PRE-IMPLANTATION GENETIC DIAGNOSIS AND ASSISTED REPRODUCTIVE TECHNOLOGY IN HAEMOPHILIA

DR PENELOPE FOSTER

WHAT IS PGD ?

- early embryo diagnosis
- allows identification of gender or abnormal gene
- can select which embryos to transfer to patient
- an alternative to antenatal testing and termination of affected pregnancy

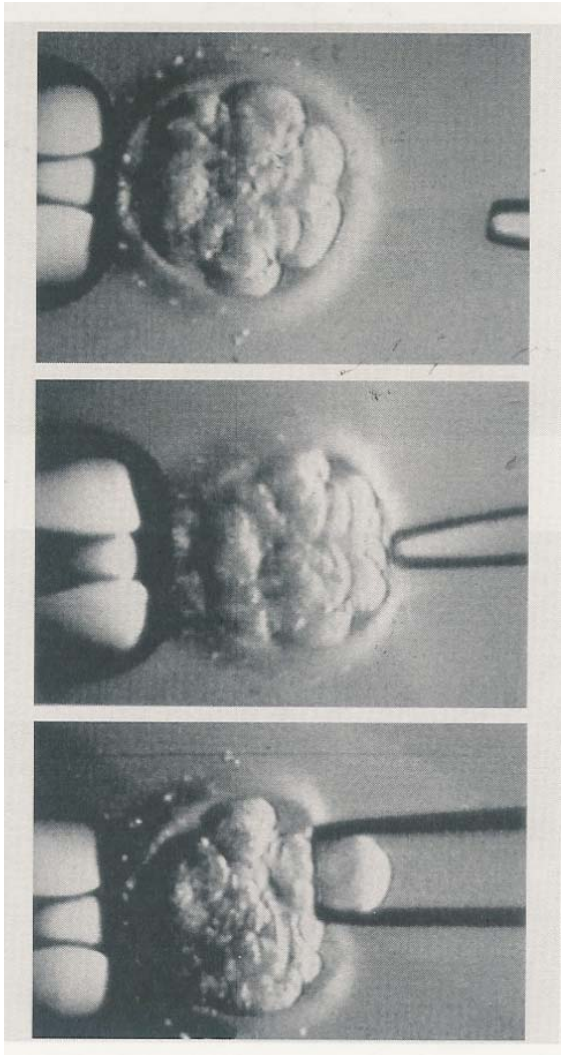
TECHNIQUE OF PGD

.standard IVF cycle

.biopsy of 1 or 2 cells from day 3 embryo

.diagnostic testing on biopsied cells

.selection of embryos for transfer



PGD IN HAEMOPHILIA

OPTIONS (1)

GENDER SELECTION

If male partner has haemophilia:

(all male offspring unaffected, all female offspring carriers)

Gender selection enables transfer of male embryos only, excludes all carriers

If female partner is carrier:

(50% male offspring affected, 50% female offspring carriers)

Gender selection enables transfer of female embryos only,

but 50% discarded male embryos unaffected, 50% of transferred female embryos carriers

PGD IN HAEMOPHILIA

OPTIONS (2)

SPECIFIC GENE DETECTION

(identifies embryos with the X chromosome mutation assoc with haemophilia)

.more embryos available for transfer (~60%)

.avoids discarding unaffected male embryos

.avoids transfer of carrier female embryos

GENDER SELECTION - FISH

FLUORESCENT IN-SITU HYBRIDISATION

.detects the presence and number of particular chromosomes inc X and Y

.single cell from embryo fixed to slide

.apply FISH probes

  .labelled DNA probes which bind to complementary sequences on specific chromosomes

  .probes labelled with coloured fluorochromes

  .coloured spots indicate presence of sequence

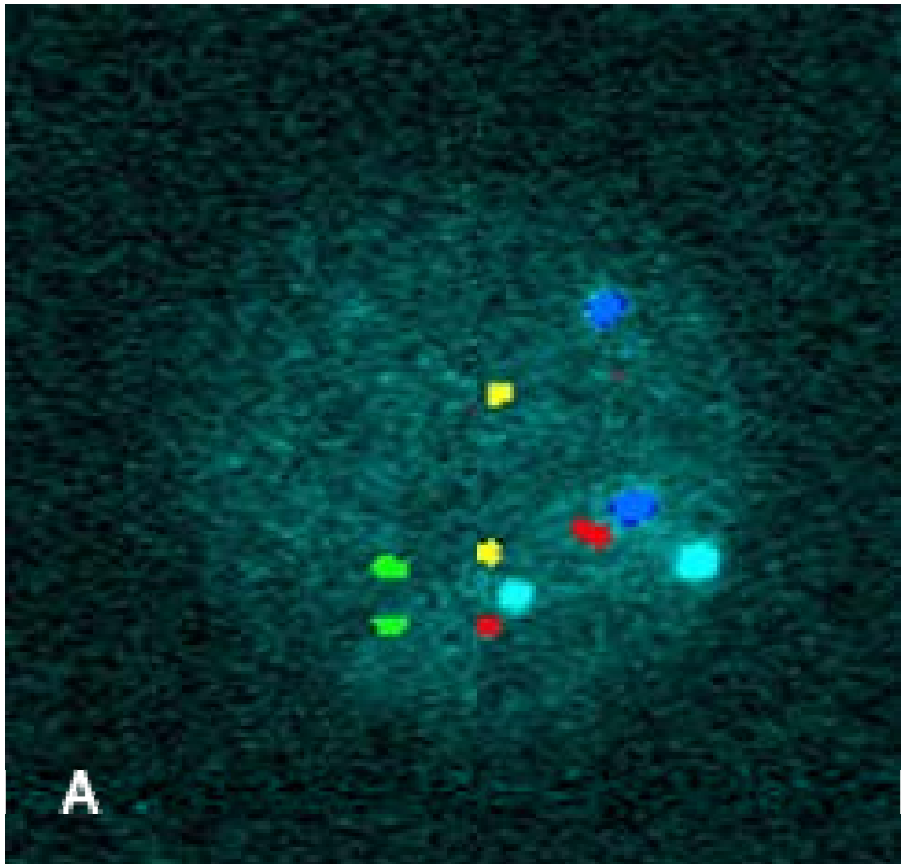
.8-probe FISH – chromosomes 4,13,16,18,21,22,X,Y

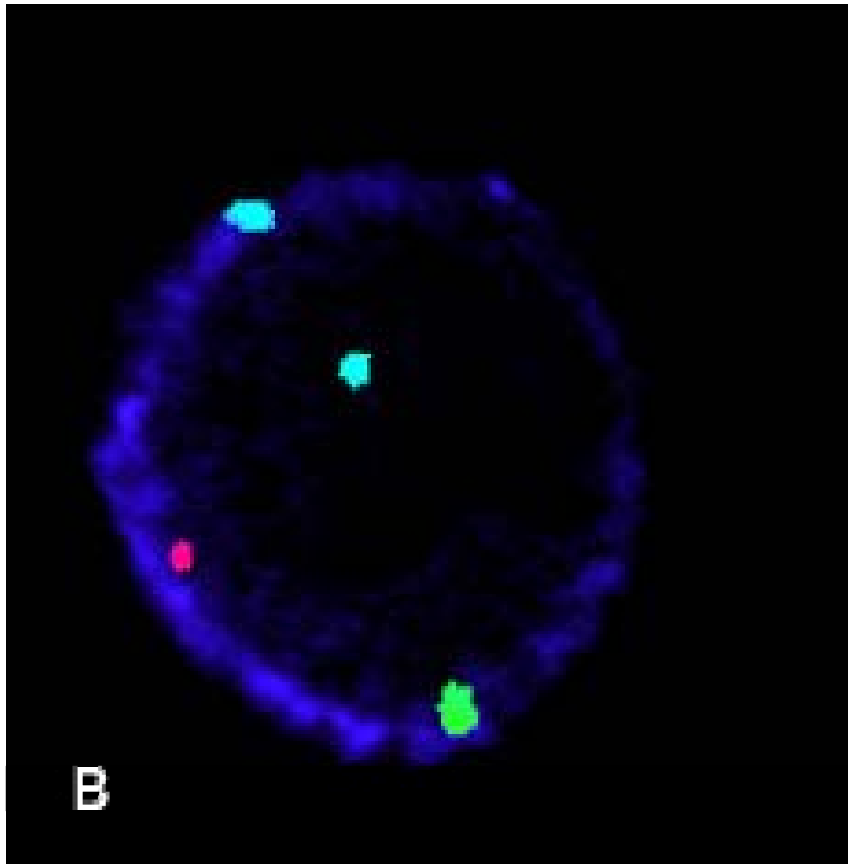
.select euploid XX or XY embryos for transfer

.only ~15% of embryos suitable for embryo transfer

.60% patients will not have embryo transfer

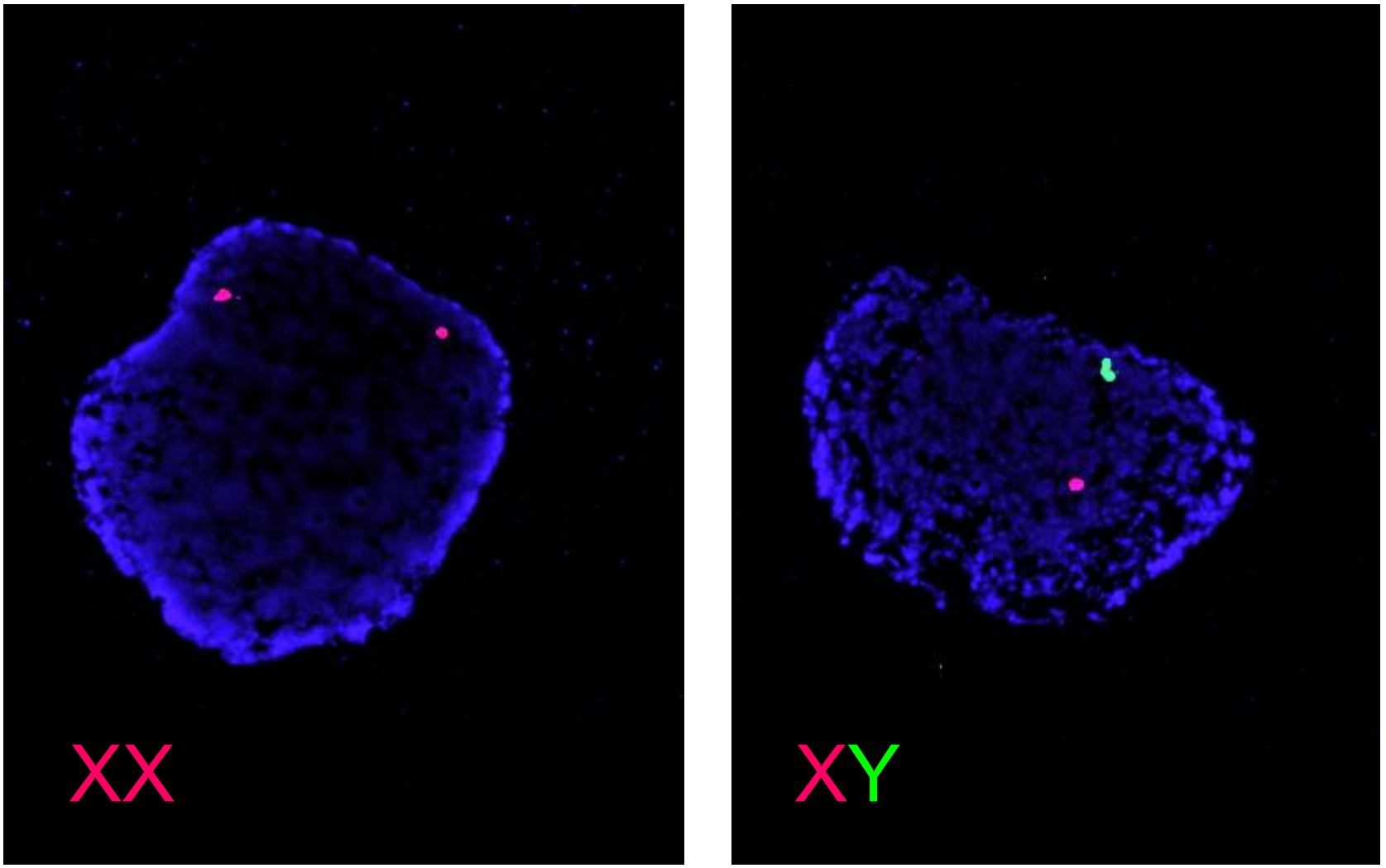
FISH ON BLASTOMERES





13, 16, 18, 21, 22

X, Y, 4


XX
XY

PGD FOR SPECIFIC GENE DETECTION

.looking for the presence or absence of the haemophilia mutation in each embryo tested

.2 cells from each embryo

. DNA amplification (by PCR)

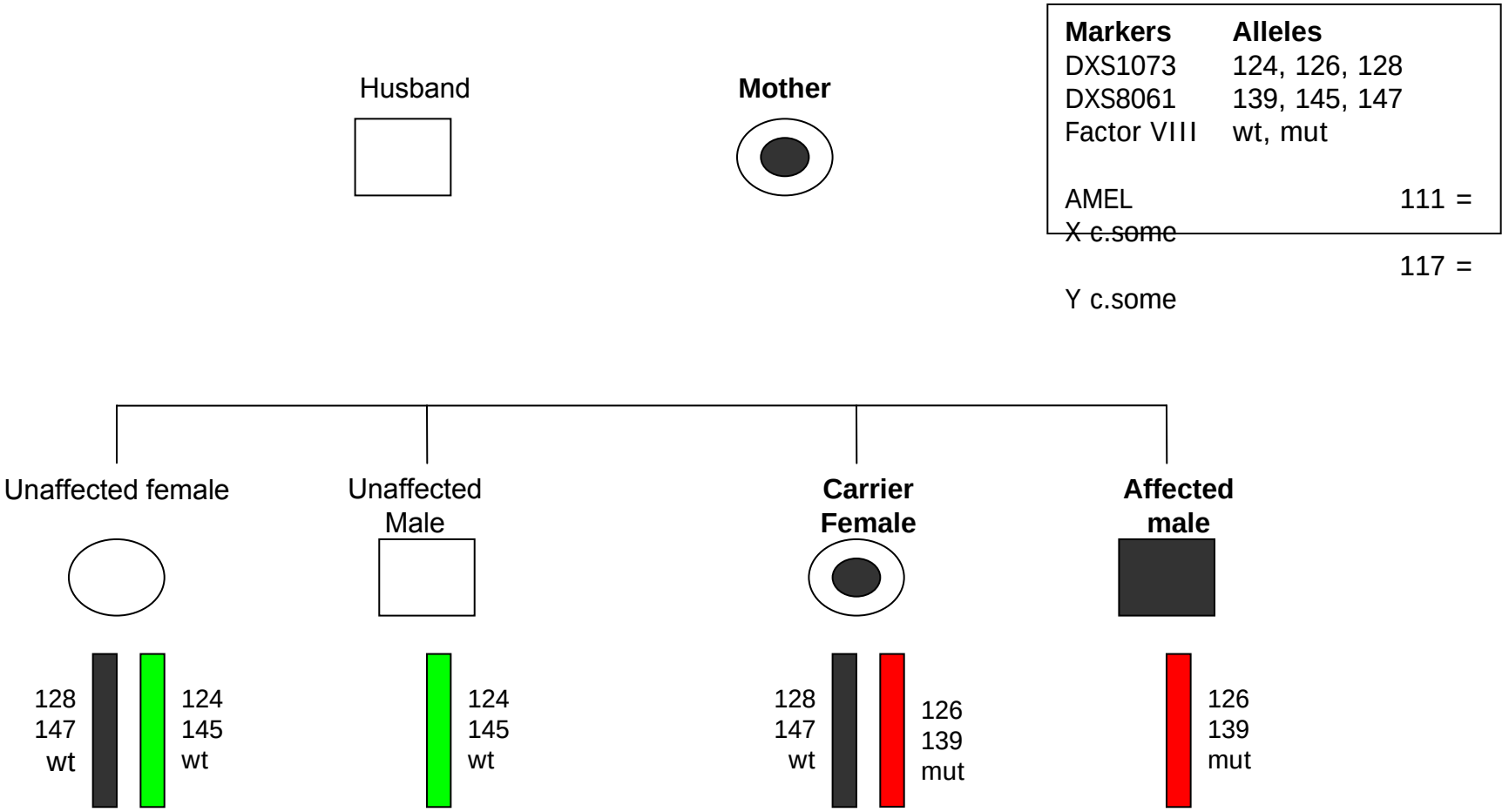
.fragment analysis on DNA sequencer

.analyse polymorphic markers along the F8 gene

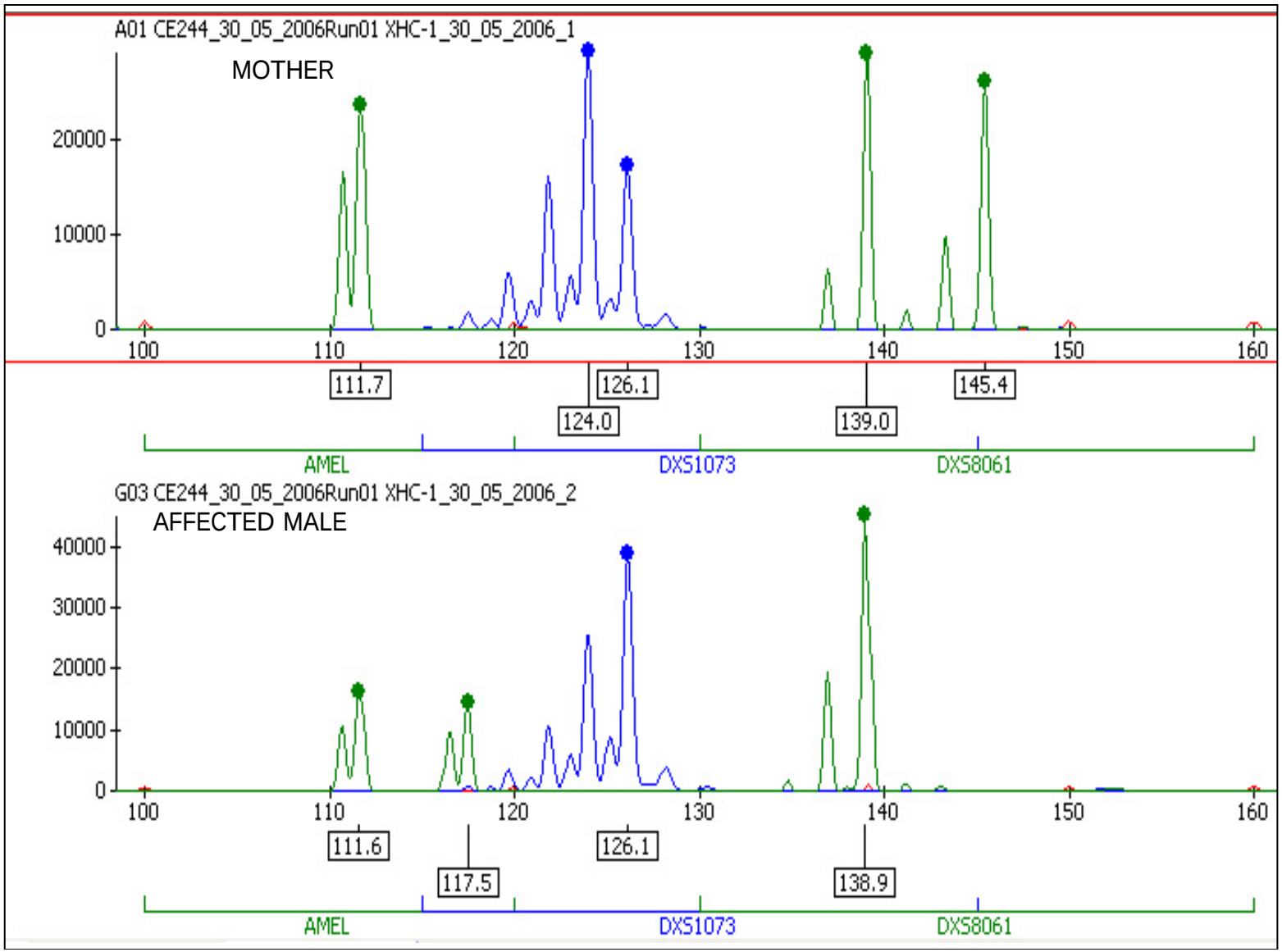
.inclusion of informative markers (belt and braces)

.individualised tests for each couple

.significant time and effort required for each test


Husband
Mother
Markers Alleles
DXS1073 124, 126, 128
DXS8061 139, 145, 147
Factor VIII wt, mut
AMEL 111 =
X c.some
117 =
Y c.some
Unaffected female
Unaffected Male
Carrier Female
Affected male
128
147
wt
124
145
wt
124
145
wt
128
147
wt
126
139
mut
126
139
mut

RESULTS OF PCR ANALYSIS FOR HAEMOPHILIA A



PGD options in Haemophilia

If man has haemophilia:

gender selection (for male embryos)
but with 8-probe FISH
only ~ 10 -15% embryos suitable for transfer
in 60% cycles there will be no transfer

gene detection
no value as all males unaffected, all females carriers

If female is carrier:

gender selection (for female embryos)
but with 8-probe FISH
only ~10 -15% embryos suitable for transfer
in 60% cycles there will be no transfer
½ discarded males unaffected, ½ transferred females carriers

gene detection
select unaffected male embryo or non-carrier female embryos
88% of cycles will have embryo transfer

PGD OUTCOMES

(MIVF data 1997 - 2008)

	GENDER SELECT			MONOGENIC		
CYCLES	56			128		
AGE	35.3			35.3		
%genetically suitable EMBRYOS	11.9			47.6		
% NO ET	62.5			11.7		
% CLIN PREG	33.3			24.8		
IMP. RATE	30.8			21.0		

MONOGENIC PGD AT MELBOURNE IVF

Tests developed to date:

- Huntington disease (direct)
- Huntington disease (exclusion)
- Cystic fibrosis
- β-thalassaemia
- α-thalassaemia
- Duchenne muscular dystrophy
- α-1-antitrypsin deficiency
- Kennedy disease
- Fragile–X
- Motor neurone disease (exclusion)
- Neurofibromatosis type 1
- Hirschprung's disease
- X-linked hydrocephalus
- Myotonic dystrophy
- Chronic granulomatous disease
- Niemann-Pick type C
- Opitz syndrome
- Leigh syndrome
- Multiple exostosis
- Rapp-Hodgkin ectodermal dysplasia
- Tuberous sclerosis
- X-chromosome deletion
- Menkes disease
- Treacher-Collins syndrome
- Retinoblastoma
- WHIM syndrome
- Mucopolysaccharoidosis IIIB
- Congenital disorder of glycosylation
- Incontinentia pigmenti
- Epidermolytic hyperkeratosis
- Congenital adrenal hyperplasia
- Hereditary deafness DFNA17
- BRCA1
- Von Hippel-Lindau syndrome
- Walker-Warburg syndrome
- BRCA2
- Familial adenomatous polyposis (FAP)
- X-linked lissencephaly
- Hunter syndrome

Multiple cases for many of these

Tests in development:

- Complex 1 deficiency
- Spinal muscular atrophy (SMA)
- Ataxia telangiectasia
- Congenital amegakaryocytic thrombocytopenia
- TTR amyloidosis
- Generalised arterial calcification of infancy (GACI)
- HNPCC (hereditary non-polyposis colon cancer

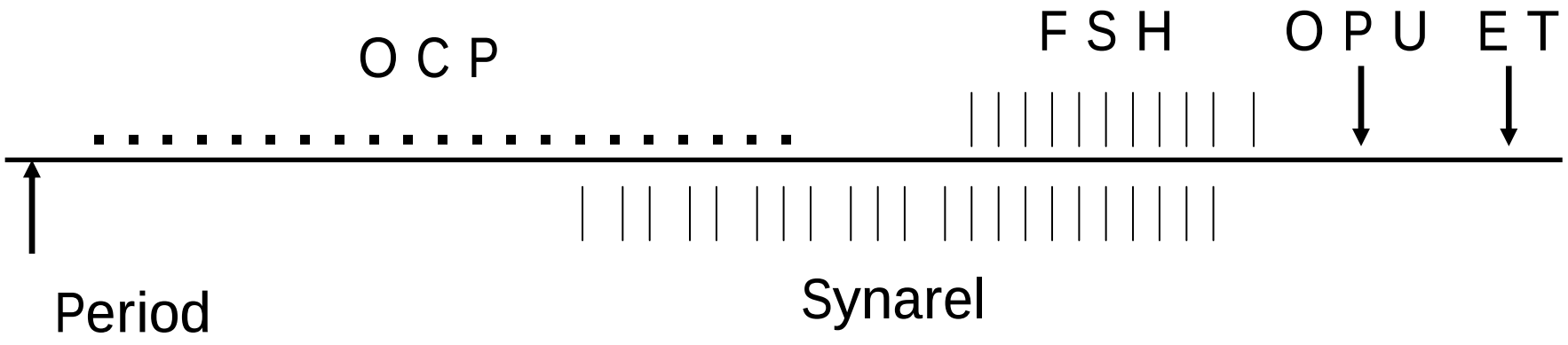
Conditions that have been diagnosed by PGD – worldwide

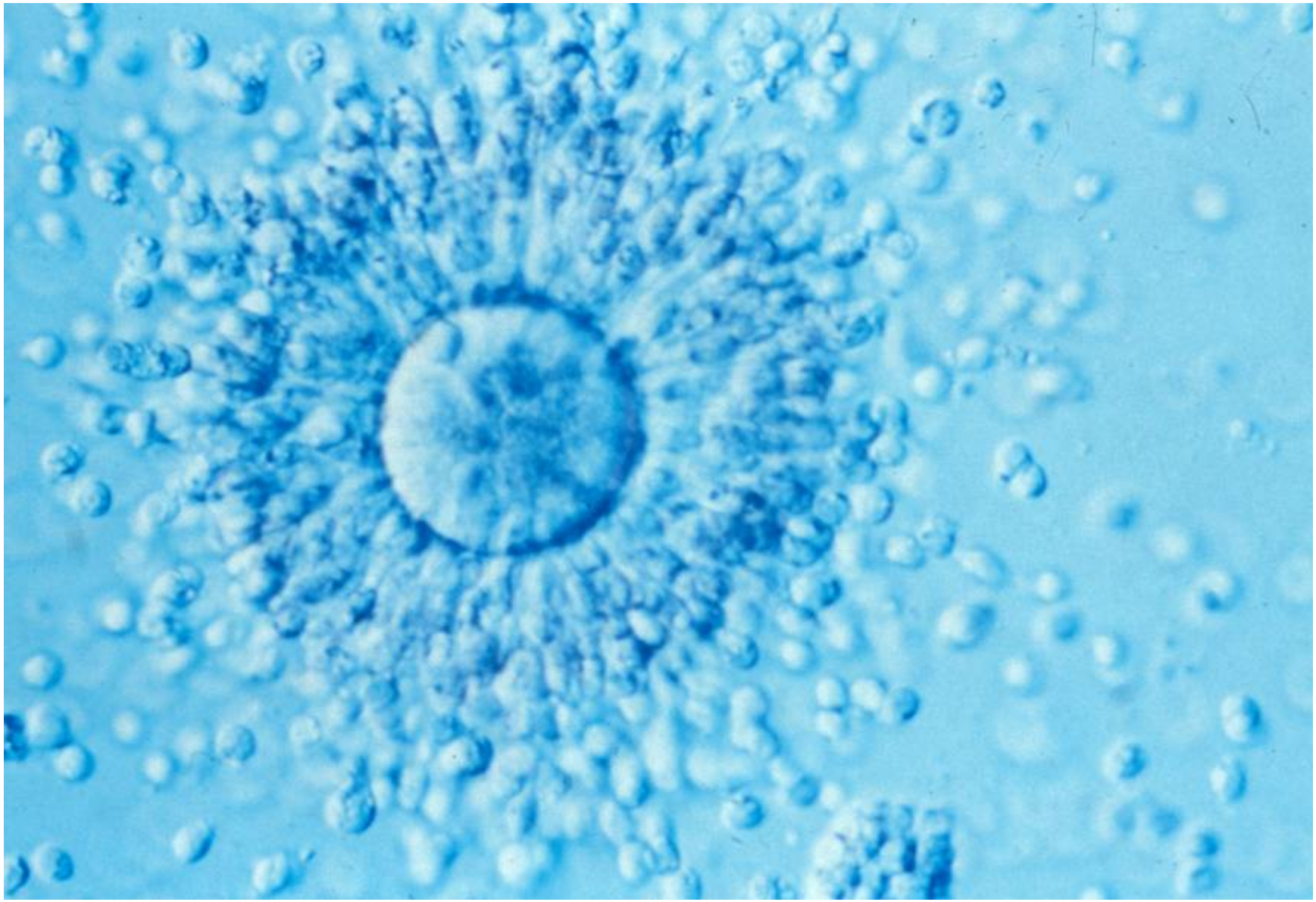
- Cystic fibrosis
- Tay Sachs disease
- β-thalassaemia
- Sickle cell anaemia
- Rh blood typing
- Spinal muscular atrophy
- Adrenogenital syndrome
- Congenital adrenal hyperplasia
- Plakophilin-1 (PKP1)
- MCAD
- CDG1C
- Epidermolysis bullosa
- Gaucher's disease
- Hyperinsulinemic hypoglycemia PHH1
- Fanconis anemia
- HLA matching
- Fragile X
- Myotonic dystrophy
- Huntingtons
- Wiscott-Aldrich syndrome
- Incontinentia pigmenti
- Ornithine transcarbamylase def.
- Myotubular myopathy
- Hunter syndrome
- Fabry disease
- Choroideraemia
- Kallman syndrome
- Coffin-Lowy syndrome
- Barth syndrome
- Hypospadias
- Golabi-Rosen syndrome
- Marfans syndrome
- Charcot-Marie-Tooth disease (type 1A)
- Amyloid polyneuropathy
- Crouzons syndrome
- NF2
- Osteogenesis imperfecta I and IV
- Stickler syndrome
- Tuberous sclerosis
- Central core disease
- Familial adenomatous polyposis coli
- Li Fraumeni syndrome
- Lesch Nyhan syndrome
- Duchenne muscular dystrophy
- Becker muscular dystrophy
- Haemophilia A
- Charcot-Marie-Tooth disease
- Retinitis pigmentosa
- Ornithine Transcarbamylase Deficiency
- Agammaglobulinemia
- Alport syndrome
- Hunter's syndrome MPSII
- Oro-facial-digital syndrome type 1
- Adrenoleukodystrophy
- Chronic granulomatous disease
- Menkes disease
- Lowe syndrome
- Ectodermal dysplasia
- Epilepsy
- BRCA1
- Ataxia
- Renal agenesis
- Norrie disease

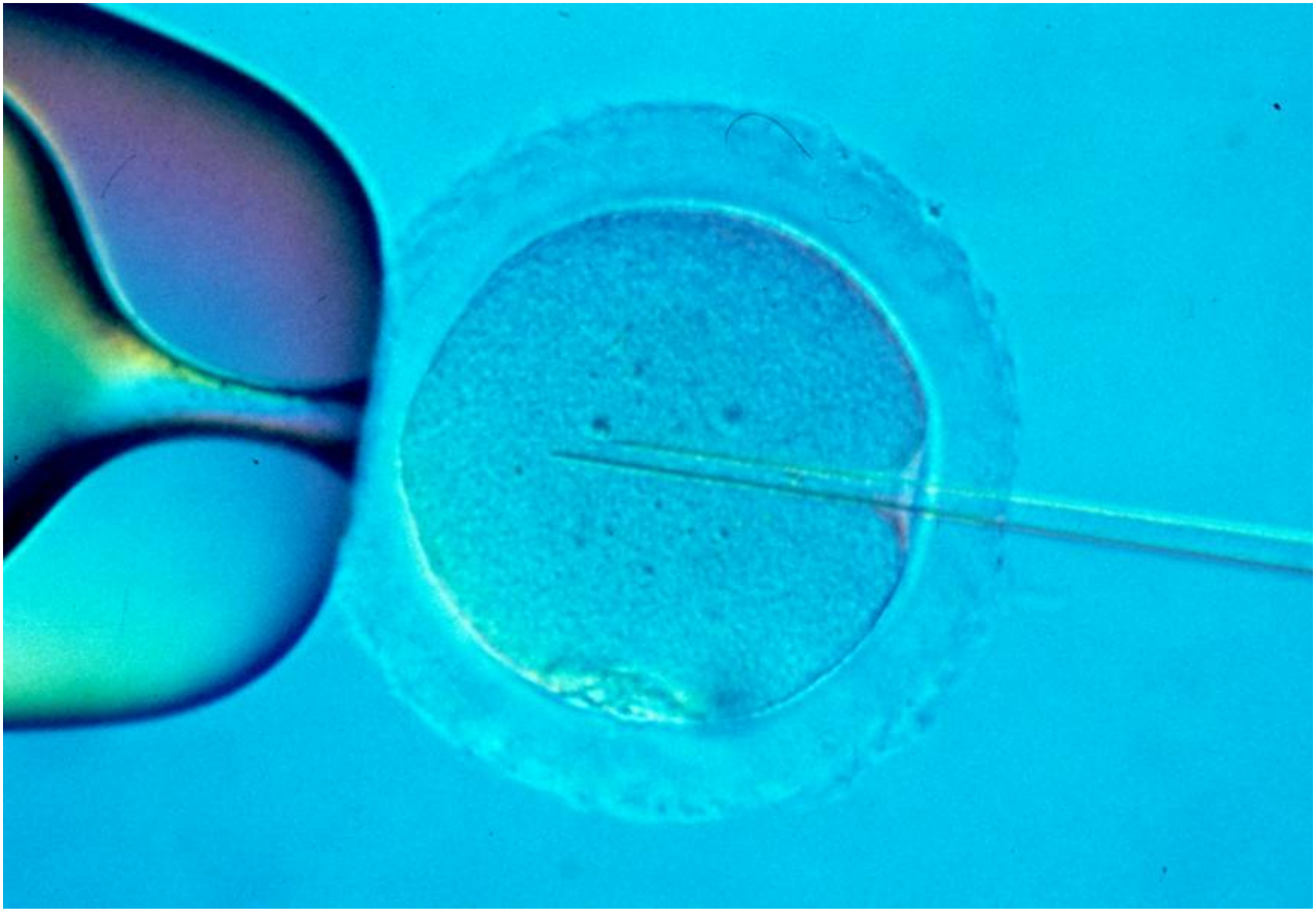
IVF Cycle

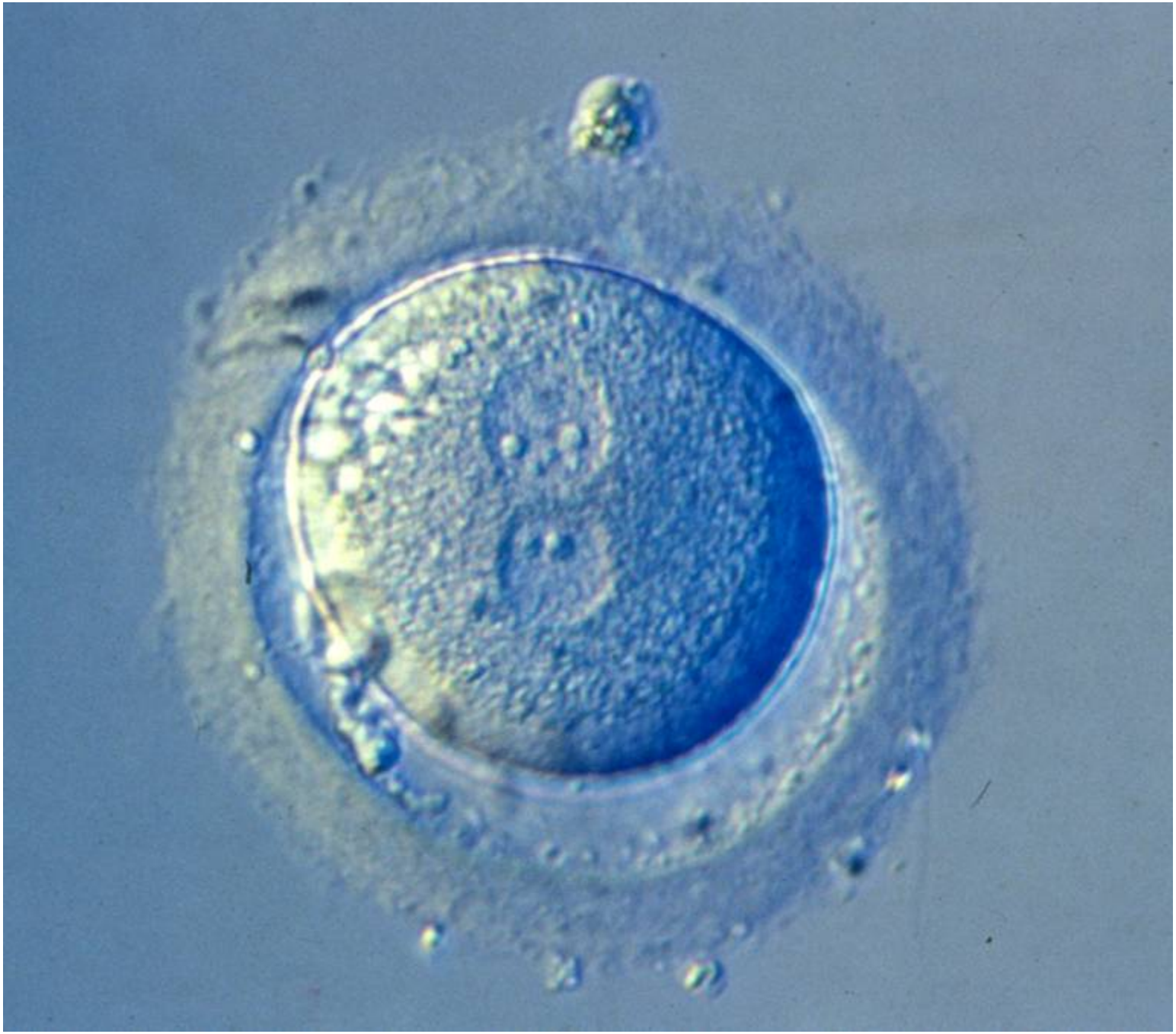
- Pituitary down – regulation with OCP & GNRH agonist (gonadotrophin –releasing hormone)
- Ovarian stimulation with r FSH (follicle stimulating hormone)
- hCG trigger
- Vaginal ultrasound – assisted OPU (ovum pick up)
- Embryo transfer (ET) 2 or 3 days after OPU

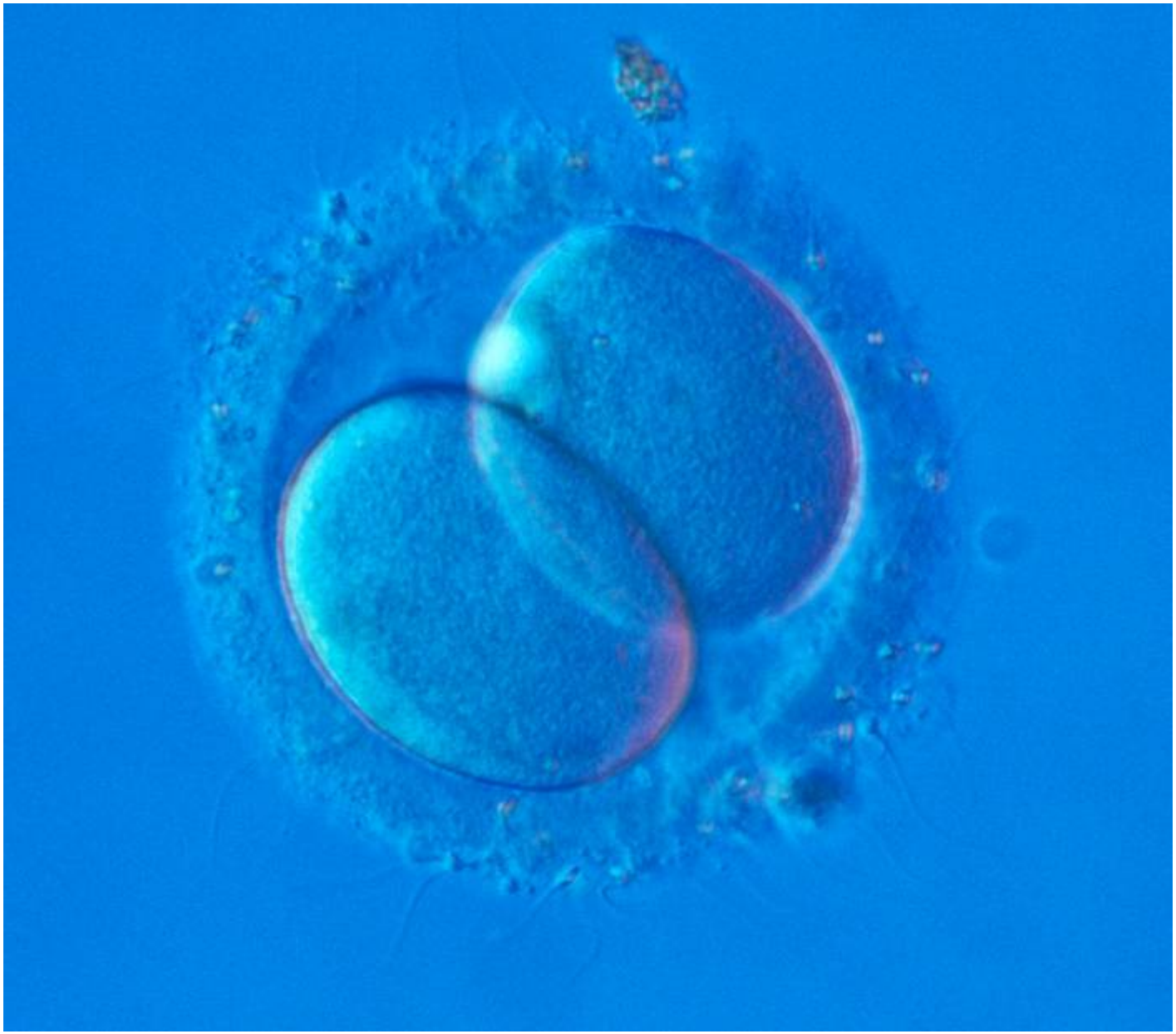
- Monitor follicular maturation with vaginal ultrasound
- Aim for cohort of “leading follicles” of 18-20mm diameter
- Average egg no / OPU = 11
- Fertilisation ~60%

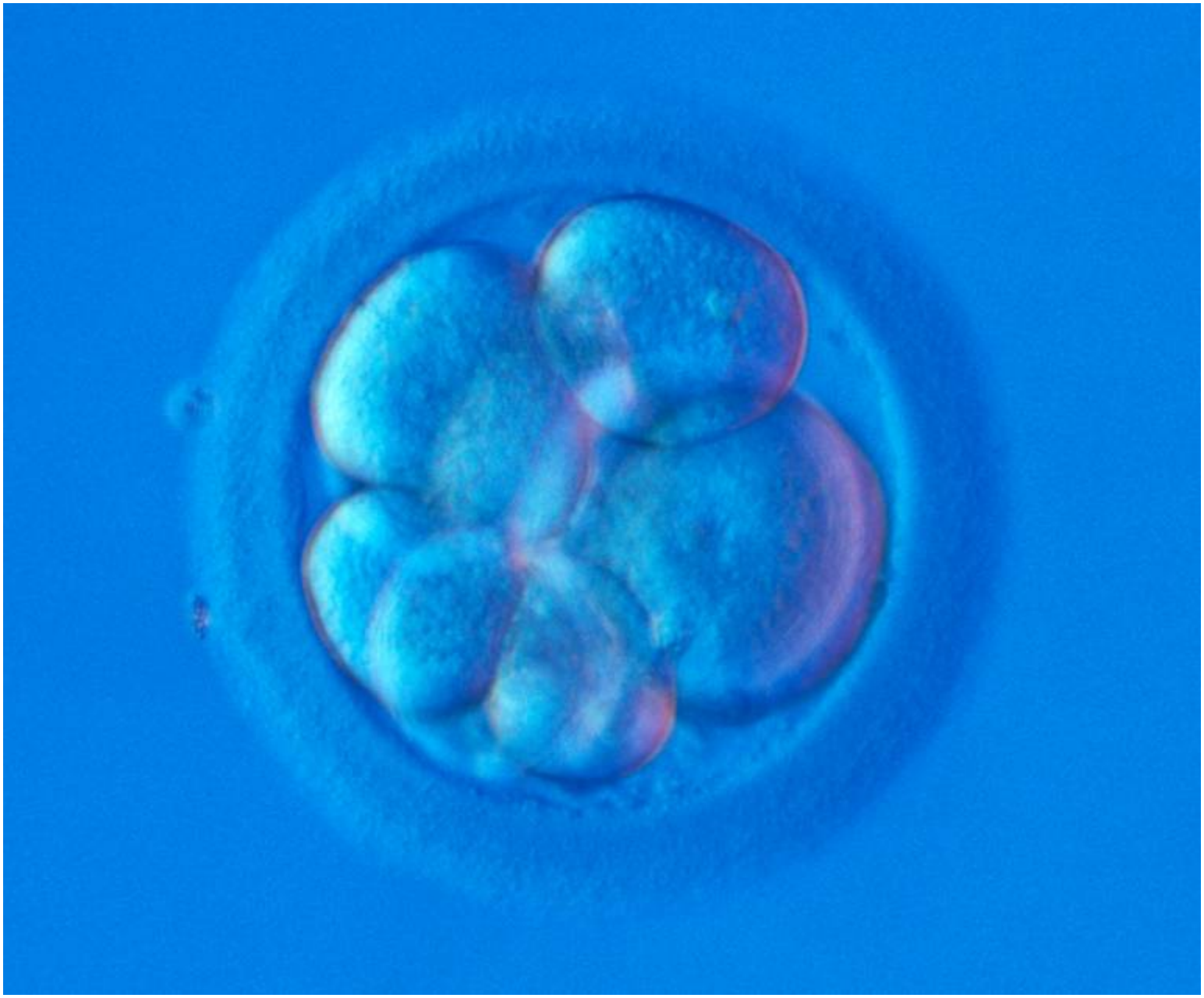

O C P
F S H
O P U
E T
Period
Synarel



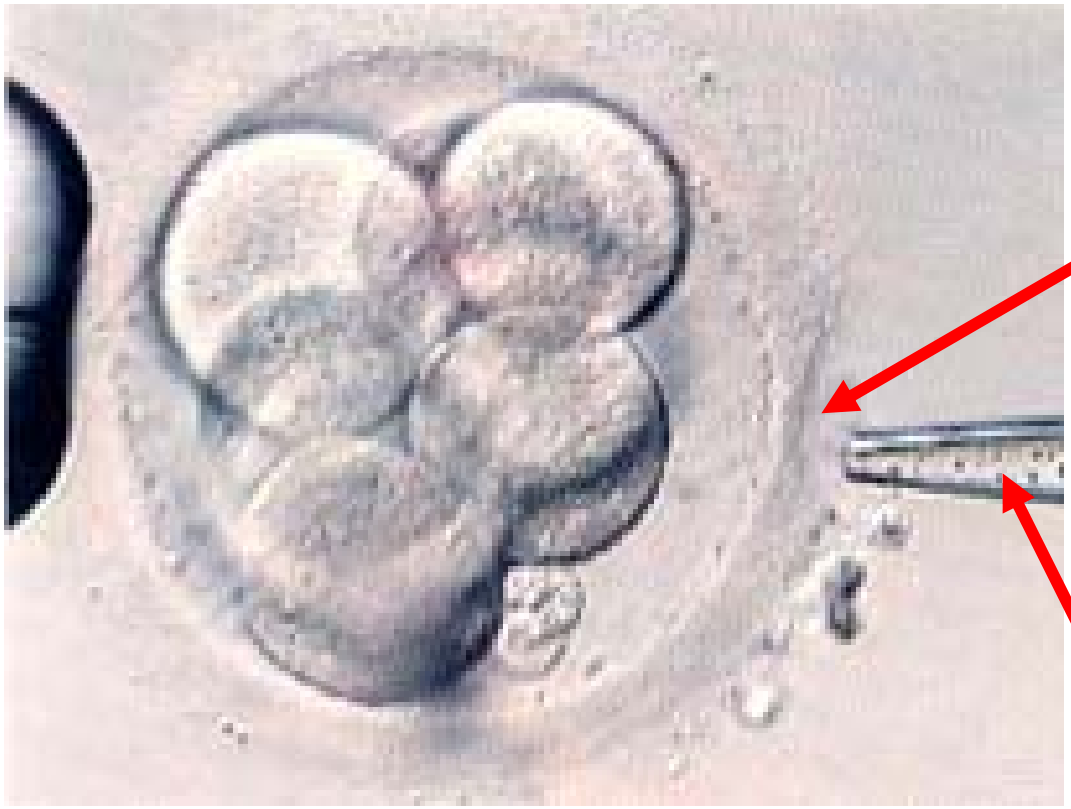




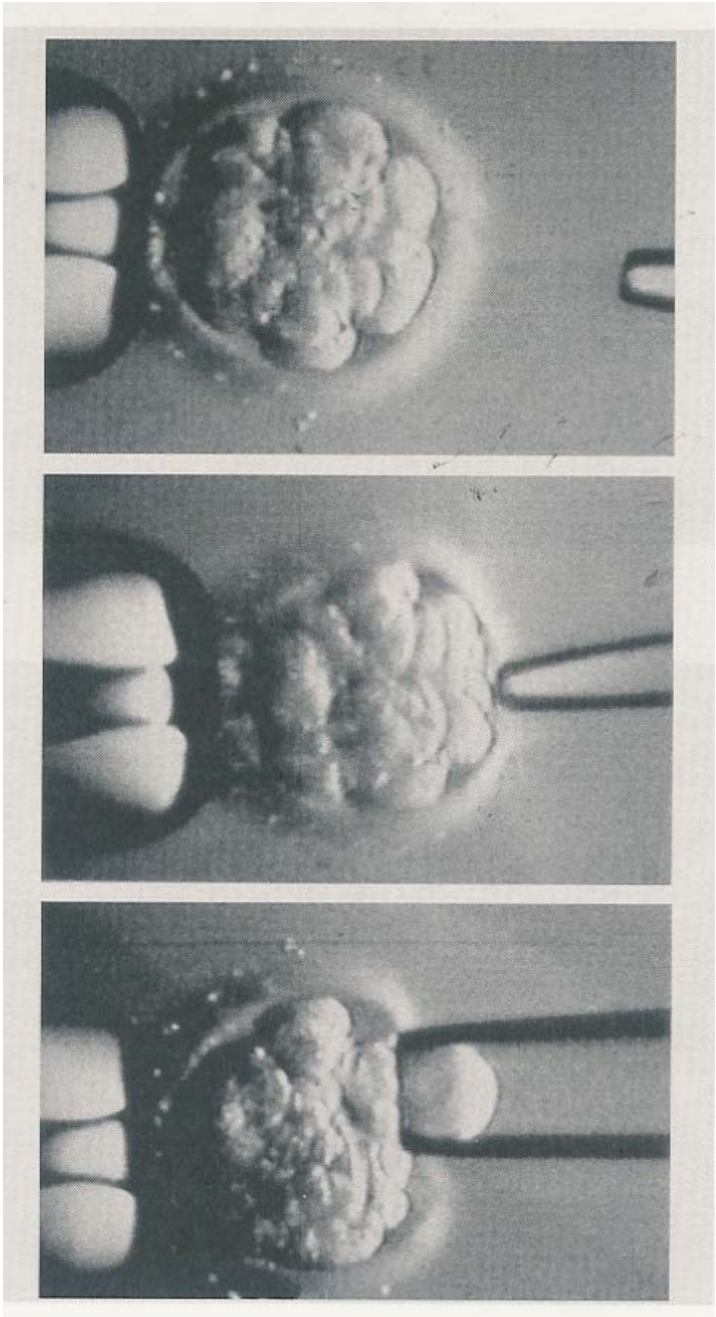




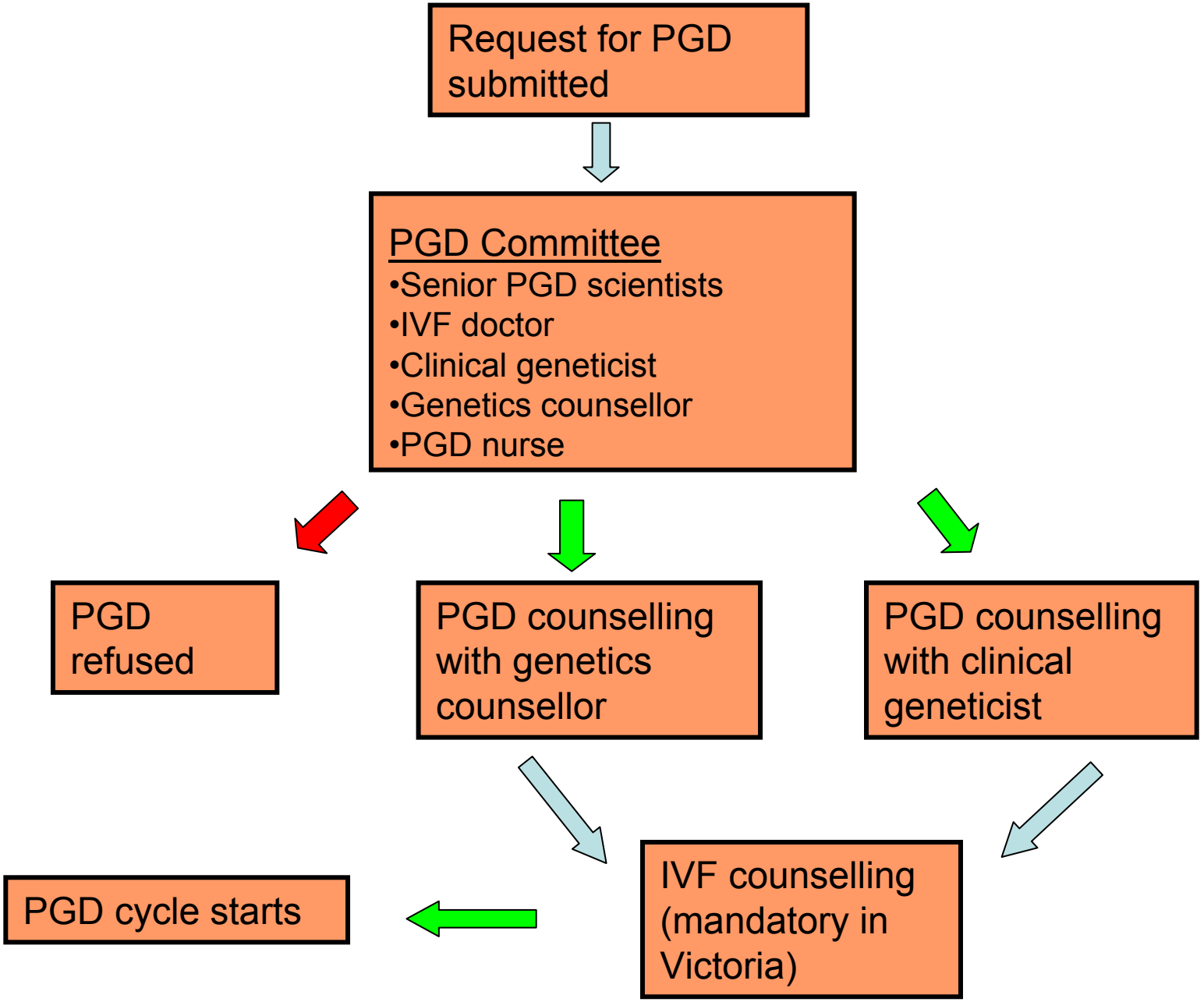
Acid drilling



Pipette loaded with acidified culture media – pH 2.4



ACCESS TO PGD AT MELBOURNE IVF



CONSENT TO PGD

- Although the degree of accuracy of these tests is high, all tests have a failure rate, and the test results could be wrong.
- A full genetic analysis is not being carried out and there are many other genetic conditions that are not being analysed or tested for.
- Finding a normal cell using FISH testing does not mean that a baby resulting from the embryo will have the normal number of chromosomes or be of the expected sex.
- In single gene defect testing, we cannot guarantee that the embryo will not have the disorder being tested for.

- It is strongly recommended that all women with PGD pregnancy consider DNA testing in early pregnancy (CVS or amniocentesis) to confirm the early embryo diagnosis.

- Spontaneous conception may occur during a PDG cycle, and all couples having PGD should avoid any form of unprotected sex during the treatment cycle.

- Rarely, some embryos may be destroyed during the biopsy procedure.

- Rarely, it may not be possible to obtain a result on an embryo.

- Embryos that are very poor quality will not be subjected to embryo biopsy and will be discarded.

PGD - BENEFITS

RELIABLE

97% embryos diagnosed

ACCURATE

misdiagnosis rate ~ 2%

RAPID

embryo biopsy and diagnostic testing completed
8 – 30 hours

TREATMENT OPTION

alternative to antenatal testing and TOP

PGD – PITFALLS

- Invasive
- Highly medicalised, requires IVF
- Expensive
- Specific feasibility testing can take months
- No guarantee of pregnancy

HIV AND ASSISTED REPRODUCTIVE TECHNOLOGY

- Chronic Viral Illness Clinic at Royal Womens Hospital Melbourne established 2002
- principle of harm minimisation (reduced risk of HIV transmission to partner and baby)
- Use of assisted reproductive technology (intra-uterine insemination or IVF)

HIV +ve MALE

.good health

.undetectable viral load (blood) for 2 months

.semen screening for HIV

2 successive samples <50 copies

=semen storage for IUI /IVF

(all semen samples tested for HIV RNA and DNA)

.risk of transmission to partner : <1/4000

CVI PROGRAMME RWH 2002 - 2009

~70 couples referred
~30 couples treated
(majority HIV-positive males)

Total number of babies born	16 inc 2 sets of twins
	1 miscarriage
	1 ectopic
	4 ongoing
no of patients on treatment	8
no of patients pre-treatment	6

There have been no cases of transmission of HIV to partner or baby

ACKNOWLEDGEMENTS
MIVF PGD TEAM

Leeanda Wilton
Sharyn Stock-Myer
Pam Matthews
Mirjana Marti
Kay Oke
Angie Giasli
Rebecca Cameron
Sophie Falle
Peter Coleman
David Amor
Fleur Cattrall
Andrea Twomey
Paisu Tang
Celine Lawler
Riddhi Marfatia
Penelope Foster

ACKNOWLEDGEMENTS
RWH CVI COMMITTEE

Michelle Giles
Gordon Baker
Harold Bourne
Suellen Peak
Gary Clarke
Suzanne Crowe
Penelope Foster
Rachael Knight
Jenny Hoy
Sam Perna
Sepehr Tabrizi
Vicky Greengrass
Gayle de Bruyckere
Suzanne Garland