PGD: the more, the better?

Edith Coonen
Centrum voor Voortplantingsgeneeskunde
Maastricht UMC

VERENIGING VOOR KLINISCHE EMBRYOLOGIE

GROW School for Oncology & Developmental Biology

academisch ziekenhuis Maastricht azM

Outline

- what is PGD/PGS
- how we do it
- how we might do it
- whom do we use it for
- whom might we use it for
- dilemma's
- concluding remarks

What is

Preimplantation Genetic Diagnosis

Identification and transfer of embryos

free from a particular genetic disease or

chromosomal aberration to obtain a healthy child

Preimplantation Genetic Diagnosis

- patients at high risk
- asked for by patients aware of their status
- mainly fertile patients (65%)
- prenatal diagnosis as alternative
- diagnosis just for the disorder
- no transfer of undiagnosed or affected embryos
- current techniques uncontroversial

What is

Preimplantation Genetic Screening

Selection and transfer of embryos with best chromosomal status to improve IVF delivery rate

Preimplantation Genetic Screening

- infertile or subfertile patients
- offered to patients previously unaware of their status
- improve quality of future child's life
- transfer of undiagnosed or 'inconclusive' embryos possible
- current technique controversial

Is it new?

Preimplantation embryo selection

Observational

- pronuclear status
  - 1PN embryo ≠ viable
  - 3PN embryo = common cause of miscarriage
- multinucleated blastomeres

Omics technology

Embryo selection

Genomics

- invasive test (biopsy)

  genes, chromosomes,

  transcripts (epigenetics)

Proteomics

- non-invasive test (media)

  protein secretion

Metabolomics

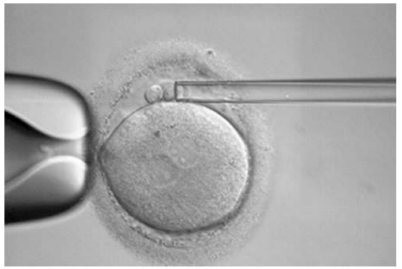
- non-invasive test (media)

  products secreted or taken up

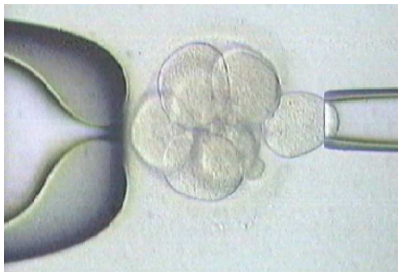
pgd/pgs

Biopsy stages

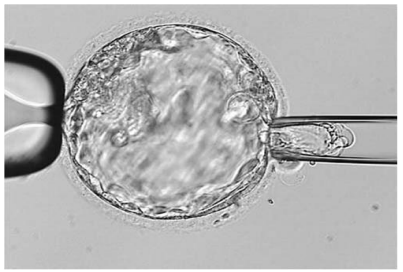
polar body



cleavage stage



blastocyst



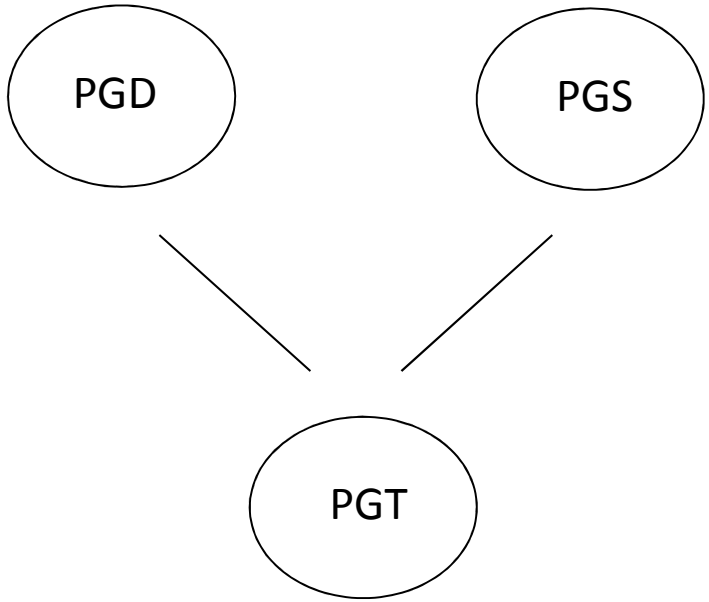
Mosaicism

- biological phenomenon
- cells from one embryo have different chromosomal content
- present in all stages of preimplantation development
  - ~ 50% at cleavage stage
  - ~ 30% at blastocyst stage (Wells et al.)
- serious consequences for PGS (PGD)

PGD

The *more*, the better?

from targeted to comprehensive testing

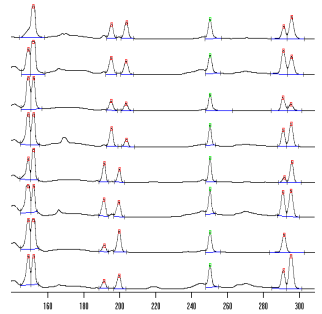


PGD

Targeted testing

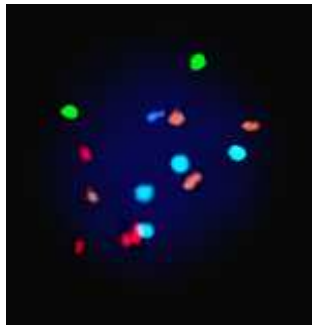
Polymerase chain reaction (PCR)

- analysis of specific mutation or linked markers
  - single gene defects



Fluorescent in situ hybridisation (FISH)

- analysis of chromosomes
  - sexing for X-linked disorders
  - structural chromosome abnormalities
  - PGS (copy number)



PGD

Comprehensive testing

Comparative Genomic Hybridisation

analysis of chromosomes and/or genes

array-CGH (chromosomes)

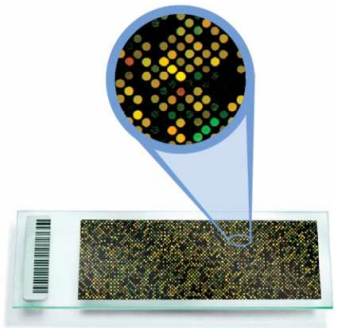
SNP array (chromosomes and genes)

karyomapping

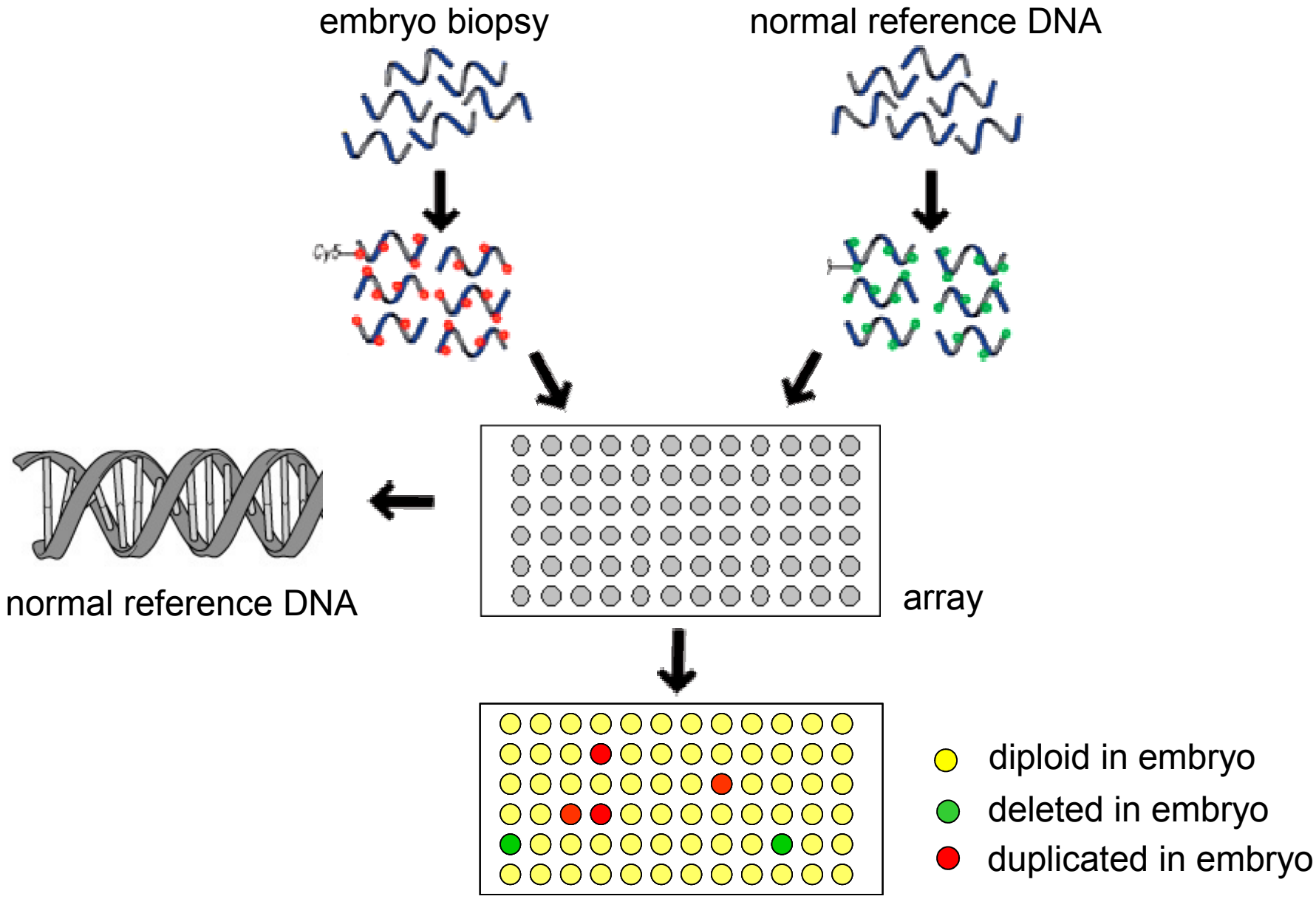
Whole genome sequencing

next generation sequencing (NGS)

high-throughput genomic analysis



Array-based Comparative Genomic Hybridisation

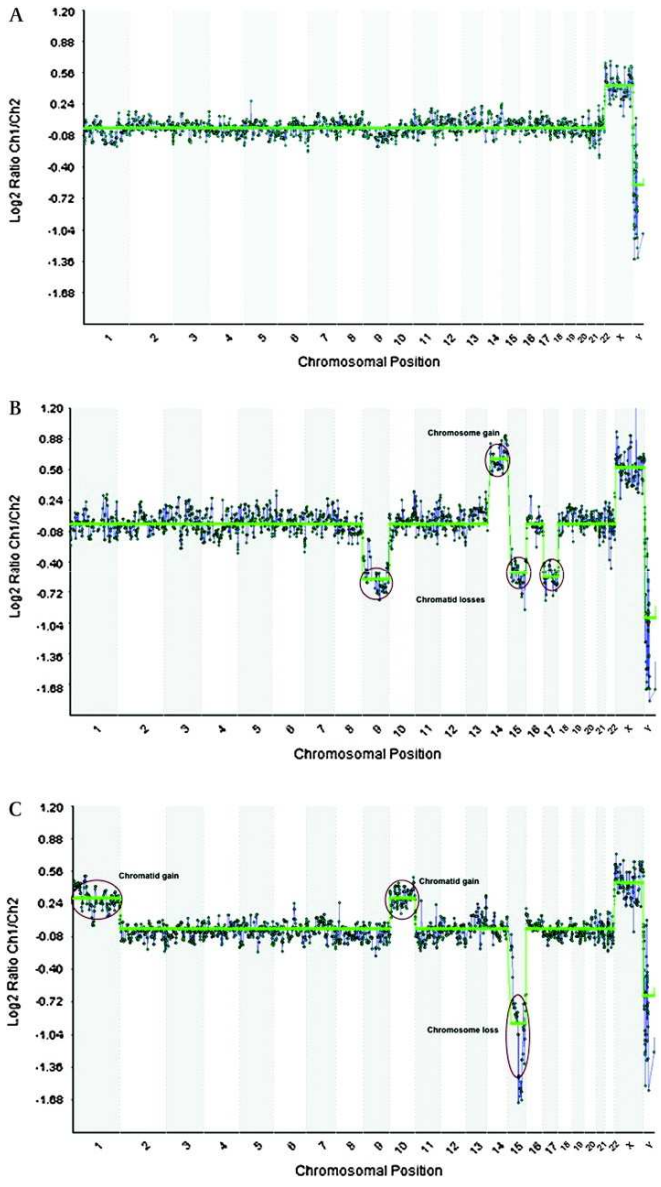


Array-CGH

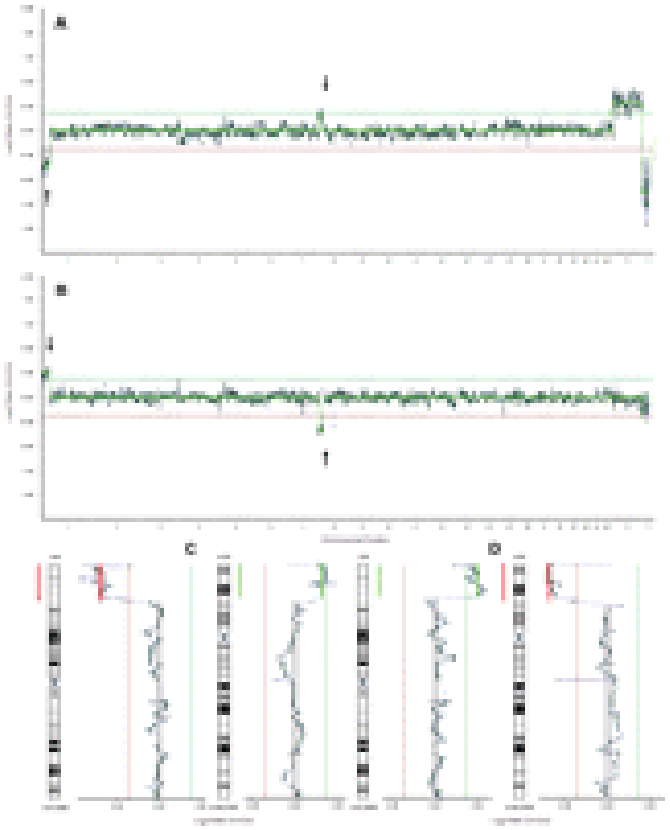
- measures DNA copy number
- only relative changes (aneuploidy)
- resolution ~ 1MB

+	-
quick procedure (fresh transfer) relatively cheap	only chromosomes

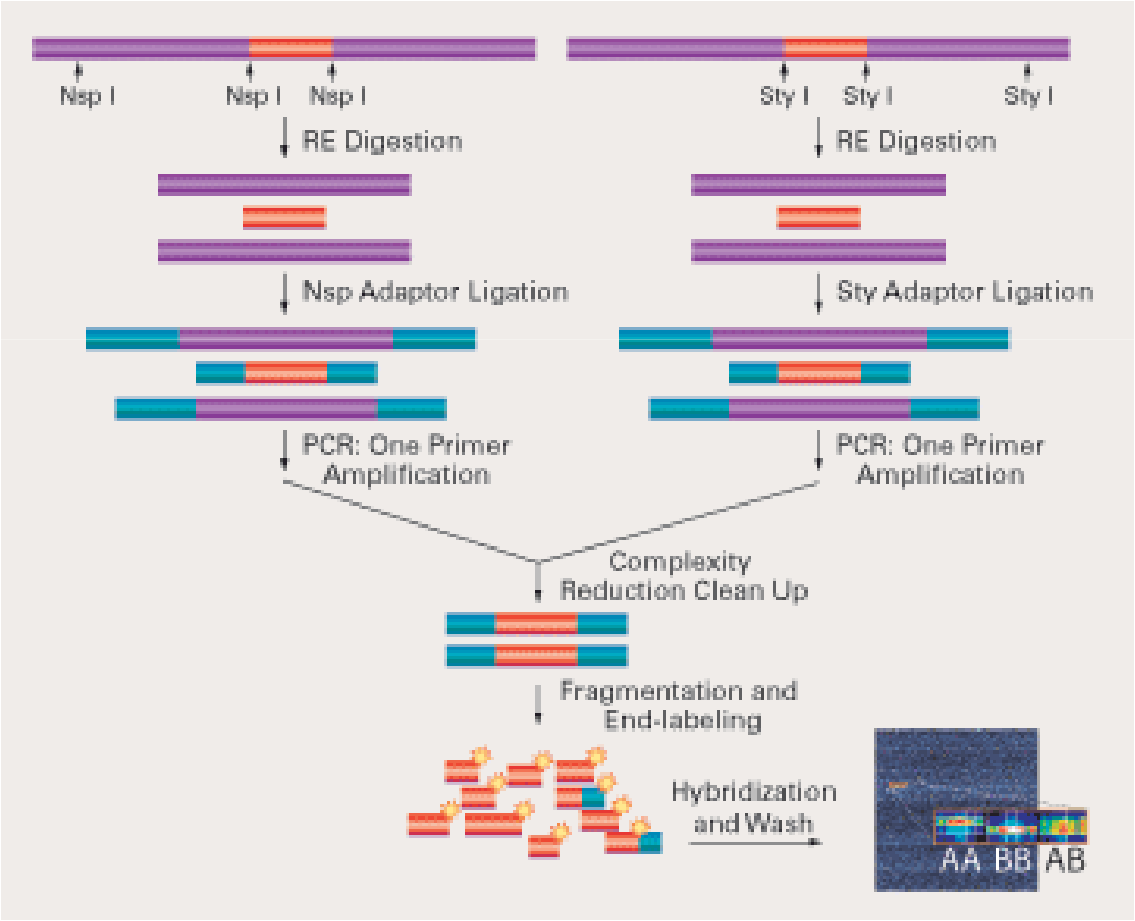
Array-CGH for aneuploidy screening



Array-CGH for detection of translocations



Single nucleotide polymorphism (SNP) array



SNP array

- 10^7 SNP's across genome
- measures single base pair changes
- detects

  aneuploidy (parent of origin)

  single gene and complex disorders

  copy number variants

  mitochondrial disorders

SNP array

-

long protocol (embryo cryopreservation)

expensive

limited clinical application

Karyomapping

Interpreting SNP data

Handyside et al (2010) J Med Genet 47, 651-658

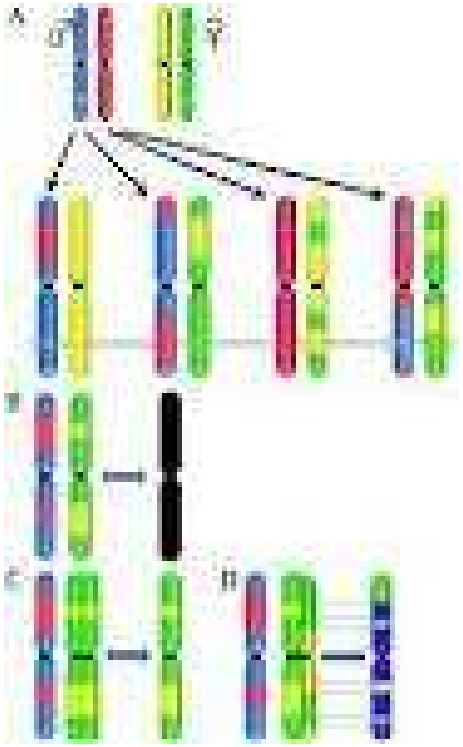
Karyomapping

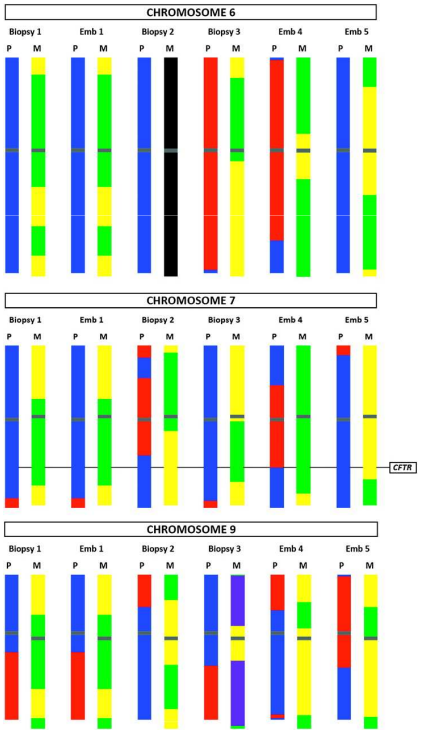
- genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes

- high density genome wide SNP genotyping of proband, parents and appropriate family member(s) to establish phase

- Mendelian analysis and karyomapping of the parental and grandparental haplotypes for each chromosome or chromosome segment in recombinant chromosomes

Karyomapping





Whole genome sequencing

analysis of both coding and non-coding sequences

Next generation sequencing (NGS)

whole exome sequencing

analysis of all coding sequences (genes)

-
not (yet) on single cell level

High throughput sequencing

personal genomics

James D Watson

May 31st , 2007

Dilemma's

- informed consent

  clinically unproven technology

  specific

  explicit

    information of uncertain or no clinical significance

    unexpected findings

    right not to know

    decision needed quickly

    vulnerable patient group

Dilemma's

- whose embryos are they anyway?

  who is to decide which is the best embryo for transfer

  geneticist, embryologist, gynaecologist, future parents

- the best embryo refers to 'best of'?

  this batch of embryos

  the best embryo to be expected after 'n' IVF cycles

PGD: the *more*, the better?

- from targeted to comprehensive testing
- from couples at risk to general population

PGD in the Netherlands

- since 1995
- one licensed centre

  Maastricht UMC (Genetics & IVF)
- transport PGD (IVF)

  UMC Utrecht

  UMC Groningen

  AMC Amsterdam

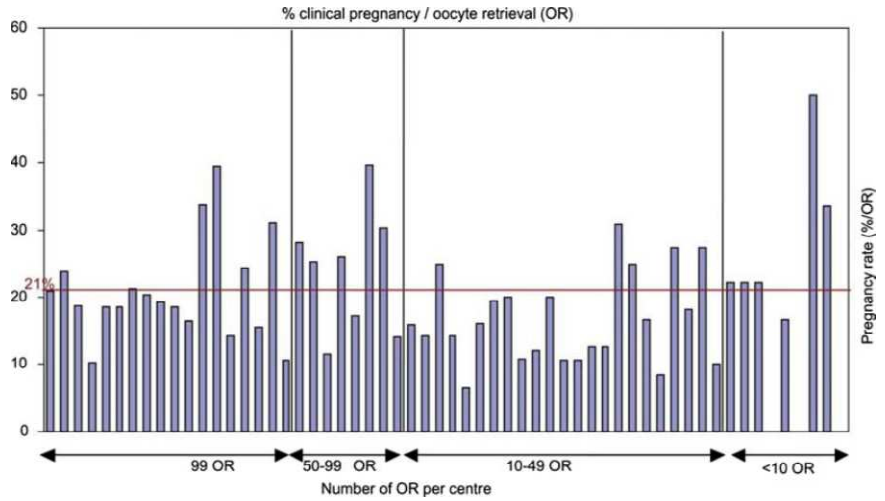
PGD in the media

Ons PGD avontuur!!

Op 15 februari 2011 i
zoon van . Ha
gefeliciteerd!
Hieronder het ervaringsverhaal van die een traject van preimplantatie genetische diagnostiek (PGD) hebben doorlopen.

PGD in Europe

ESHRE PGD consortium



Future Preimplantation genetic testing

- new technologies
- blastocyst biopsy and vitrification

However ….

Combination of factors will limit the application of genome wide testing of embryos

- PGD is an inefficient procedure

  150.000 embryos diagnosed for 5000 babies born
- methods are costly and lab protocols complex
- whole genome amplification from single or small cell numbers has serious flaws

  amplification bias, incomplete coverage, errors
- the incidence of most aberrations in embryos is too low
- we get more information than we can handle

Future

preconception screening (array, NGS)

- whole genome testing of parents
  - more cost effective
  - likely to identify risk of serious disease
- targeted testing in embryos
  - karyomapping

PGD: the more, the better?

Not everything that counts can be counted

And not everything that can be counted, counts

(albert einstein)

THANK YOU