Postgraduate Course in Reproductive Medecine and Reproductive Biology - Geneva, March 2003

The Genetic Consultation in Ob-Gyn : Reproductive Pathologies and Prenatal Diagnosis

Monica Gersbach-Forrer, MD
Division of Medical Genetics
Geneva University Hospital

The Genetic Consultation in Ob-Gyn :

I - Reproductive Pathologies

- Primary Sterility / Infertility and Genetics
- Secondary Sterility / Infertility and Genetics
- Genetic Causes of Male / Female Reprod. Problems
- Genetic Causes of Recurrent Fetal Loss
- Genetic Consultation Related to Reprod. Problems
- Importance of Family History
- Pre FIV, Pre ICSI Genetic Counseling

(ICSI = Intra-Cytoplasmic Sperm Injection)

The Genetic Consultation in Ob-Gyn :

II - Prenatal Diagnosis

- Etiology of Congenital Pathologies
- Genetic Resolution Levels
- Karyotype
- What Can Be Detected Today ?
- Maternal Serum Screening : II, I Trimester
- Invasive PND : Amniocentesis, CVS
- FISH Technique
- PGD = Preimplantation Genetic Diagnostics
- Conclusions

Primary Sterility / Infertility and Genetics

- **Sexe chromosome anomalies**
  - Numerical : ex : 47,XXY Klinefelter / 46 XO Turner
  - Structural : ex : deletions of X or Y
- **Autosomal chromosome anomalies**
  - Structural : ex : Translocations
- **Single Gene disorders**
  - autosomal dominant : Steinert Myotonic Dystrophy in males
  - autosomal recessive : Cystic Fibrosis, Immotile Cilia Syndrome
  - X-linked : Androgen Resistance

Secondary Sterility / Infertility and Genetics

- **Sexe chromosome anomalies**
  - Numerical : ex : mosaics XY/XXY or XX/XO
- **Autosomal chromosome anomalies**
  - Structural : ex : translocations, inversions
- **Single Gene disorders**
  - autosomal dominant : Steinert Myotonic Dystrophy in females
  - autosomal recessive : Sickle cell anaemia
  - X-linked : Focal dermal hypoplasia, FraX syndrome

Genetic causes of Male and Female Infertility: IV Compartments : examples

I Hypothalamic

→ KAL1 gene (XLR hypogonadotrophic hypogonadism in males)

→ AHC gene (XLR cong.adrenal hypoplasia in males)

II Pituitary

→ GNRHR gene (AR gonadotrophin releasing hormone receptor)

→ FSHβ gene, LHβ and hCGβ gene complexe

Successful therapy for pituitary causes = replace missing trophic factor (LH, FSH)

III Gonadal (major factor)

→ Genes

involved in Gonadotrophin receptors, steroid hormone receptors, steroid synthesis (poor prognosis → donor), autosomal genes (SOX9, WT1 can cause sexual ambiguity+infert.),…..

→X Chromosomal causes :

- whole X deletions (45,X cell line with/without mosaicism (46,XY/ 46,XX/ 47,XXX), when fertile beware POF - partial X deletions (Xp11, Xp21, Xq13 putative POF1 region, Xq26 putative POF2 region

- X;autosome translocations (rare)

III Gonadal (cont'd)

→ X Chromosomal causes (cont'd) :

- Single gene X disorders : FMR1 gene (fragile X syndrome) → POF in premutation carrier women (but not in full mutation carriers!)

→ Y Chromosomal causes :

- 46,XXY (Klinefelter) or 46,XX men (unclear physiopath)
- Translocations (rare, but risk of unbalanced offspring)
- Single gene Y disorders : SRY gene (sex determining region on Y), AZF (azoospermia factor) : AZFa,b,c,d regions : numerous genes

IV Outflow tract

→ Androgene receptor gene (AR)

46,XY male with androgene insensitivity → female phenotype)

→ CFTR gene: cystic fibrosis (AR)

congenital bilateral absence of vas deferens found in 1-2 % infertile males, around 90% of which carry one or two CFTR mutations : normal but immotile testicular sperm → reproduction by biopsy+ICSI.

Test partner + Genetic counseling, other family members at risk?

→ HOXA 13 gene

only known single gene causing uterine anomalies

Sperm parameter reminder :

Azoo-spermia	= absent sperm
Oligo-	= < 20 mio/cc
Astheno-	= < 50% motility
Terato-	= < 30% normal sperm (WHO)
	= < 6% normal sperm (Kruger morphology)

Main Causes of Decreased Parameters :

- Chromosomal abnormality → 15 % (azoo), 5 % (oligo)
- De novo del of azoosp factor region (AZF) → 13% (a/oligo)
- Cong. Bilat. Abs. of vas deferens (CBAVD) → 1- 2% (azoo)

Currently Gene and Chromosomal abnormalities are known to affect count and motility, yet unknown gene mutations are expected to affect morphology

Genetic Causes of Recurrent Fetal Loss

- ≈ 50 % of all first trimester spontanous abortions show a chromosomal abnormality (most de novo)
- ≈ 5 % abnormal parental karyotyp, esp. translocations
- Provide empirical risks for offspring (theoretical and empirical data)
- PND Options
- Discuss implications for other family members

Genetic Consultation in Sterility / Infertility

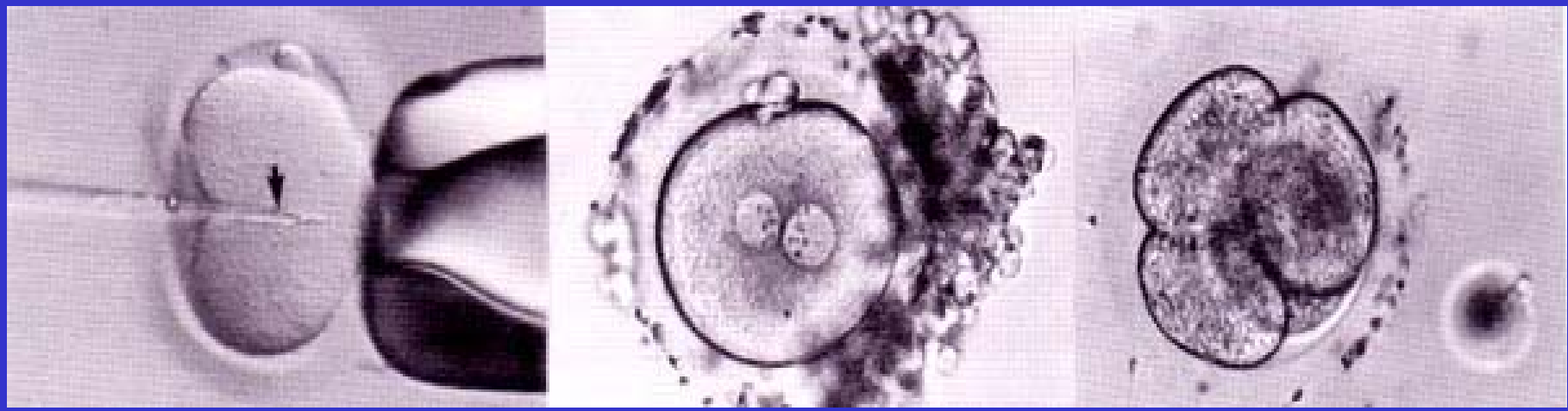
I Genetic testing

- Identify the etiology
- Identify syndromic causes of reproduction failure

II Genetic counseling

- Implications of syndromic causes
- Other family members at risk? Offer counseling
- Expose reproduction options (donor, adoption,..), ART methods (Artif.reprod.techniques): IVF, ICSI, chances of success, technique, limits, genetic risks…
- Prenatal Diagnosis
- Risk / implications of transmission (ex. Y microdeletion)
- Psychological and ethical implications

ICSI = Intra-Cytoplasmic Sperm Injection



Genetic Consultation in Reproduction Medecine : Importance of Personal and Familial Medical History

Pulmonary/digestive symptoms

→ Cystic fibrosis (AR)

→ Imotile cilia syndrome (AR)

Hypogonadism / Sexual ambiguity

→ Kallmann syndrome (XLR, rarely AD,AR)

→ Partial androgen resistence (XLR)

Neuromuscular symptoms

→ Kennedy disease (XLR)

→ Steinert myotonic dystrophy (AD)

Pre-IVF, pre-ICSI counseling while awaiting more definitive data…

Before treatment starts every couple should receive updated data about :

- Risk of transmitting parental chromosomal aberrations
- Risk of de novo chromosomal (sex/autosomal) – Gene aberrations (manipulation, frozen storage, etc…)
- Risk of transmitting fertility problem to offspring
- Potential increase of other pathologies after IVF / ICSI

II – Prenatal Diagnosis

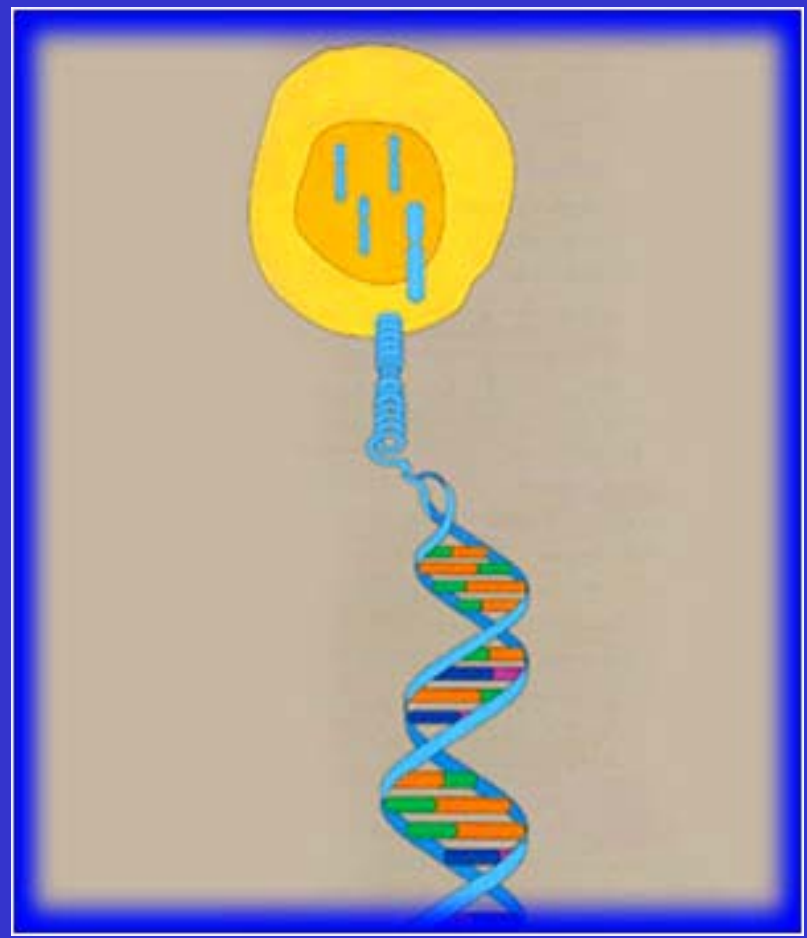
Etiology of congenital pathologies :

- Chromosomal changes (1/200)
- Monogenic disorders (single gene) (1/100)
- Multifactorial disorders (5-10/100)
  (polygenic + exogenous factors)

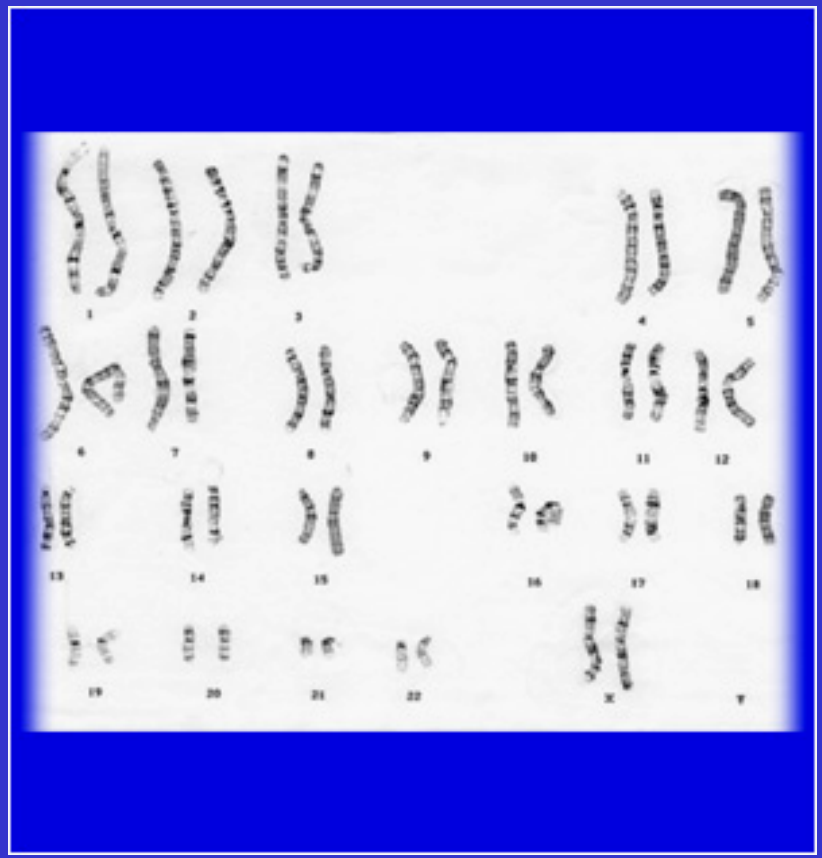
- Exogenous causes (?)
- Mitochondrial mutations (?)
- Imprinting and uniparental disomy (rare?)

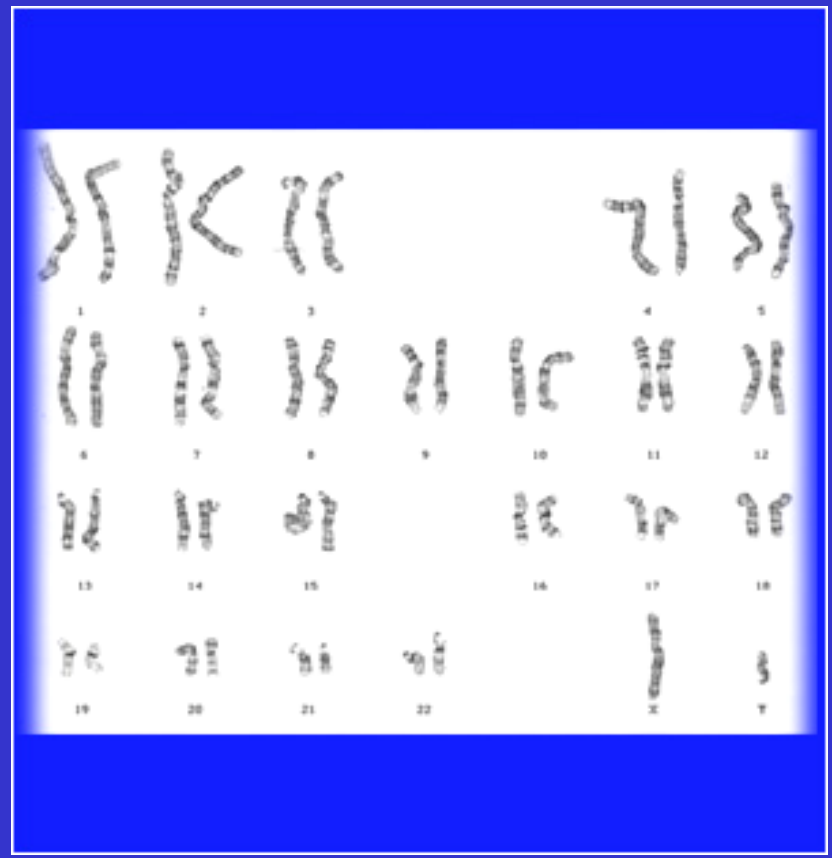
Genetic disorders → Genetic resolution levels

- Human gene
  = DNA sequence of several kb
- Human metaphase band
  = 100 genes (7500 kb)
- Human chromosome (average)
  = 1500-2000 genes (150'000 kb)
- Human genome
  = 22 pairs of autosomes + one pair of sex chromosomes (XX/XY)
  = around 35'000 genes
  = 3'500'000 kilobases of DNA



Human karyotype → metaphase bands





Prenatal Diagnosis
What can be detected today ?

- Chromosomal disorders
  - detection dependant on level of resolution
- Monogenic/polygenic disorders
  - direct analysis (gene cloned, mutation defined)
  - indirect analysis (gene located, linkage analysis)
- Multifactorial disorders
  - detection by indirect means (US, markers in maternal serum, amniotic fluid..)

PND always = attempt to answer a specific question.

No screening of genes !

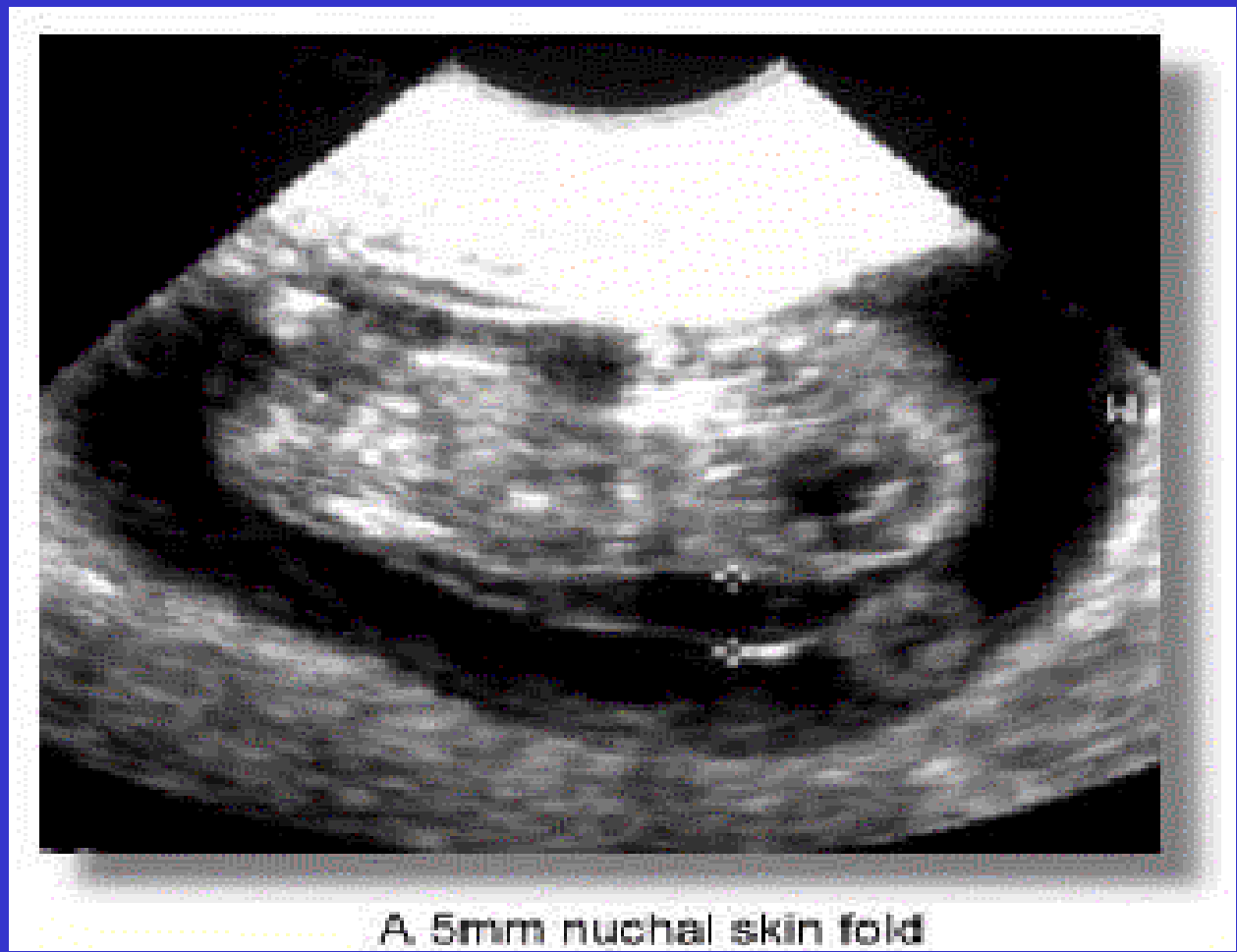
Prenatal Diagnosis Methods

- Non-invasive
  - Ultrasound
  - Maternal serum screening
- Invasive
  - Amniocentesis
  - Chorionic villous sampling (CVS)
  - Cord blood sampling (cordocentesis)

Ultrasound markers

- Down syndrome
  - Nuchal translucency (end first trim.), hydrops
  - Duodenal, esophagal atresia
  - Skeletal signs (humerus, femur short), growth
  - Malformations (esp. heart, kidney, CNS,..)
- Chromosomal abnomalies in general
  - Growth
  - Malformations (heart, kidney, CNS,intest (CF)..)
  - Amniotic fluid: oligamnios, hydramnios
  - Fetal mouvements

Nuchal Fold 3 mm → Risk T21 3 X incid. by maternal age
Nuchal Fold 4 mm → Risk T21 18 fold
Nuchal Fold 5 mm → Risk T21 28 fold
Nuchal Fold 6 mm → Risk T21 36 fold


A 5mm nuchal skin fold

Ultrasound examinations

- « Routine » : first trimester
- « Morphologic » : second trimester
- « Specialized » : third trimester

Maternal Serum Screening

- Screening ≠ diagnosis !
- Is an Option, not a standard of care!

- Second trimester
- First trimester
- Combined

Second Trimester Maternal Serum Screening

- 15-16th week
- Specifically developped for detection of T21

Serum Markers:

- Alpha-fetoprotein (AFP) reduced in T21 / increased in open neural cord/abdominal wall defect
- Human Chorionic Gonadotropin (hCG) increased in T21 / reduced in T13 + T18
- Unconjugated Estriol (uE3) reduced in T21

Second trimester maternal serum screening

(cont'd)

- Maternal age + biochemical serum screening $\rightarrow$ risk for ongoing pregnancy
- If risk found > risk at age 35 (cut-off)$\rightarrow$ amniocentesis is offered
- Identifies $\approx$ 65% of T21

First trimester screening

- 11-14th week
- Specific for detection of T21, T13 and T18

Markers:

- PAPP-A (Pregnancy Associated plasma protein-A) reduced in T21, T13 and T18
- Free sub-unit of β-Human Chorionic Gonadotropin increased in T21 / reduced in T13 + T18
- Nuchal Translucency > percentile 95 in T21. Importance of quality of image and of measure (sagital axe)

First trimester screening (cont'd)

- Maternal age + PAPP-A + β-hCG + NF → risk for ongoing pregnancy

- If risk found > theoretical risk at age 35 (cut-off) → choriocentesis is offered and result can be obtained before end of 12th week
  (Interruptio possible by curetting)

- Identifies ≈ 90 % of T21
- Cave: No measure of AFP → combined with second trimester mesure of serum AFP

Invasive PND : Amniocentesis

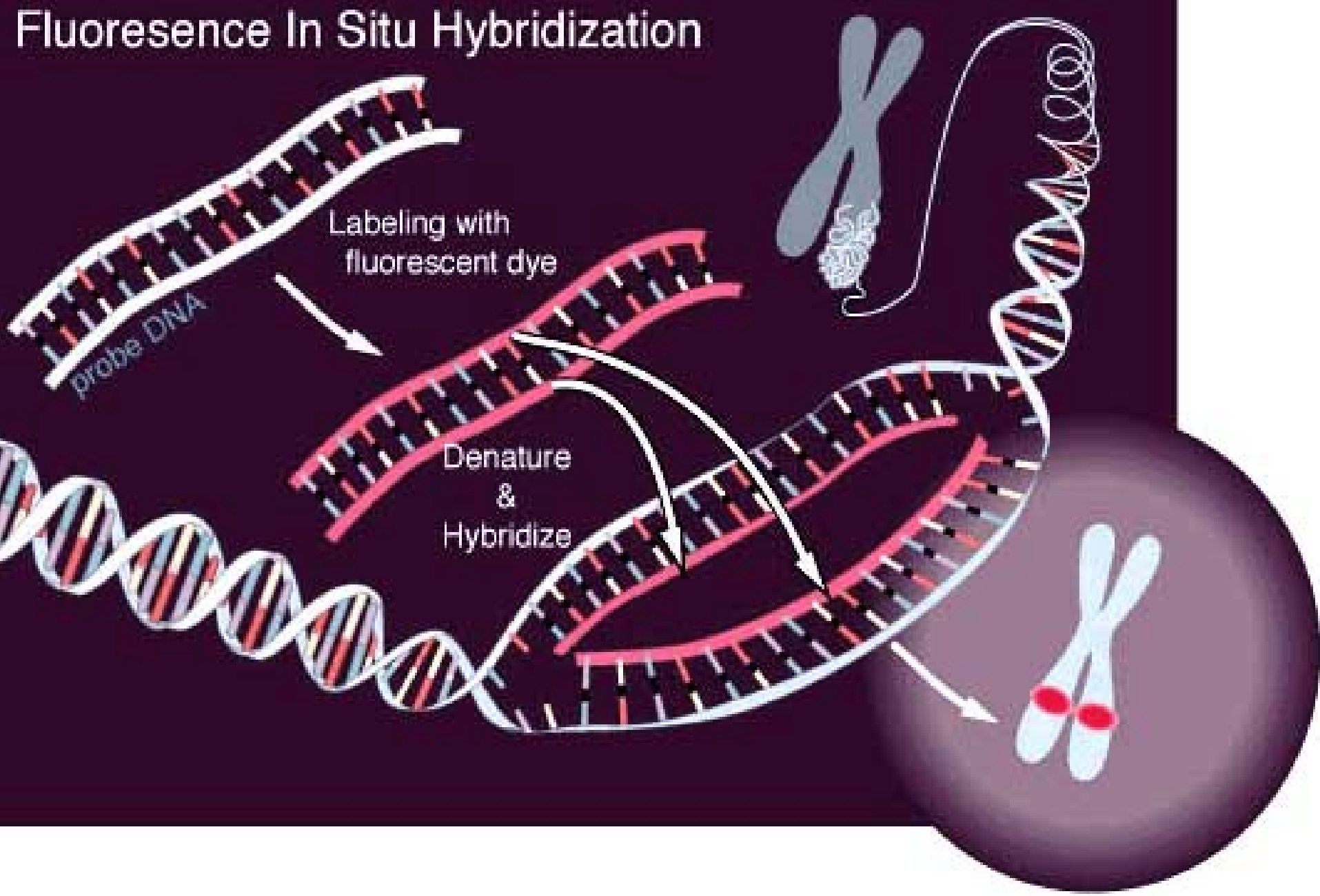
- Technique → Course Dr Ph.Extermann
- 15-20 ml amniotic fluid
- 14th-18th week (if enough liquid → term)
- 0.5 % induced misscarriages
- Few cells → culture (7–10 days) → analysis of 16-20 cells of diff. cultures (cave mat. contamination!) → result 10 -14 days
- In ≈1 % failure of culture or result uncertain
- Limitations : no DNA analysis but FISH analysis is possible

Invasive PND : Chorionic Villous Sampling (CVS)

- Technique → Course Dr Ph.Extermann
- 30 mg chorionic villosity (trans-cervical, -abdominal)
- 10th-11th week (as soon as placenta large enough)
- 1 % induced misscarriages
- Very rich in cells → short or no culture necessary → preparation 2 days + analysis of 16-20 cells of diff. cultures (cave mat. contamination!) → chromosomal result 4-7 days
- 1-2 % Mosaicism : confined to placenta? → Amniocentesis
- In ≈1 % failure of culture or result uncertain
- Limitations : no DNA analysis but FISH analysis is possible

FISH = fluorescent in situ hybridisation

- Identifies number of Chromosomes X, Y, 13, 18, 21
- Rapid = result within 24 – 36 hours
- Make sure patient understood limitations of test!


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

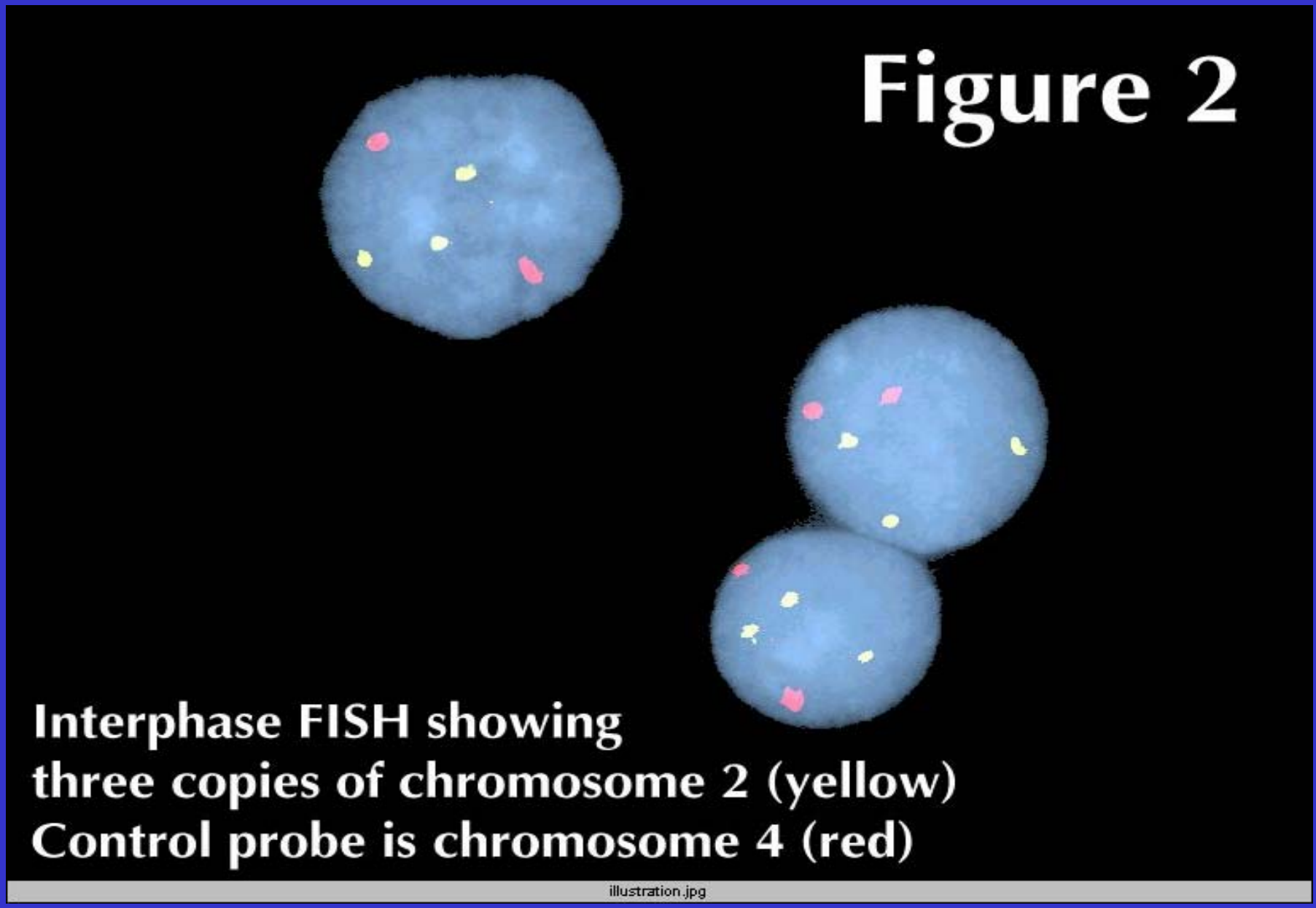


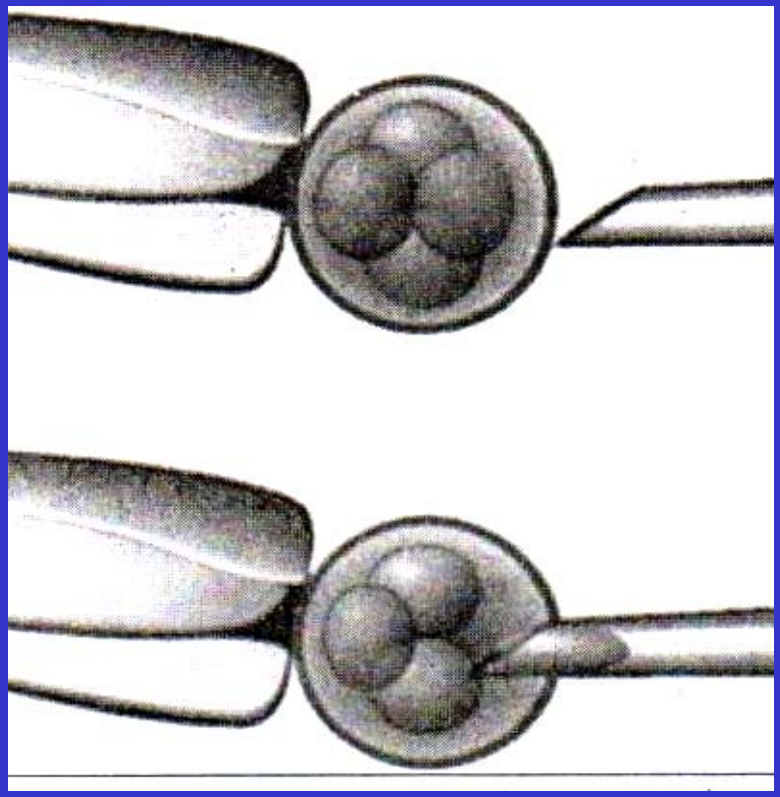
Figure 2
Interphase FISH showing
three copies of chromosome 2 (yellow)
Control probe is chromosome 4 (red)
illustration.jpg

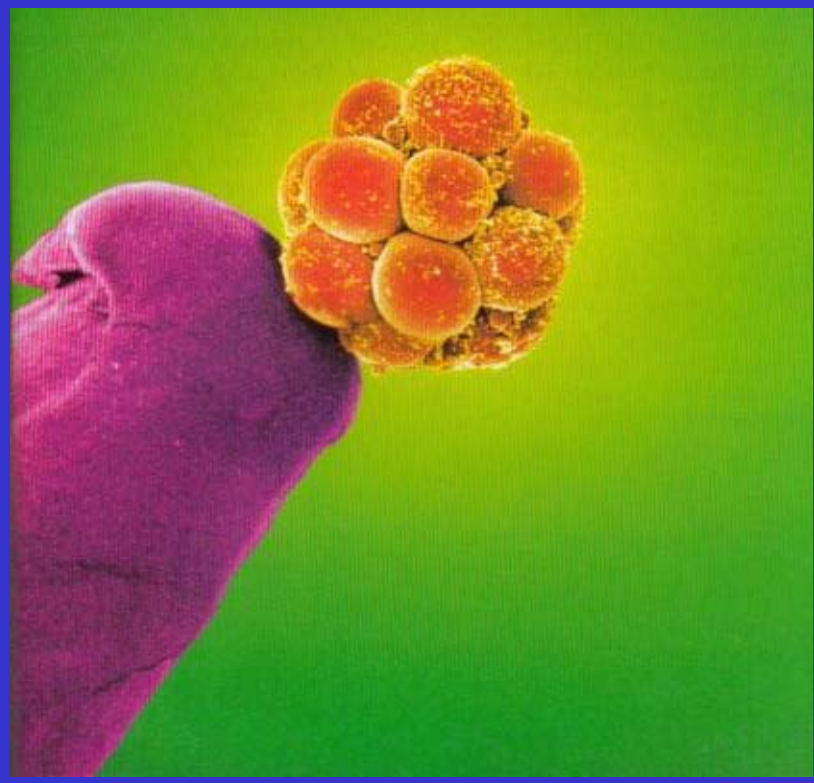
Preimplantation Diagnosis (PID)

- implies IVF (medically heavy procedure)
- 8 cell stage (blastomere - day 3) → set apart 1-2 cells (embyo biopsy) which will be tested (FISH)
- Implantation of embryos with favorable test
- Important ethical implications, only taken into consideration for serious major pathologies.
- High costs, long waiting lists
- Different legislations around the world

Preimplantation Diagnosis (PID)

(implies IVF)





Not all that can be done must be done or is good to be done!

PND is always a couple's (and in the end the pregnant woman's) free choice

Information must be neutral and complete about all available options

The ethical aspects must be addressed thoroughly

Each case must be an individual one