imprinting disorders such as Beckwith Wiedemann, Angelman and retinoblastoma (Sutcliffe 2006, Maher 2003, Moll 2003)

What can we learn from ART (2)

"Time to pregnancy" (with & without fertility treatment) is implicated in:

- maternal health factors e.g. pre-eclampsia
- birthweight- 2 fold increase in low or very low BW
- Perinatal/neonatal death and SCBU admission odds ratio 2.9.

Review ESHRE 2008 data

(Goossens et al 2009 in press)

ESHRE data collection from January 1997 to December 2006 (& subsequent pregnancies)

- Data available on 3303/3841 babies
- No data available on 538/3841 babies (14%)
- Multiple birth rate 23% *(ESHRE 2009)*
- Malformations reported in 132/3303 babies
  Overall malformation rate = 3.99%
  Major malformations= 68
  Minor malfomration= 65 (1 baby had more than 1 malformation)

Difficulties with PGD paediatric collection?

- Numbers per centre are small- 700 children needed for power to detect major abnormalities *(Desmyttere 2008)*
- Easy to lose contact with families and fail to collect complete data
- Funding of paediatric follow up
- Distance to travel to PGD Centre & secrecy of PGD
- Ascertainment bias, requires control population

Number of variables to control for:

- 1 versus 2 cell biopsy
- Singletons versus multiples
- PGD vs PGS as background reason for treatment is different
- Fresh vs cryopreserved embryos

GSTT paediatric follow up

- Introduced at the beginning of the PGD service- (1997)
- Encouraged all couples to participate
- Committed paediatrician as part of the PGD team

Follow up process

- Discussed with couples at the outset of treatment
- Pregnancies managed by local obstetric teams.
- Multiple pregnancies referred to Fetal Medicine Unit for chorionicity scan.
- Birth outcome questionnaire sent to couple at 30/40
- Follow up appointments arranged at 1 and 2 years of age

Birth outcomes 1997-September 2007

Total babies born = 126
Total deliveries = 93

- 63 singletons-
- 51 twins (26 x 2)- 1 IUD @ 29/40
- 12 triplets (4 x 3)

Singletons at birth 63

- 62/63- reviewed (1 no details)
- 49/62- no problems reported (79%)
- 5 neonatal complications

Major abnormalities

- 1 hypothyroidism
- 1 hydronephrosis

Minor abnormalities

- 2 positional talipes (resolved)
- 1 undescended testes (resolved)
- 1 laryngomalacia (resolved)
- 1 microcephaly & meconium aspiration
- 1 cardiac murmur

Singletons at 1 year
42

29/42 reviewed
21/29- no problems reported (72.4%)

New problems

- 1 capillary haemangioma (27/40)
- 1 congenital hip dysplasia
- 1 convergent squint
- 1 lactose intolerance
- 1 LF hearing loss (+++URTI)
- 1 torticolis

Continuing problems

- 1 hypothyroid- treatment in progress
- 1 pyeloplasty for hydronephrosis

Singletons at 2 years
31

12/31 reviewed
9/12 no problems reported

New problems

- 1 Congenital hip dysplasia

Continuing problems

- 1 Squint
- 1 Congenital hip dysplasia

Twins at birth
51 (26 x 2)

- 51/51 reviewed (1- IUD @ 29/40)
- Zygosity
  - DZ 18 (sets)
  - MZ 1
  - unknown 7 (all 2ET)
- 43/51 no problems reported (84%)
- 1 minor abnormality- hydrops (resolved)
- 7 neonatal complications

Twins at 1 year
40

- 30/40 reviewed
- 22/30 no problems reported

New problems- minor abnormalities

- 1 motor delay
- 1 floppy larynx
- 2 squints 1 microcephaly
- 1 2/3 syndactyly
- 1 skin tag
- 1 microcephaly
- 1 hearing deficit

Twins at 2 years
22

- 16/22 reviewed
- 14/16 normal

Abnormalities- no new abnormalities

- 1 motor delay (as before)
- 1 floppy larynx (as before)

Triplets
12 (4 x 3)

At birth

- 12/12 reviewed
- MZ twins 2 sets
- 8/12 no problems reported
- 1 minor abn- undescended testes
- 3 neonatal complications

At 1 year

- 7/12 reviewed
- 3/7 no problems reported
- 4 abnormal
  - 1 deceased SID cardiac abnormality (29/40)
  - 2 patent foramen ovale (29/40)
  - 1 multiple abns. including NEC (30/40)

At 2 years

- 6/9 reviewed
- 5/6 normal
- 1 dev delay & behavioural problems (new)

Summary of GSTT data

(Lashwood 2007)

Major abnormalities (3.9%)

- 2 major at birth
- 3 more by 2 years

Minor abnormalities (15.8%)

- 8 at birth (most in singletons, 1 preterm)
- 12 more by 2 years

Neonatal complications

- 15
  - 11/15 preterm
  - 2/15 maternal pre eclampsia

New studies

EU 8th Framework PGD study

Multi faceted study. The main goals of the study are:

- to facilitate the collection of data relating to the use of reproductive technologies and their outcomes in EU member states through the use of common terminologies and data registers (the 'technical strand')
- to facilitate the referral of patients from one centre to another, particularly for patients at risk of rare monogenic diseases (the "clinical strand")
- ***to enable the efficient follow-up of patients and their off-spring so as to monitor safety and efficacy through data collection and a data register (the 'monitoring strand')***
- to examine the relevant requirements of the EU Human Tissue and Cells Directive (2004/23/EC) and other relevant legal requirements at EU and member state level, and to address related ethical issues (the 'legal and ethical strand').

The history

ESHRE steering committee committed to retrospective data collection.

2005- 2006

- questionnaires sent to centres asking their views and willingness to participate in study.
- 2006- Responses indicated that in first instance at least a retrospective follow up study could be done.

Centres were invited to participate if they met study criteria which were:

- must have 10 or more live births
- patients must be able to read and write 1 of 6 languages, English, German, Flemish, French, Spanish, Czech
- Babies born up to and including 30/10/07

Study size

- 16 centres agreed to participate
- Approximately 2000 babies
- May be able to increase numbers if database allows

Study process

- Parental questionnaire based study.
- Non case controlled, one time period study
- Managed centrally by CI Alison Lashwood
- Local centre collaborator to administer questionnaires.
- Returned to CI with unique identifier

Data management

- Data to be stored on a new paediatric web based database created as part of the EU project (Consortium Database Working Group)
- This database will form a prototype for possible future PGD data collection.
- Database still being created therefore data will be entered centrally.

Ethics

- UK centres require ethics approval- complex process- granted in September 2008
- All centres asked about local requirements for ethical approval- many centres indicated this was not required.

Current progress

- Participating centres in process of despatching questionnaires
- Data entry hopefully will start in 2-3 months
- First reports due by October 2009

Summary

- Short and long term data on PGD babies is limited.
- At present the overall abnormality rate at birth appears to be no higher than abnormality rates for other ART procedures
- In future studies a number of variables will need to be considered and controlled for.
- A large collaborative, prospective case controlled study is required.
- Such a study is complicated by multicentre participation (in multiple countries), language, standardisation of terminology and paediatric review.

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What have we learned from 10 years of the ESHRE PGD Consortium

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Overview

- Why collect data?
- What has the data revealed?
- Is there still a need to continue collecting the same data?
- Should the reporting emphasis of reporting change?
- What has the consortium achieved?

Who wants the data

- **Involved Profession:**
  - Is PGD effective and how are they doing in comparison?
  - Who is offering PGD?
  - What new diseases are being tested?
  - What are the trends in disease diagnosis technologies?
  - Is the procedure safe?
  - What are the risks?
- **Media and patients**
  - Information - who needs PGD and how its done?
  - Does it work for my disease?
  - What is on offer and where?
  - Is it safe?
  - What's new?

Aims of the ESHRE PGD Consortium

- To survey the availability of PGD
- To collect prospectively and retrospectively data on the accuracy, reliability and effectiveness of PGD
- To initiate follow-up studies of pregnancies and children born
- To produce guidelines and recommended PGD protocols
- To formulate a consensus on the use of PGD

Different parts to the collection and reports

- Referrals - why are patients being referred?
- Cycles- how many, how and for what?
- Pregnancies - how many, outcome?
- Children - outcome and early health
  - long term health

In the beginning - Data collection I Jan 97- Sept 98

- Joep Geraedts
- Alan Handyside
- Joyce Harper
- Inge Liebaers
- Karen Sermon
- Catherine Staessen
- Alan Thornhill
- Anna Vanderfaeillie
- Stephan Viville

PGD Data collections I-IX

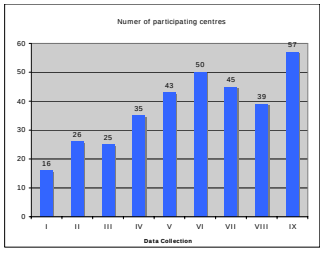
- Series I Jan 1997-Sept 1998 [16 centres]
  (120 OR; 82 Children)
- Series II Oct 1998 - May 2000 + from 1993 [26 centres]
  (196 OR;162 children)
- Series III May 2000-May 2001 + any from before [25 centres]
  (426 OR; 279 Children) Social sexing included
- Series IV May 2001 - Dec 2001 [36 centres]
  (1819 OR; 243 babies) Major increase PGS
- Series V Jan 2002 - Dec 2002 (children to Oct 2003) [43/66 centres]
  2219 cycles; 485 pregnancies; 382 babies

PGD Data collections V-IX

Links babies to the cycles

- Series V Jan 2002 - Dec 2002 (children to Oct 2003) [43/66 centres]
  2219 cycles; 485 pregnancies; 382 babies
- Series VI Jan 2003 - Dec 2003 (children to Oct 2004) [50 centres]
  2984 cycles; 501 pregnancies; 373 babies
- Series VII Jan 2004 - Dec 2004 (children to Oct 2005) [45 centres]
  3358 cycles; 679 pregnancies; 528 babies
- Series VIII Jan 2005 - Dec 2005 (children to Oct 2006) [39 centres]
  3488 cycles; 845 pregnancies; 670 babies
- Series IX Jan 2006 - Dec 2006 (children to Oct 2007) [57 centres]
  5858 cycles; 1437 pregnancies; 1206 babies

Effectiveness of PGD - trends in use
Centres participating in data collection



- Steady increase in number of centres participating
- Not all registered centres send in data; varies year on year
- Number of centres limited- does not include some of the busiest USA centres

93 Members by country I-IX (full + associate)

Argentina:	1	Netherlands:	5
Australia:	3	Poland:	1
Brazil	1	Portugal:	1
Belgium:	6	Russia:	1
Bulgaria:	1	Serbia:	1
Czech Republic:	2	Singapore:	1
Denmark:	3	South Africa:	1
Egypt:	1	Spain:	11
Germany:	9	Sweden:	2
Japan:	3	Thailand:	2
Finland	2	Taiwan:	1
France:	3	Turkey:	3
Greece:	3	UK:	4
India:	2	Ukraine:	1
Israel:	4	United Arab Emirates:	1
Italy:	4	USA:	5
Korea:	2		

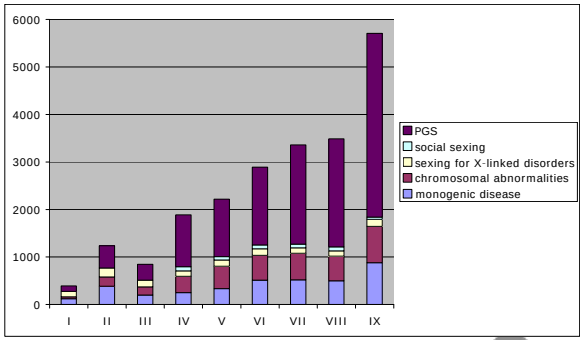
Two types of membership

- Full
  - Send in full data
- Associate
  - Send in summary data

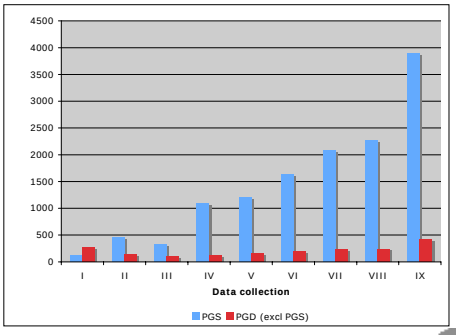
All receive / share communication

- Newsletters
- Surveys
- Meetings
- Information on web site

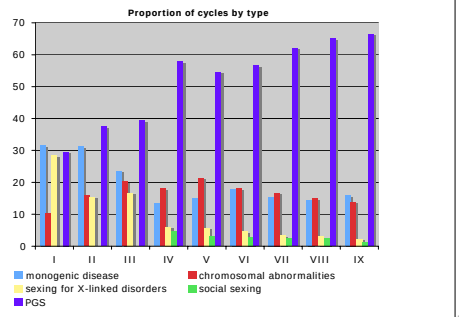
Effectiveness of PGD - trends in use
Number of cycles



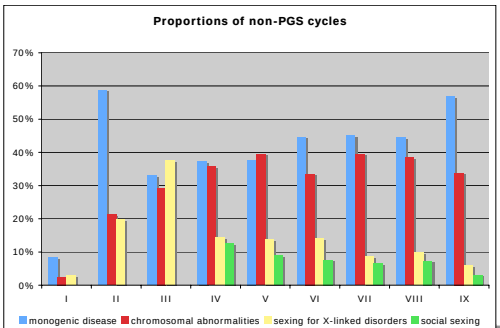
Effectiveness of PGD - trends in use
Rising number of PGS cycles



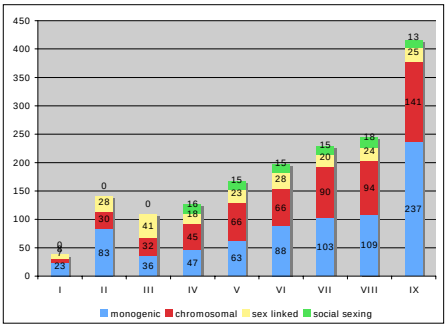
Effectiveness of PGD - trends in use
proportion by type of condition



Effectiveness of PGD - trends in use
Percentage by type of condition (non PGS)



Effectiveness of PGD - trends in use
Number of clinical pregnancies by type of condition



Effectiveness of PGD - outcome I-IX percentage by type of condition

	Cycles to OR	No. embryos biopsied	Embryos transferred	Transfer procedures	Pregnancy rate (per OR / per ET)
Monogenics (Rec, Dom)	3481	19988	5296	2730	21% per OR **27% per ET**
Chromosome Rearrangements	3476	22878	4045	2254	16% per OR **25% per ET**
Sexing X linked	1072	6691	1458	798	19% per OR **26% per ET**
Social sexing	542	3317	815	395	21% per OR **29% per ET**
Aneuploidy PGS	13020	71119	16897	9394	18% per OR **25% per ET**

Headline data not useful information for patients

Cumulative data I-IX: PGS

- **Almost all patients infertile** –13020 to OR
- 12597 cycles to biopsy, Majority cleavage stage aspiration + PB biopsy
- 9394 cycles to ET (72%)
- 2390 clinical pregnancies (18% per OR, 25% per ET)

Differences from PGD:

- Not about recurrent genetic disease - appropriate comparator IVF/ICSI
- Indications variable and often unclear
- High proportion go ahead with 1 embryo
- Although gives additional information about safety of biopsy and early outcome of children, provides little helpful information about usefulness
- To date no account has been taken of lack of efficacy shown in randomised trials

PGD for Monogenic Disease

- Recessive
  - β-thalassaemia
  - Cystic Fibrosis
  - Spinal muscular atrophy
  - Sickle cell disease
- Dominant
  - Huntington's disease
  - Myotonic dystrophy
  - Charcot-Marie-Tooth disease
- Sex linked (specific diagnosis)
  - Duchenne muscular dystrophy
  - Haemophilia
  - X-linked mental retardation

Headline rates unhelpful as disguise true information based on type of inheritance

PGD for Monogenic Disease
Dominant conditions : Outcome I-IX

Collection I-VIII			Collection IX	
MD	HD		MD	HD
294/393	252/326	Reaching	80/98	72/98
74%	77%	ET	81%	73%
Clinical pregnancy			Delivery rate	
21%/ET	23%/ET		26%/ET	34%/ET
16%/OR	18%/OR		19%/OR	27%/OR

PGD Chromosome rearrangements:
Outcome I-VIII

Robertsonian translocations			Reciprocal translocations	
Male	Female		Male	Female
331/450	234/315	Reaching	430/737	470/768
73%	74%	ET	58%	61%
CPR/ET 28%			CPR/ET 22%	
CPR /OR 21%			CPR /OR 13%	

PGD Chromosome rearrangements: Outcome: Collection IX

Robertsonian translocations			Reciprocal translocations	
Male	Female		Male	Female
116/161	54/83	Reaching	112/208	139/260
72%	65%	ET	53%	53%
33%/ET	18%/ET	Delivery rate	26%/ET	28%/ET
24%/OR	12%/OR		14%/OR	15%/OR

Sexing for X-linked disease Cumulative data I-IX:

- 1072 cycles to OR
- 1060 to biopsy – most cleavage/aspiration
- 798 cycles to ET
- 204 clinical pregnancies (19% per OR, 26% per ET)

Findings

- Similar results to recessive conditions
- More cases now being done by molecular means to allow unaffected males to be diagnosed.

Social sexing: Cumulative data I-IX

- 542 cycles to OR – 43 were infertile
- 526 cycles to biopsy

- 3317 biopsied, 99% successful
- 395 cycles to ET, 72%
- 116 clinical pregnancies (21% per OR, 29% per ET)

Findings

- Similar results to monogenic disease and PGS as proportion to ET similar
- Relatively few cases proportionally 3% (USA not reported)
- What does this add to knowledge of PGD/PGS safety?

Outcome: Pregnancies
Cumulative data I-IX:

- **3703 clinical pregnancies**
  - 2726 singletons
  - 858 twins (23%)
  - 90 triplets (2.4%)
  - 6 quads
  - 23 unknown
- **482 losses**
  - 1st trimester: 355 miscarriage, 37 ectopic, 8 TOP, 3 unknown
  - 2nd trimester: 60 miscarriage, 19 TOP

Early collections do not take account of these losses inflating success of PGD?PGS

Safety: Deliveries and complications
Cumulative data I-IX:

- 3075 deliveries:
  - 2351 singletons
  - 697 twins (22%)
  - 27 triplets (0.9%)
  - 47 % C-section (40 % singletons, 67 % twins, 74 % triplets)
  - 28 % pre-term (mainly twins and triplets)
- 511 pregnancies with complications
  - Minor (emesis): Major (abruptio placentae)
  - More frequent in twins (17%) and triplets (20%) than in singletons (12 %)

Safety: Malformations
Cumulative data I-IX:

133/3240 malformations (4%)

Minor malformations: 61 cases (1.8%)
Major malformations: 69 cases (2.1%)
- Impairing normal function or necessitating surgery
- From haemangioma to serious heart defects

- Unknown malformations: 3 cases

Safety

Safety: PCR misdiagnosis Cumulative data I-IX

Monogenics		
Myotonic dystrophy type 1	PND	TOP
SMA	Post	Born
ß-thalassemia	PND	TOP
ß-thalassemia	PND	TOP
Familial amyloid polyneuropathy	PND	Born
Cystic fibrosis	PND	Born
Cystic fibrosis (1 of twins)	Post	Born
CMT1A	PND	Born
CMT1A (twins)	PND	TOP of both twins
Fragile X	PND	Born
Sexing for X-linked disease		
46,XY in retinitis pigmentosa	PND	Born
46,XY in Duchenne muscular dystrophy twin	PND	TOP of one twin

Safety: FISH misdiagnosis Cumulative data I-IX:

Sexing for X linked disease		
45,XO Haemophilia A	PND	TOP
46,XY Haemophilia A	Post	Born
46,XY Retinitis Pigmentosa (twins)	Post	Born
Translocations		
T13 after 45,XY,der(13;14)(q10;q10)	Mis	Mis
47,XX,+der(22)t(11;22)(q23.3;q11.2)	PND	TOP
46,XY,der(15)t(13;15)(q25.1;q26.3)pat	PND	TOP

Safety: FISH misdiagnosis (PGS / SS) Cumulative data I-IX:

PGS		
T16 after 1st PB biopsy only	Mis	Mis
T16 after 1st PB biopsy only	Mis	Mis
47,XX,+16	Mis	Mis
47,??,+16	Mis	Mis
47,XX,+21	Post	Born
47,XXX	PND	Lost to follow-up
46,XY/47,XY,+18	PND	TOP
47,XY,+21	PND	TOP
Social Sexing		
Requested male but female foetus	PND	TOP

Conclusions and comments

Consortium and its publications have served a useful purpose

- Answered some of the proposed questions about use, types of conditions referred, types of conditions undertaken.
- Preliminary answers to early outcome
  - High incidence of multiple pregnancy and expected complications
  - No obvious concern about biopsy (large numbers)
- Brought together many unit with similar intentions to share data and ideas
- Significant moves to standardise methods and improve safety by running training courses, EQA measures, and reporting misdiagnoses

Conclusions and comments

Achieved by highly motivated [and often the same] group of individuals who have believed in its usefulness and purpose

Steering Committee 2007

Chair, Joyce Harper,
Past Chair, Karen Sermon,
Deputy Chair Alan Thornhill.
Joep Geraedts, Netherlands,
Stephane Viville, France,
Christine deDie, Netherlands,
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Sjoerd Repping, Netherlands,
Joanne Traeger-Synodinos, Greece,
Katerina Vesela, Czech Republic,
Tugce Pehlivan, Spain
Francesco Fiorentino, Italy

Conclusions and comments

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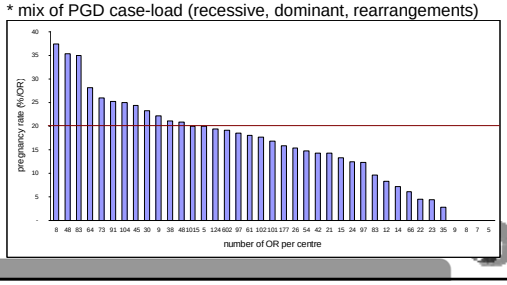
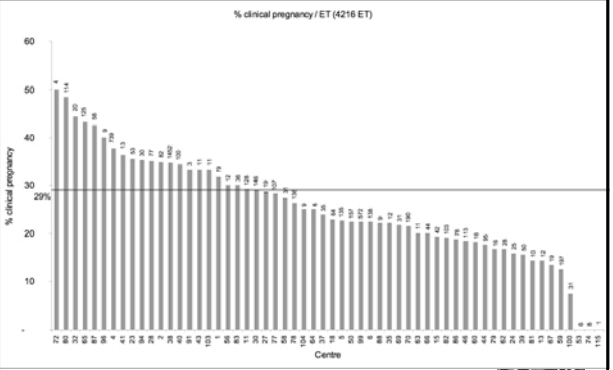
Conclusions and comments

Publication of headline CPR rates provides little benefit as is influenced by

* overall clinic ART success rates
* skill in biopsy and embryo handling
* caseload of clinic
* mix of PGD case-load (recessive, dominant, rearrangements)



Clinical pregnancy rate per ET gives fairer picture of post biopsy embryo quality



Conclusions and comments

The recording and reporting of PGS success rates with the recurrent genetic disease (PGD) figures is unhelpful, since PGS is generally offered to patients without genetic disease, and failed or compromised IVF/ICSI cycles. They should be recorded with the overall figures for ART procedures as that is what they seek to improve.

Most useful PGD outcome data will be LBR per disease type which will give patients and clinician a fair idea of how PGD can benefit them (or not)

Early outcome safety of biopsy (whatever the reasons for its undertaking) has largely been shown

The most important safety data still to be collected are

- long term health of children
- accurate figures for misdiagnosis and ways to avoid them

Data Collection – 10 years

www.eshre.com/ESHRE/English/SIG/Reproductive-Genetics/PGD-Consortium/page.aspx/201

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Further reading

www.eshre.com/ESHRE/English/SIG/Reproductive-Genetics/PGD-Consortium/page.aspx/201

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