In vitro fertilisation and genetic testing

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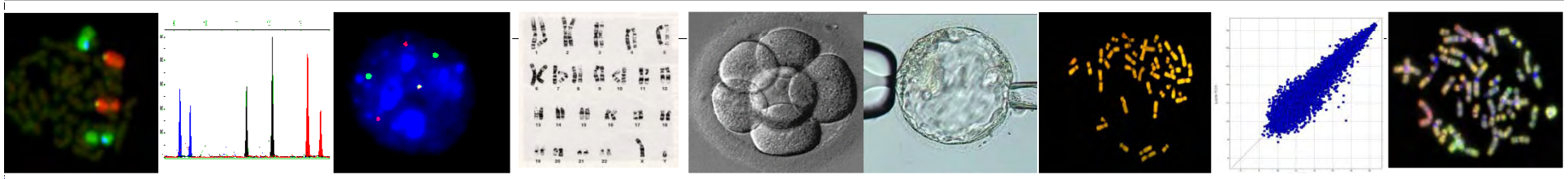
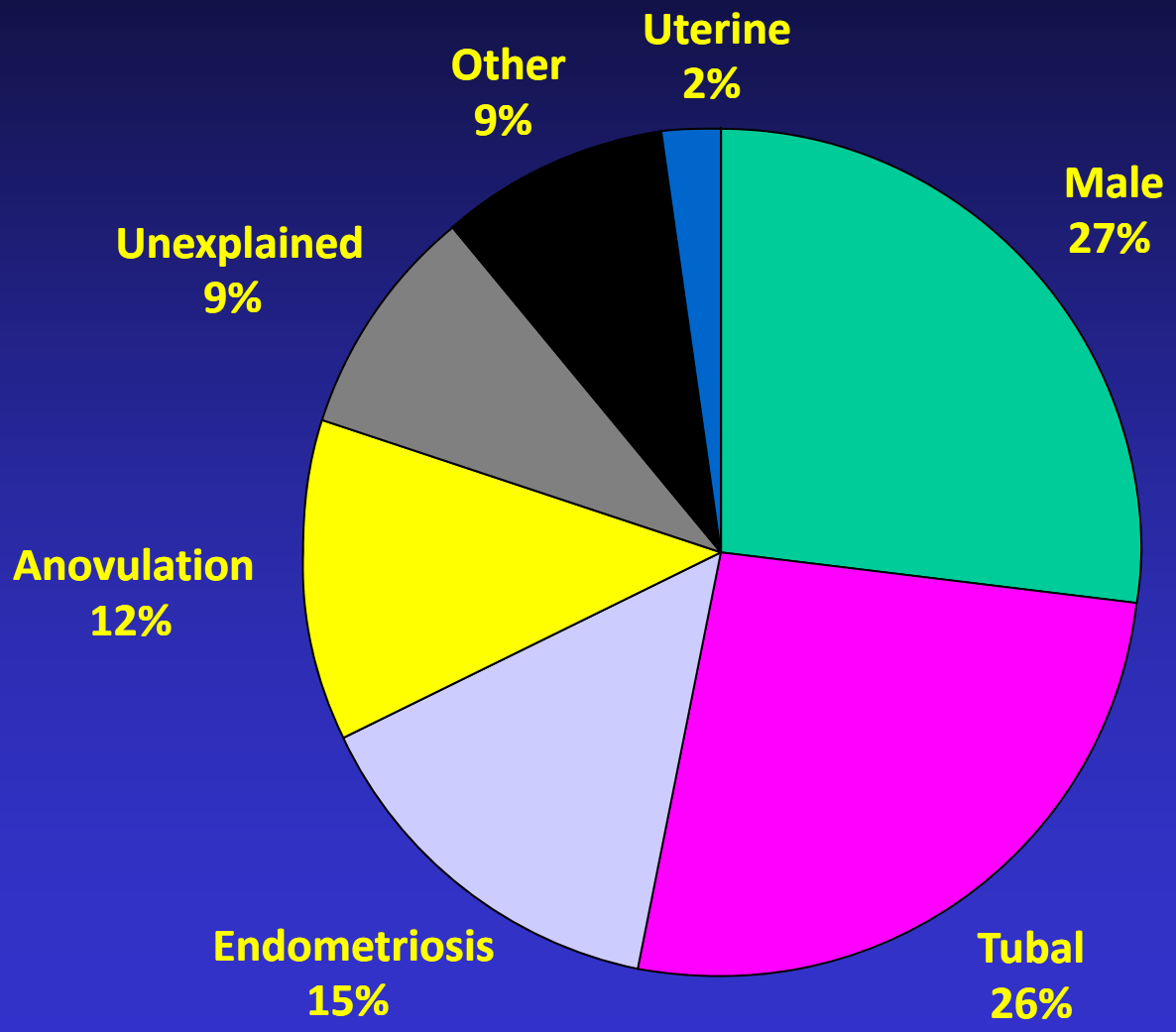


Infertility

- Unprotected intercourse for 1 year without conception
- Very common – 1 in 6 couples affected
- In vitro fertilization first birth in 1978
- More than 3,000,000 IVF babies worldwide
- 1-5% of all births in Western countries

Indications for IVF/ICSI



Ovarian stimulation

- Ideally need 10-15 high quality eggs
- 3 types of drugs
- GnRH (gonadotropin releasing hormone agonist/antagonist)

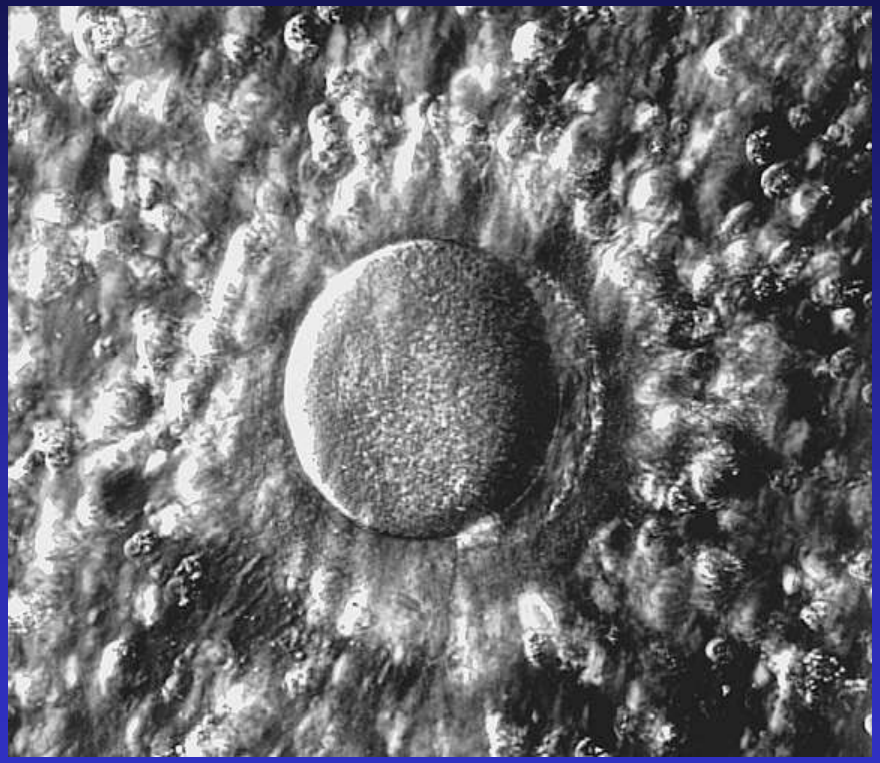
  Suppresses luteinizing hormone (released from pituitary)

  LH surge would cause premature ovulation
- Follicle stimulating hormone (FSH)

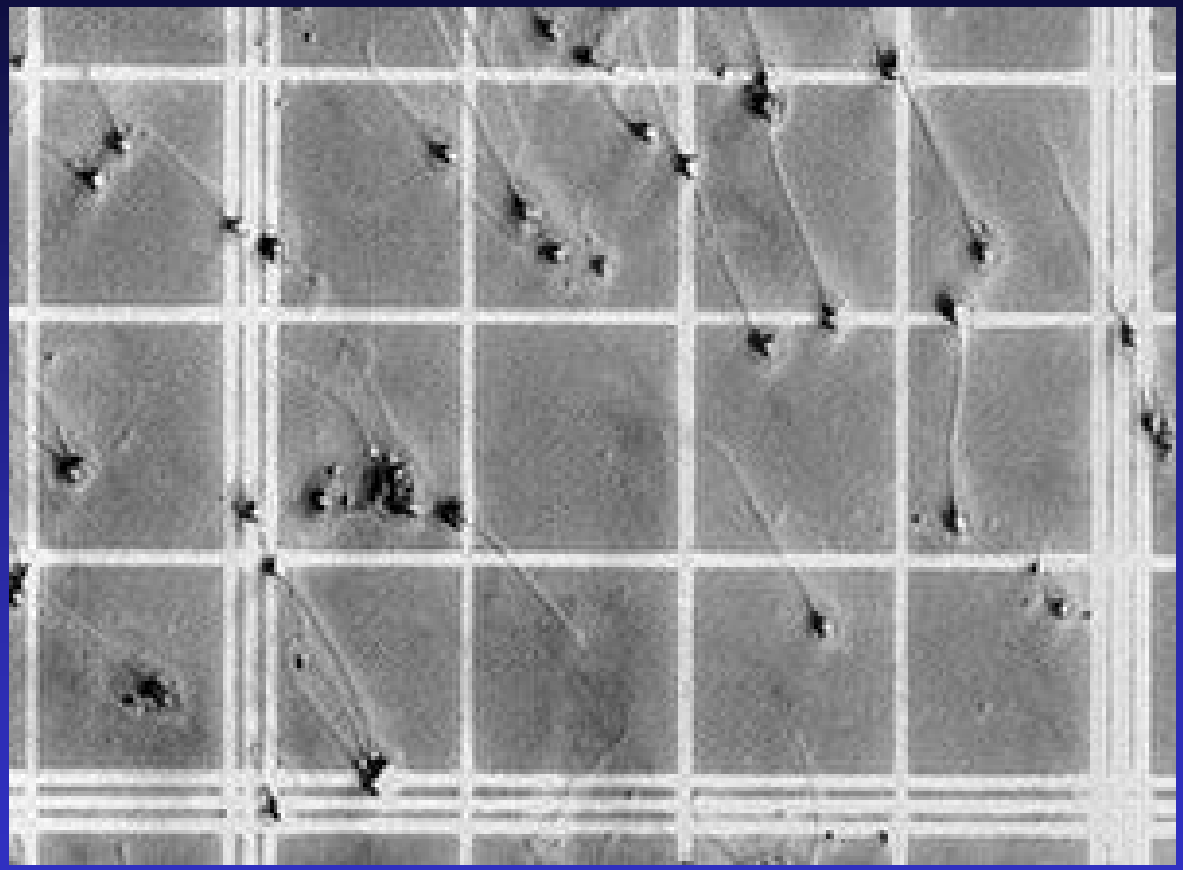
  Stimulates development of multiple follicles (structures that contain eggs)
- Human chorionic gonadotropin (HCG)

  Causes final maturation of eggs in follicles

Egg collection procedure

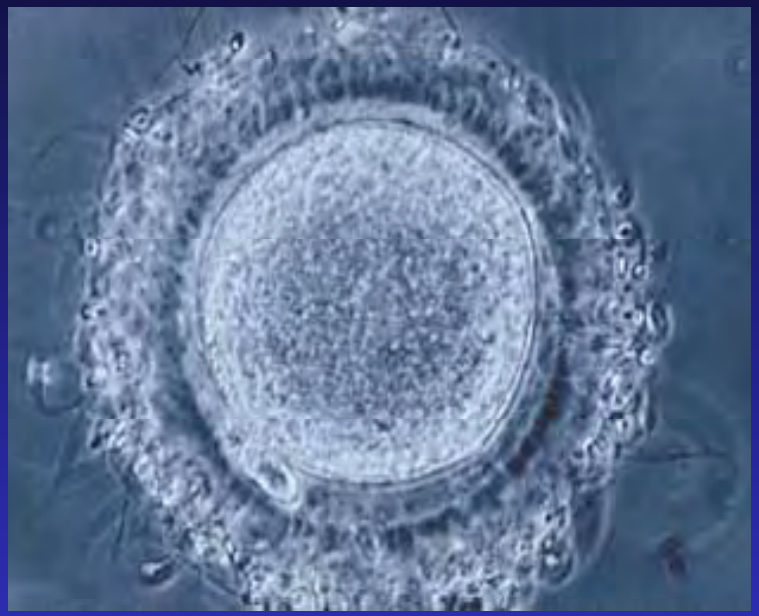


Sperm preparation



Semen analysis, wash and centrifugation

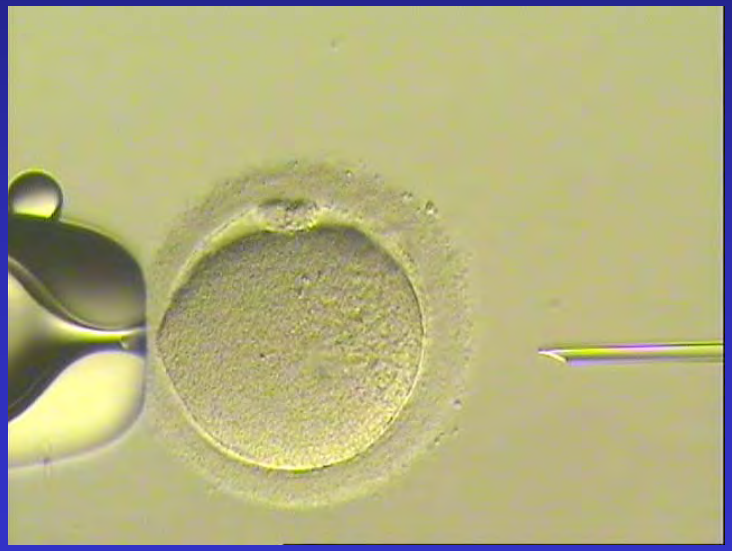
Fertilization



Mixing of sperm and eggs

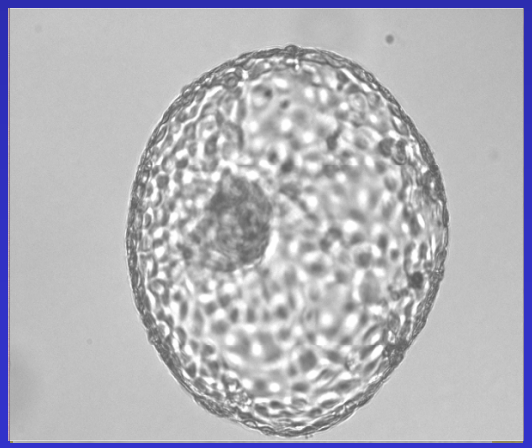
Fertilization using intracytoplasmic sperm injection (ICSI)

- Method developed for men with sperm deficiencies
  - Low concentration of sperm in ejaculate
  - Sperm with motility problems
  - Sperm unable to penetrate the egg



- First reported in 1992. Now very widely applied

Embryo development



2 PN

2-cell embryo

8-cell embryo

morula

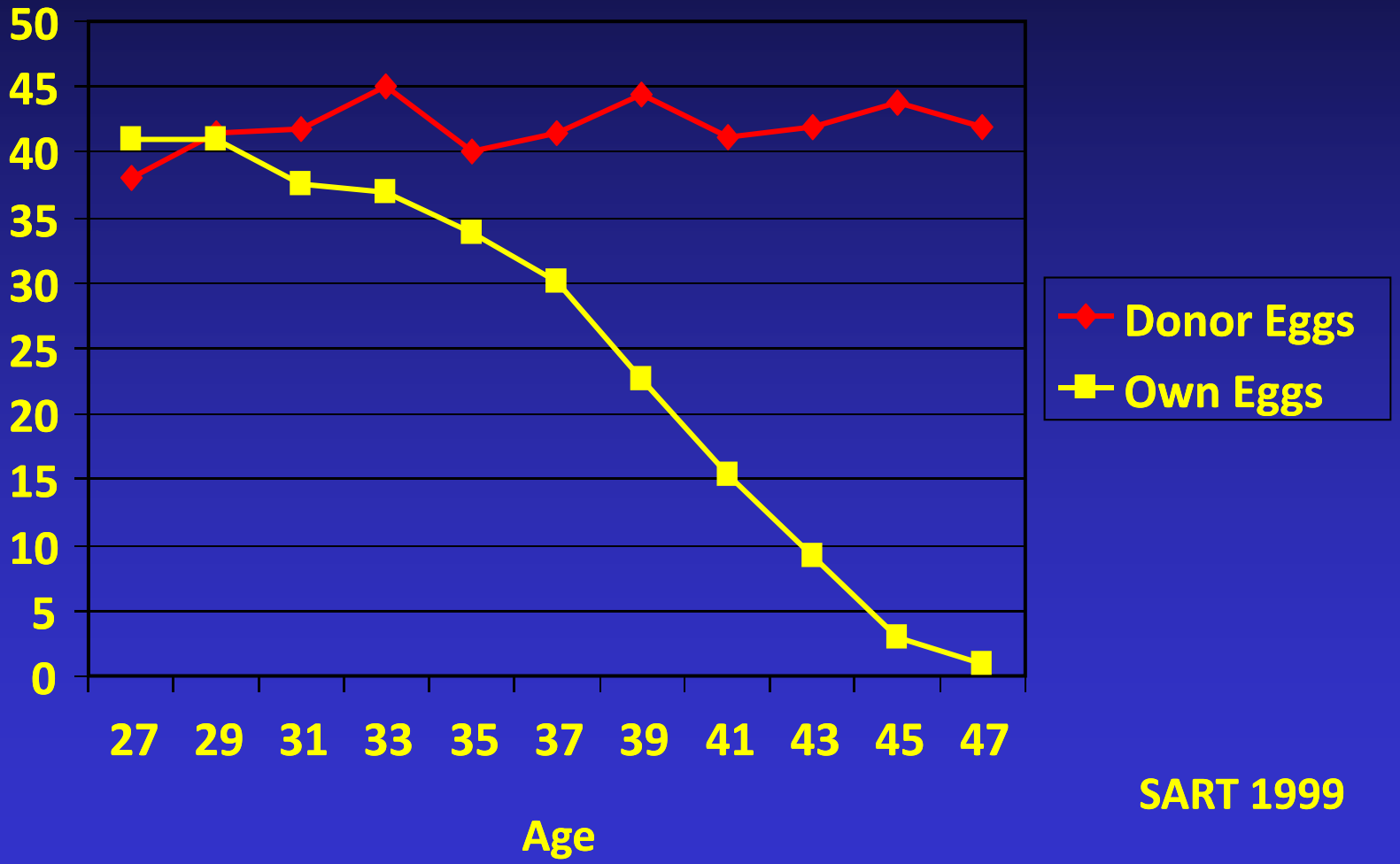
Blastocyst

Hatched blastocyst

Fallopian Tube

Uterus

Success rates of IVF – affect of maternal age



SART 1999

Genetic analysis of embryos

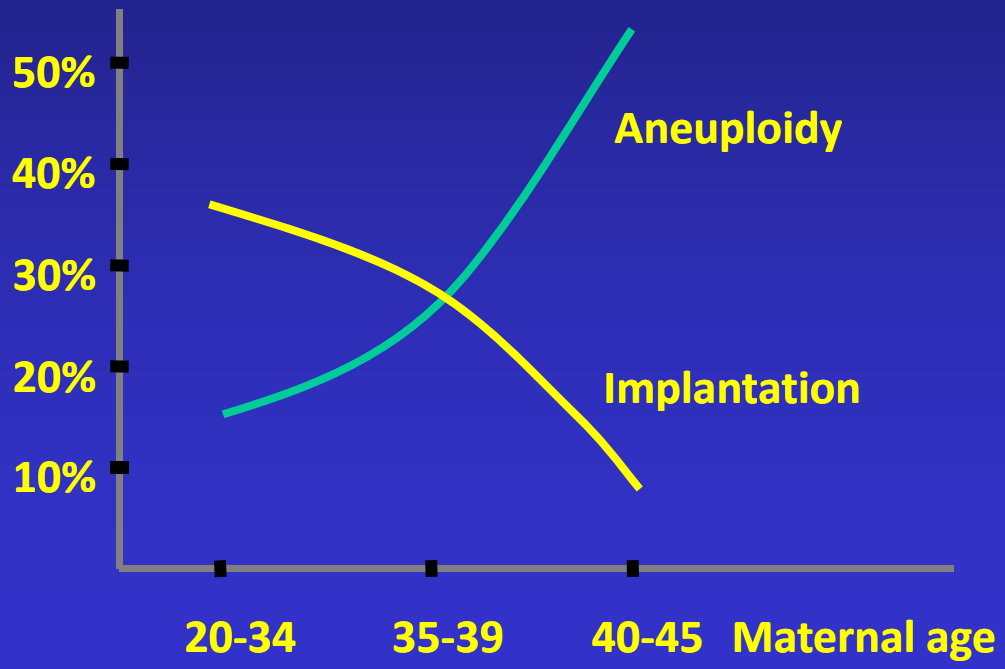
Testing chromosomes in embryos produced during IVF treatment: Why bother?

Aneuploidy and IVF failure

Chromosome abnormality is extremely common in oocytes

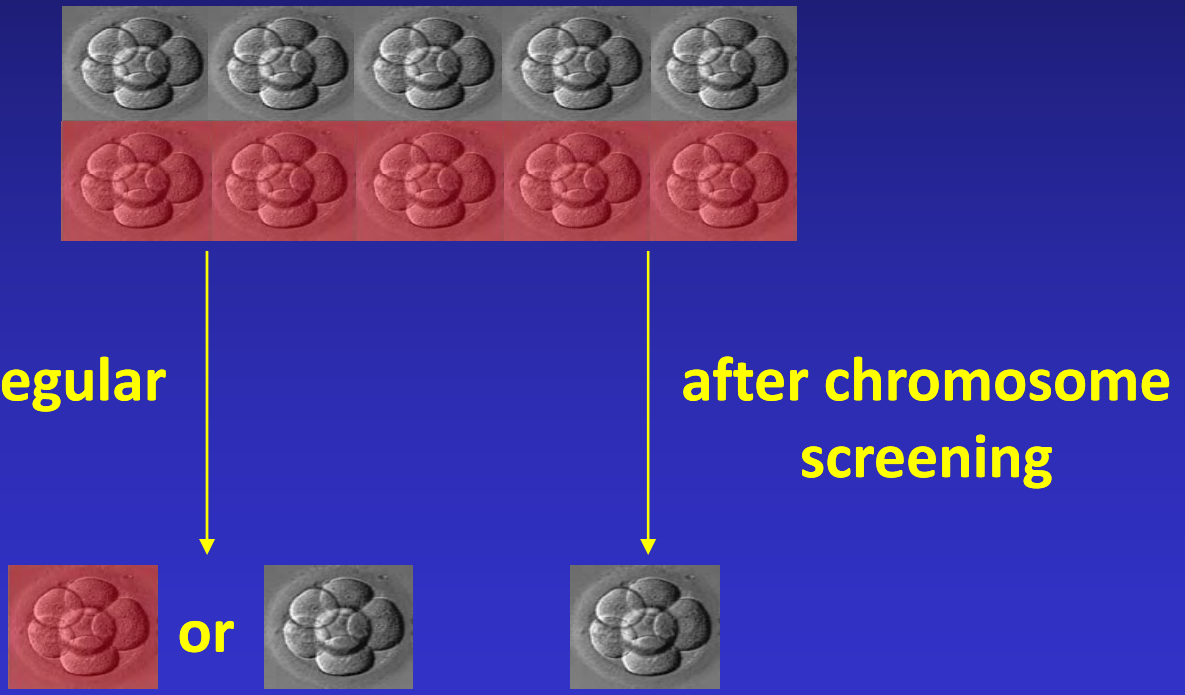
Problem increases with advancing maternal age

As aneuploidy increases age, so implantation rate decreases



Preimplantation genetic screening (PGS)

Standard embryo evaluations do not reveal embryos with the wrong number of chromosomes



Preimplantation genetic screening (PGS)

Anticipated benefits for IVF patients

Reduce aneuploid syndromes

Reduce miscarriage

Increase embryo implantation/pregnancy rate

PGS- the controversy

- It is widely accepted that PGS reduces the risk of Down syndrome

BUT…

- Several randomised trials have shown no improvement in pregnancy rates

Increase in implantation/pregnancy- controversy

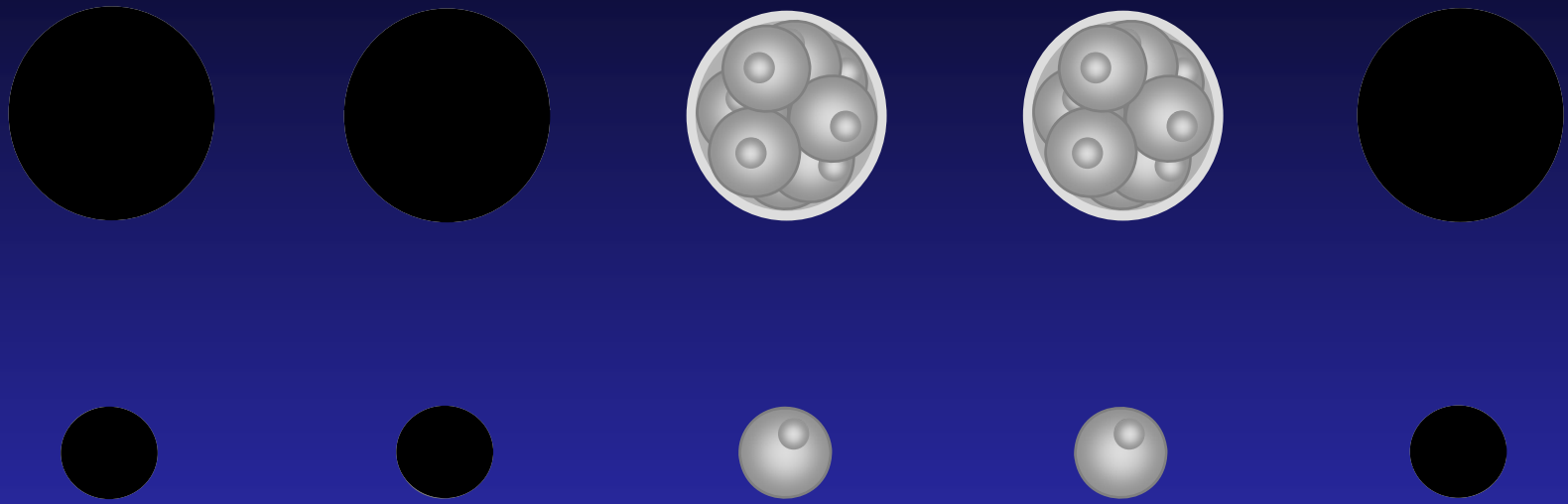
- Mastenbroek et al (2007), NEJM
- Maternal age ≥35
- 8 chromosomes assessed, randomised
- No significant improvement in implantation

Problems with negative PGS studies

BUT….

- **Many patients with <5 embryos included in study (mean 4.8)**
  Little selection possible
- **Many 4-cell embryos biopsied**
  Developmental potential reduced
- **20% of tests failed to produce a result**
  Literature 6-20 times less failure, little selection possible
- **Did not test chromosomes 15 & 22 (only 28% of aneuploidies detected)**
  Many abnormal embryos undetected , little selection possible
- **Implantation rate for biopsied, non-diagnosed embryos= 6%**
  Developmental potential reduced. Lack of biopsy experience?
- **Implantation PGS group= 11.7%**

Problems with negative PGS studies



Critically damaged by biopsy

Only 28% of aneuploidies detected

No result

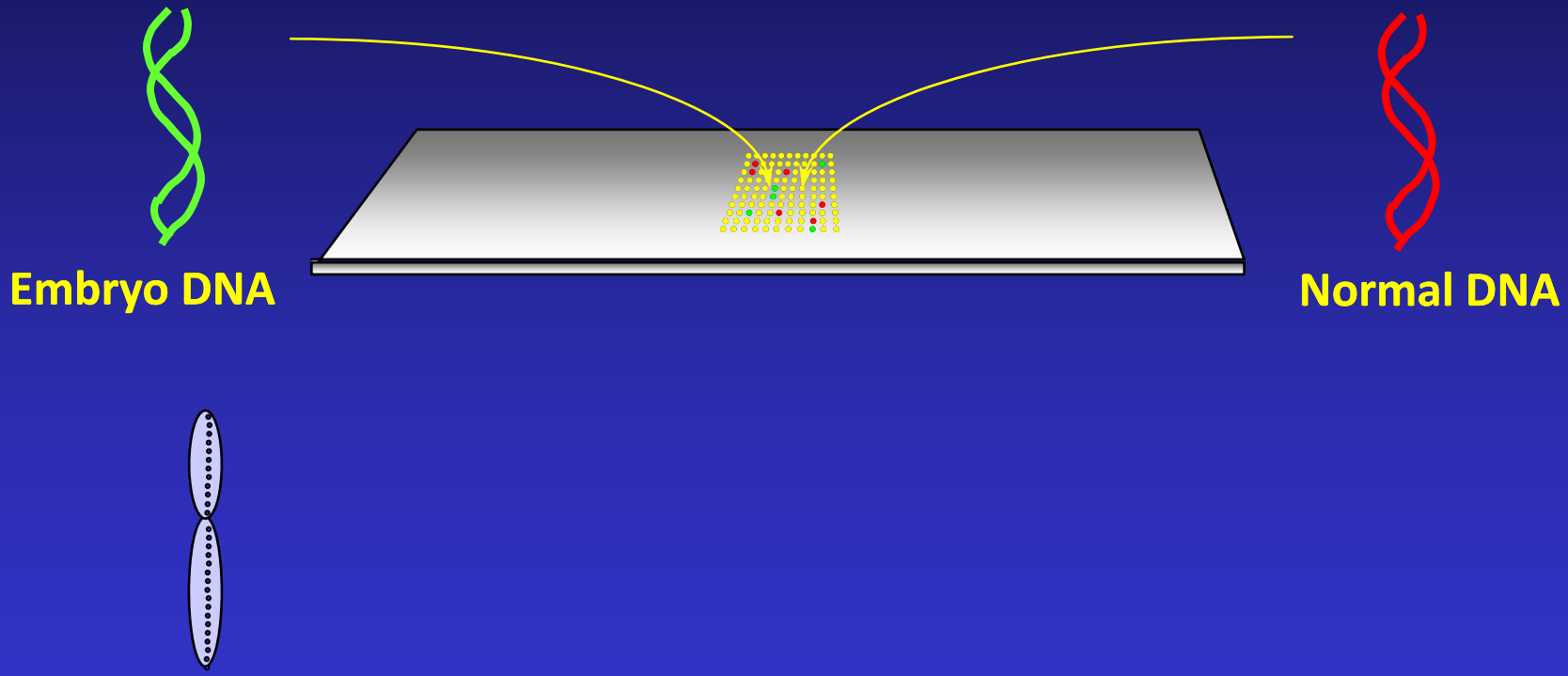
Pool of embryos reduced while little selective advantage has been gained

Legitimate criticisms of traditional PGS methods

- Methodologies are not robust, limiting application
- Biopsy can have a serious impact if poorly performed
- Mosaicism will lead to the exclusion of a small number of potentially viable embryos
- No randomized study has proven that PGS is beneficial

Microarray comparative genomic hybridization

- Rapid – results in 24 hours
- Allows the copy number of every chromosome to be determined



Gutierrez-Mateo et al., Fertility & Sterility 2010; Fragouli et al., Human Reproduction 2011

Microarray comparative genomic hybridization

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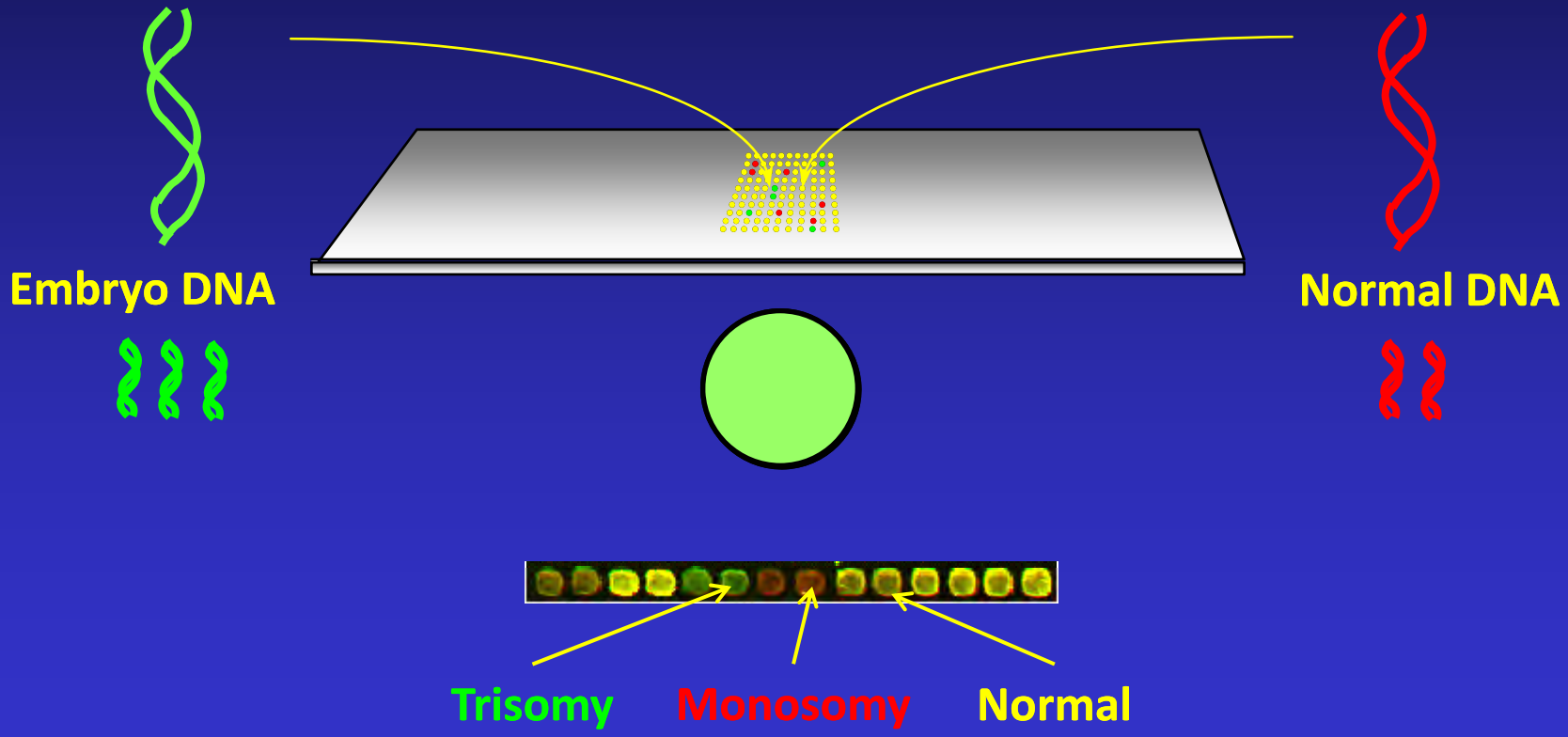
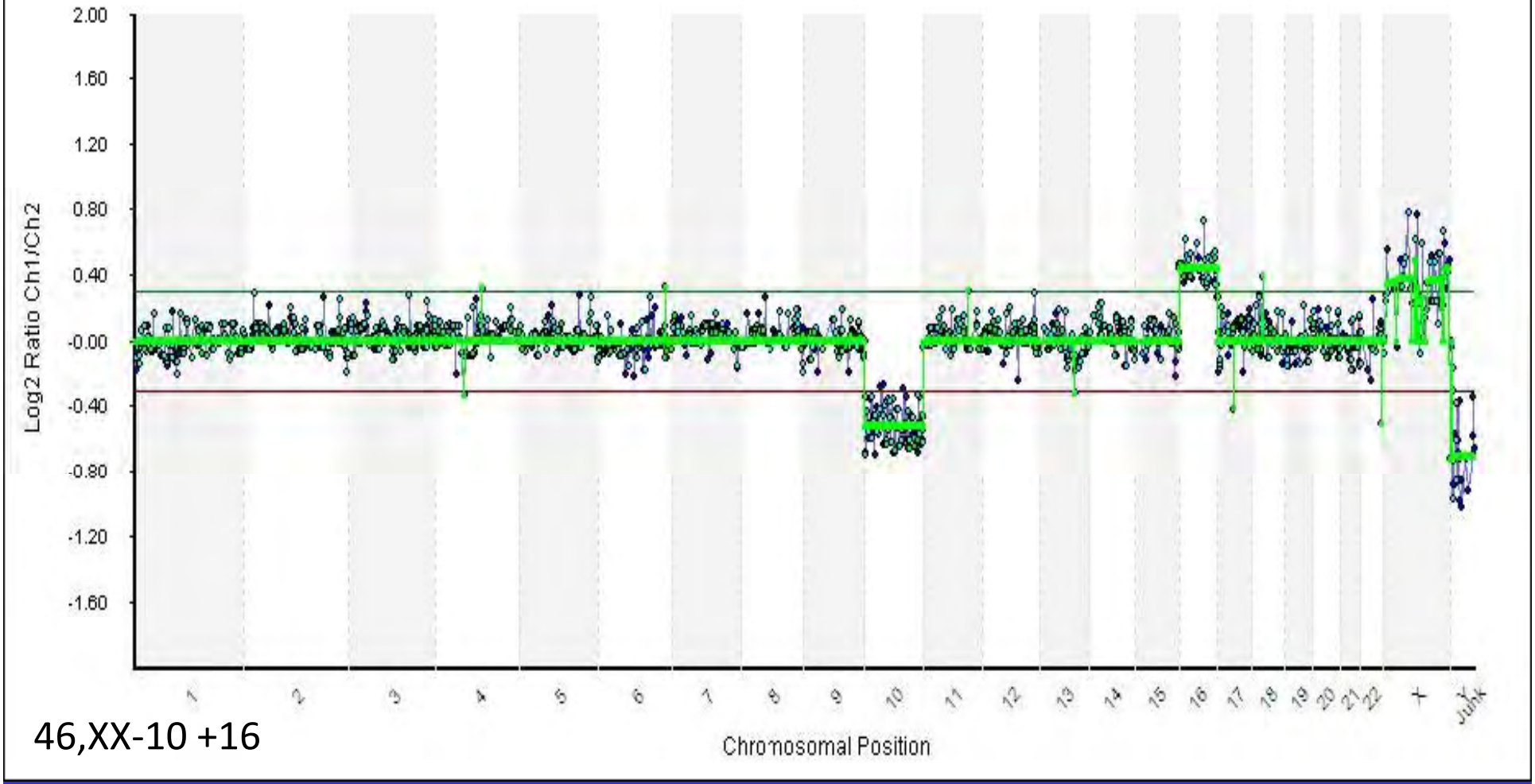


Gutierrez-Mateo et al., 2010; Fragouli et al., 2010

Microarray-CGH (array-CGH or aCGH)



46,XX-10 +16

Clinical application of CGH

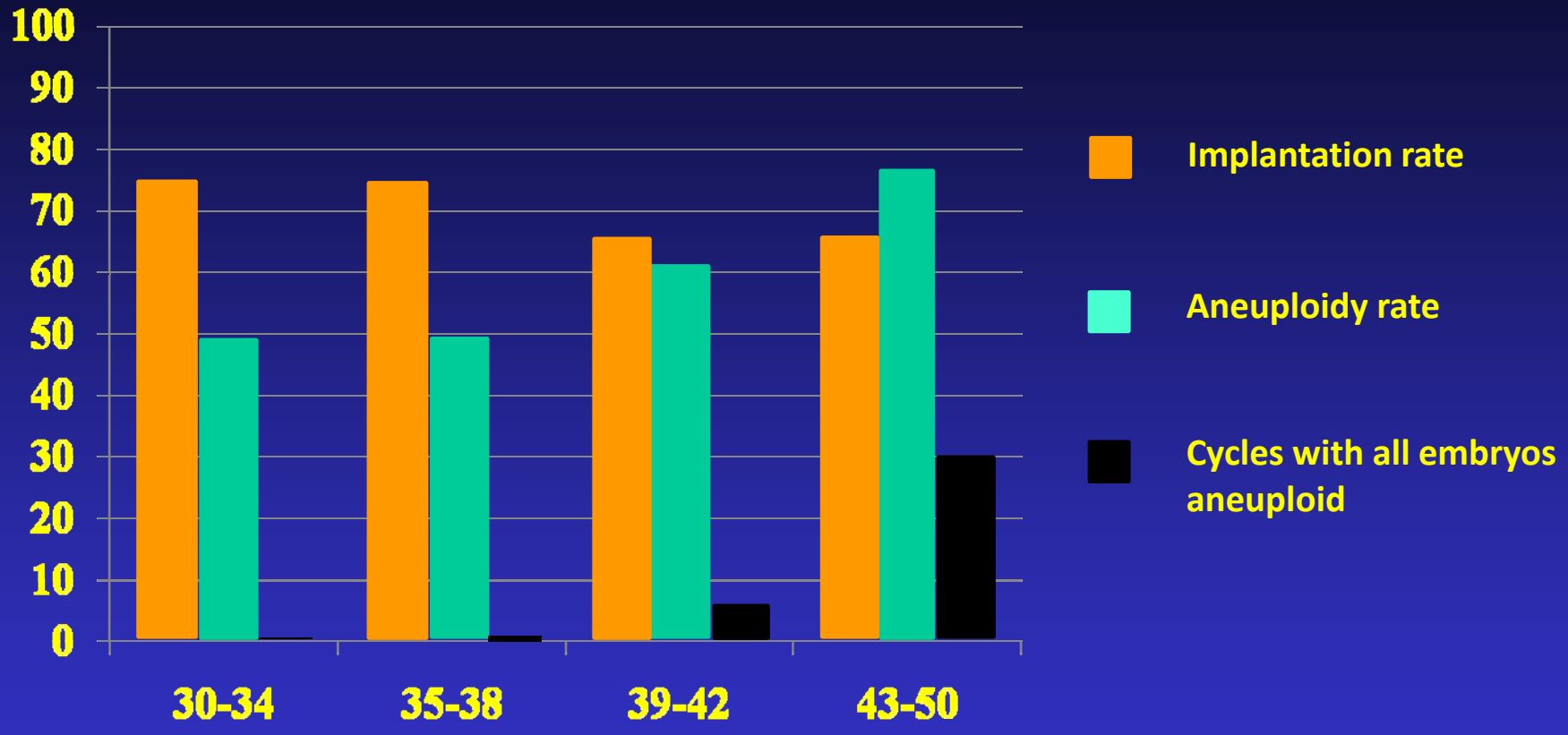
Analysis of blastocyst stage

Biopsy of several cells is possible

- Diagnosis robust and accurate
- Little or no impact of embryo biopsy

Fragouli et al., 2010; Schoolcraft et al., 2010

Blastocyst CGH- clinical results



Wells and Fragouli, unpublished

Analysis of mutations in the DNA sequence

(causing single gene disorders)

PGD of single gene disorders

Alternative to prenatal diagnosis - avoids pregnancy termination

>200 different single gene disorders diagnosed using PGD

PGD for any disease provided the causative mutation is known

First disease diagnosed – cystic fibrosis, 1992

PGD of single gene disorders

>100 diseases already approved by the HFEA

Adrenoleukodystrophy (Adrenomyeloneuropathy)
Agammaglobulinaemia
Alpers Syndrome
α thalassaemia/mental retardation syndrome
Alport's Syndrome
Alzheimers Disease - early onset
Anderson Fabry Disease
Androgen Insensitivity Syndrome
Aplastic anaemia - severe
Barth Syndrome
Battens Disease (infantile)
Beta Hydroxyisobuyryl CoA Hydrolase Deficiency (Methacryic Aciduria)
Beta Thalassaemia
Bilateral Frontoparietal Polymicrogyria
BRCA 1 (increased susceptibility to breast cancer)*
Bruton Agammaglobulinemia Tyrosine Kinase
Cardiac Valvular Dysplasia
Carney Complex
Charcot Marie Tooth Disease
Chondrodysplasia Punctata
Choroideraemia
Chronic Granulomatous Disease
Coffin-Lowry Syndrome
Congenital Adrenal Hyperplasia
Congenital Fibrosis of the Extraocular Muscles
Congenital Stationary Night Blindness
Crouzon Syndrome
Cystic Fibrosis
Cystinosis
Diamond Blackfan Anaemia
Dystonia 1 Torsion Autosomal Dominant (DYT1)
Ectodermal dysplasia (Hypohidrotic)
Epidermolysis Bullosa (Hallopeau-Siemens & Herlitz junctional)
Facioscapulohumeral Dystrophy
Familial Adenomatous polyposis coli
Fanconi's Anaemia A
Fanconi's Anaemia C
Fragile X Syndrome
Gaucher's Disease (Type II)
Gonadal mosaicism
Greig's Cephalopolysyndactyly
Haemophilia A

PGD of single gene disorders

Diseases already approved by the HFEA

Haemophilia B
Hereditary diffuse gastric cancer*
Hereditary motor and sensory neuropathies
Homozygous Familial Hypercholesterolaemia
Hunters Syndrome
Huntington's Disease
Hydrocephalus
Hydroxyisobuyryl CoA Hydrolase Deficiency
Hyper IgM Syndrome - Hypogammaglobulinaemia
Hypospadias (severe)
Ichthyosis
Incontinentia Pigmenti
Juvenile Retinoschisis
Krabbe Disease
Leber's hereditary optic neuropathy / Lebers Optic atrophy
Leigh's (subacute necrotising encephalopathy of childhood)
Lenz syndrome
Lesch Nyhan Syndrome
Leukocyte Adhesion Deficiency (Type I)
Li-Fraumeni Syndrome
Lymphoproliferative Syndrome
Lynch Syndrome (MLH 2)
Lynch syndrome (MLH 1)
Macular Dystrophy (childhood onset - variant of Retinitis pigmentosa)
Marfan Syndrome
Medium-chain acyl-Co A dehydrogenase
MELAS (Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)
Menkes Syndrome
Myoclonic epilepsy and ragged red fibres (MERFF)
Metachromatic Leukodystrophy
Multiple Endocrine Neoplasia (Type I)
Multiple Exostoses
Muscular Dystrophy (Beckers)
Muscular Dystrophy (Duchenne)
Muscular dystrophy (Oculopharangeal)
Myotonic Dystrophy
Myotublar myopathy
Neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP)
Neurofibromatosis type I

PGD of single gene disorders

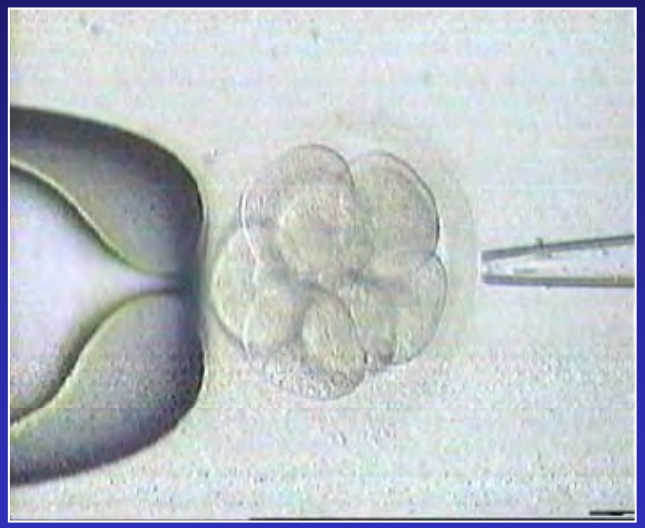
Diseases already approved by the HFEA

Neurofibromatosis type II
Niemann Pick Disease Type C
Ornithine carbamoyl transferase Deficiency (OTC)
Ornithine transcarbamylase deficiency (OTD)
Osteogenesis Imperfecta (Type II)
Ostheopathia Striata with Cranial Sclerosis
Otopalatodigital syndrome (Type 2)
Partial Lipodystrophy, Familial (Type 2)
Pelizaeus Merzbacher Disease
Phenylketonuria (PKU)
Plakophilin 1 (PKP1) associated ectodermal dysplasia syndrome
Polycystic kidney disease
Pompe Disease (early onset)
Prader Willi Syndrome
Pyrodoxine-dependent seizures
Recurrent Digynic Triploidy
Recurrent hydatitiform mole
Retinitis Pigmentosa
Retinoblastoma
Retinoschisis (Juvenile)
Sandhoff Disease
Sensorineural deafness - autosomal recessive non-syndromic
Severe Combined Immune Deficiency (x-linked)
Sickle Cell Anaemia
Spastic paraplegia
Spinal Muscular Atrophy (SMA1)
Tay Sachs Disease (infantile onset)
Torsion Dystonia
Treacher Collins Syndrome
Tuberous Sclerosis (TSC2)*
Turner's syndrome (Mosaic)
Von Hippel Lindau Syndrome*
Wiscott-Aldrich Syndrome*
Wolman's Disease (Acid Lipase Deficiency)

Chromosome rearrangements

HLA-typing

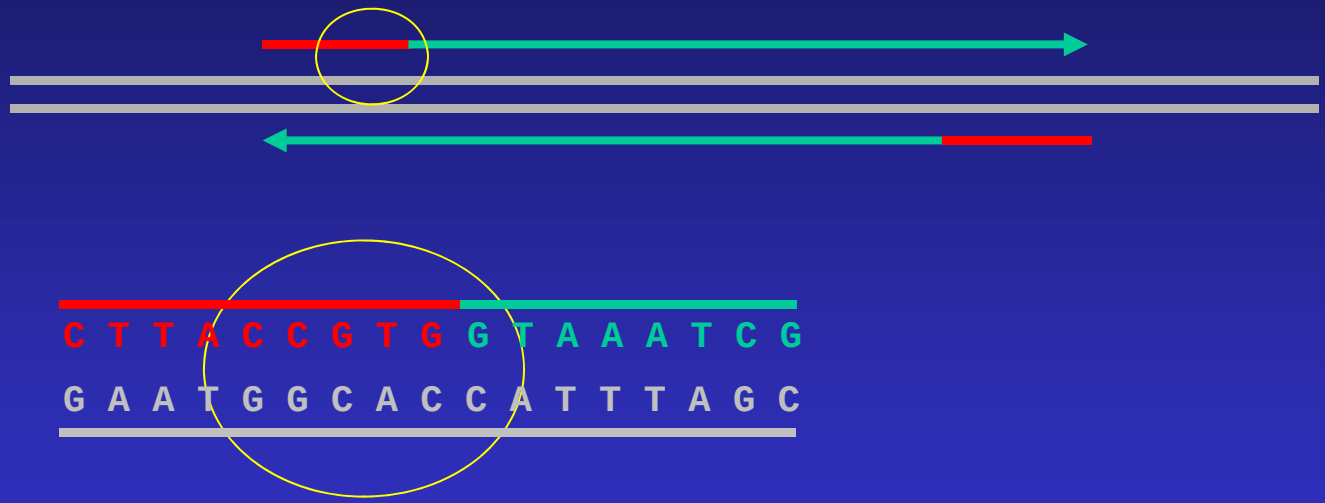
Cleavage stage biopsy



- Used by majority of PGD labs
- Biopsy at 6-10 cell stage (day 3)
- Blastomeres totipotent
- 1-2 cells for analysis

DNA amplification: the polymerase chain reaction

- Enzymatic method for copying specific DNA sequences



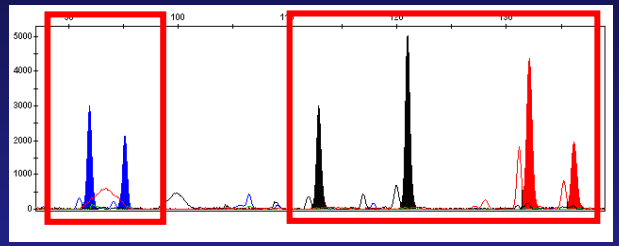
- Essential for the analysis of genes in single cells

See Wells & Sherlock 1998 for review

Generalized Diagnostic Methodology

Biopsy

PCR



Mutation detection

Polymorphism analysis

DNA fingerprint

Contamination detection

Linked polymorphisms

Diagnosis

Report

Ethical questions

Application of PGD to late onset disorders

HLA typing

Complex (polygenic) disorders