Geneva Foundation for Medical Education and Research
WHO Collaborating Centre in Education and Research in Human Reproduction

Training in Research in Reproductive Health/Sexual Health
Geneva 2005

Genetics Module

February, March, 2005

Division of Medical Genetics
Geneva University Hospital

Division of Medical Genetics, Geneva University Hospital

Who's who…

Stylianos Antonarakis	**Head of our Division, medical geneticist MD with primary interest in gene identification**
Michael Morris	**Molecular biologist clinical DNA laboratory**
Sophie Dahoun	**Medical geneticist MD clinical cytogenetics laboratory**
Monica Gersbach	**Medical geneticist MD with specialization in clinical genetics**

Genetics module

Feb 25, 2005

09 – 10 am	**The genetics consultation in Ob-Gyn**
10 – 11 am	**Genetic counseling: principles, practice, ethics** **Monica Gersbach**

March 2, 2005

11 – 12 am	**Principles of molecular diagnosis** **Michael Morris**
02 – 03 pm	**Clinical pre- and postnatal cytogenetics** **Sophie Dahoun**
03 – 04 pm	**The human genome project, its implications for the practice of medecine in the 21th century** **Stylianos Antonarakis**

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Postgraduate Course in
Reproductive Medecine and Reproductive Biology - Geneva

The Genetics Consultation in Obstetrics & Gynecology: 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 IVF, Pre ICSI* Genetic Counseling

(*ICSI = Intra-Cytoplasmic Sperm Injection)

The Genetic Consultation in Ob-Gyn cont'd :

II - Prenatal Diagnosis

- **Etiology of Congenital Pathologies**
- **Genetic Resolution Levels**

- **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 : FraX syndrome, Focal dermal hypoplasia

Genetic causes of Male and Female Infertility: 4 Compartments : examples

1. Hypothalamic
   - → **KAL1 gene** (XLR hypogonadotrophic hypogonadism in males)
   - → **AHC gene** (XLR cong.adrenal hypoplasia in males)

2. 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)

3. 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 (premature ovarian failure)

- partial X deletions

  Xp11, Xp21, Xq13 putative POF1 region, Xq26 putative POF2 region

- X;autosome translocations (rare)

3. Gonadal (cont'd)

→ X Chromosomal causes (cont'd) :

- Single X gene disorders :

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

→ Y Chromosomal causes :

- 46,XXY (Klinefelter)
- Translocations (rare, 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

4. 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)

Genetic 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

- Genetic consultation after 2-3 episodes
- ≈ 50 % of all first trimester spontanous abortions show a chromosomal abnormality (most « de novo »)
- ≈ 5 % abnormal parental karyotyp, esp. translocations:
  - **Provide risks for offspring (theoretical + empirical)**
  - **PND Options**
  - **Discuss implications for other family members**

Genetics Consultation in Sterility / Infertility

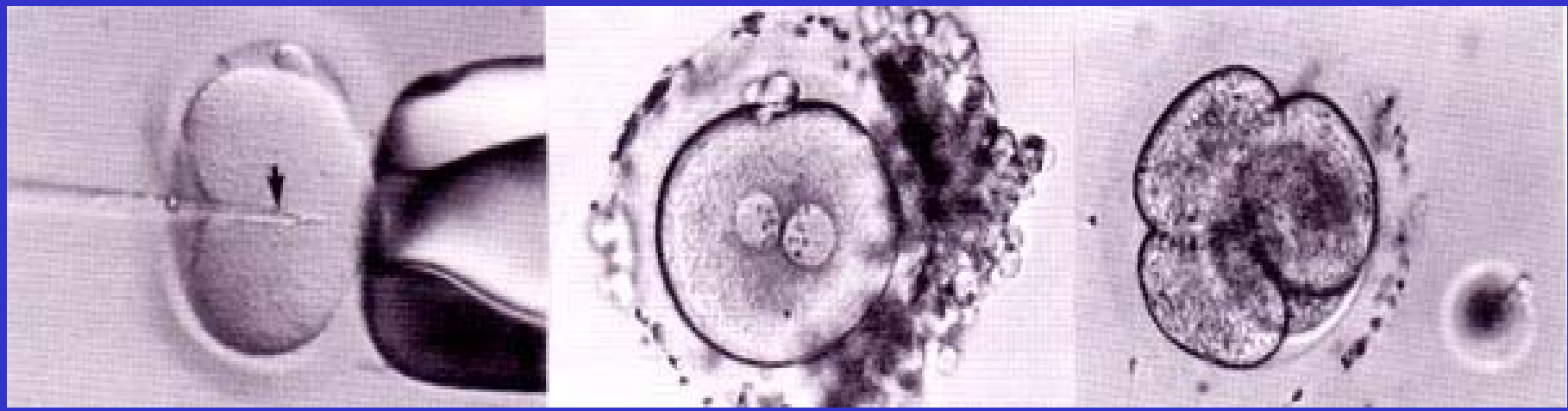
I Genetic testing

- **Identify the etiology**
- **Identify syndromic causes of reproduction failure**

II Genetic counseling

- **Implications of syndromic causes**
- **Other family members at risk? Offer counseling to them**
- **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 (Kartagener) (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 anomaly
- Risk of transmitting fertility problem (Y del) to offspring
- Risk of de novo chromosomal (sex/autosomal) – Gene aberrations (manipulation, frozen storage, etc…)
- Potential increase of other pathologies after IVF / ICSI

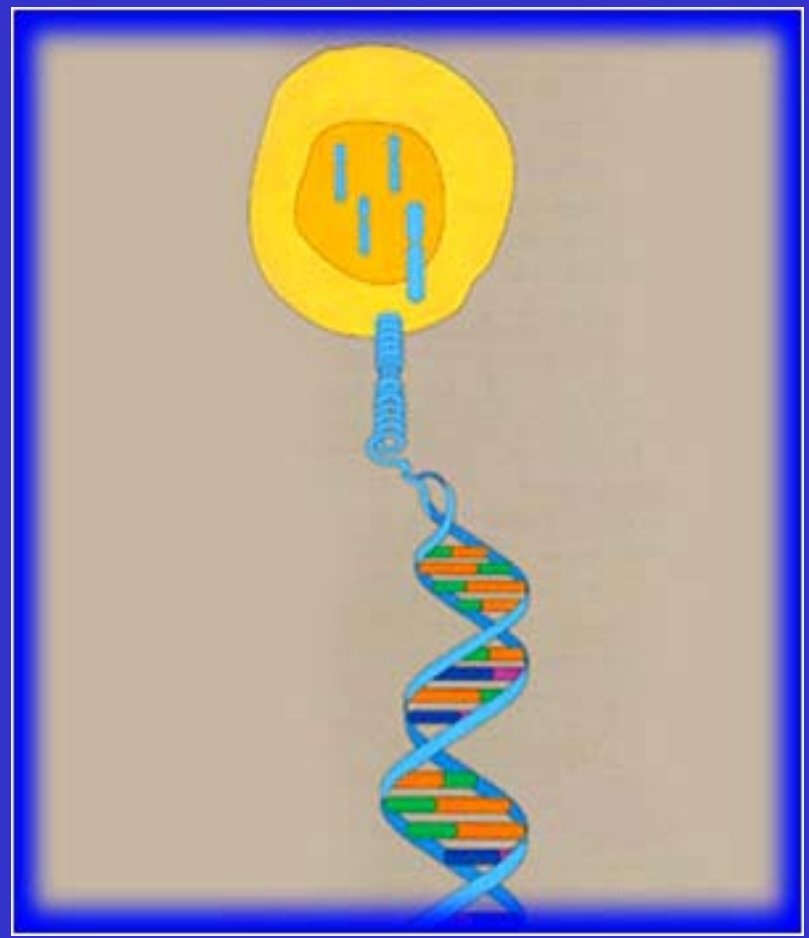
II – Prenatal Diagnosis

Etiologies and frequencies of congenital pathologies :

• Chromosomal changes	0.5 %
• Monogenic disorders (single gene)	1 %
• Multifactorial disorders (polygenic + exogenous factors)	5-10 %
• 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 32'000 genes
  = 3'200'000 kilobases of DNA



Prenatal Diagnosis
What can be detected today ?

- Chromosomal disorders

  - detection depends on level of resolution

- Monogenic/polygenic disorders

  - direct analysis (gene known in detail, 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 !

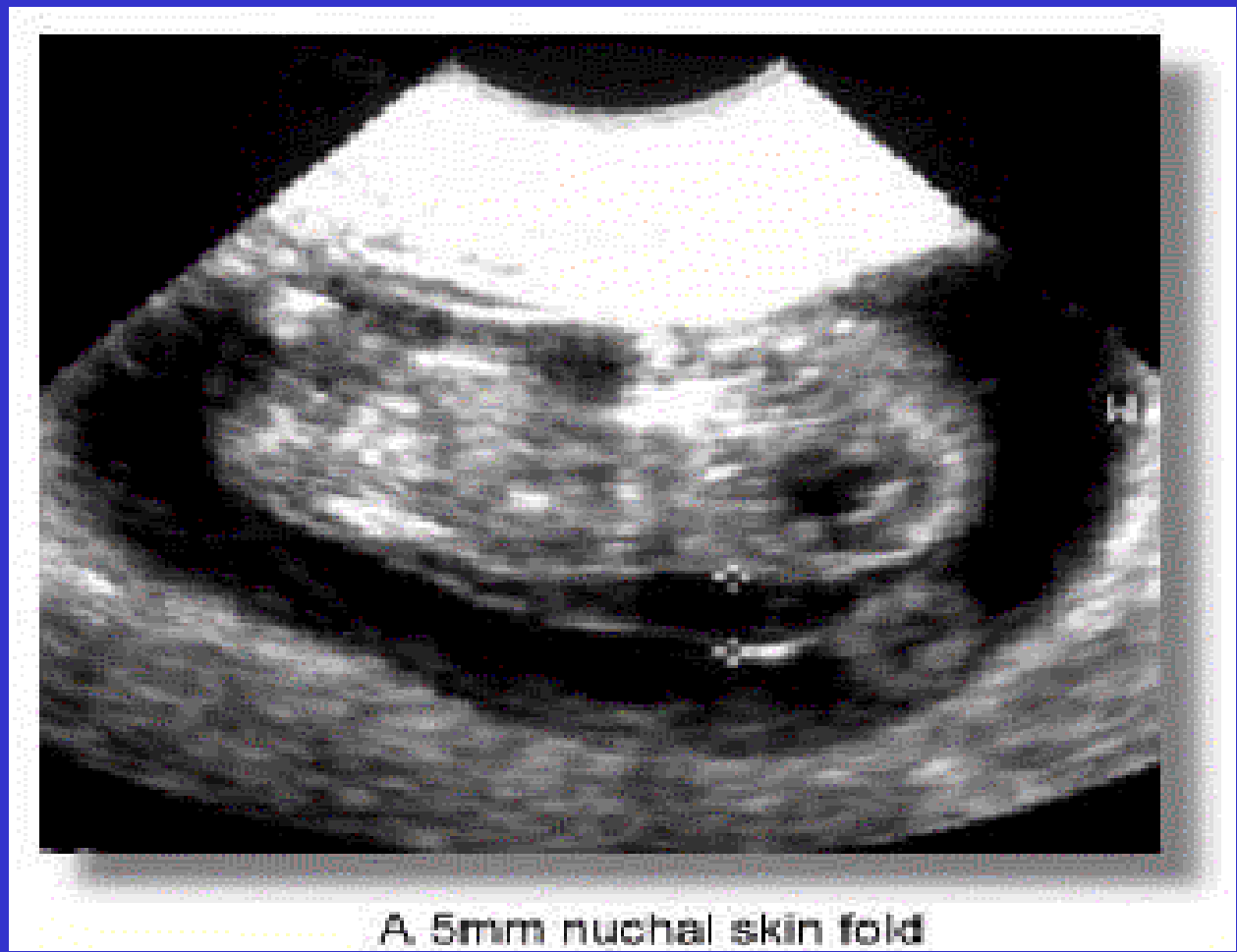
Prenatal Diagnosis
Methods

- 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, absent nasal bone**
  - **Duodenal, esophagal atresia**
  - **Skeletal signs (humerus, femur short), growth**
  - **Malformations (esp. heart, kidney, CNS,..)**
- Chromosomal abnomalies in general
  - **Growth retardation**
  - **Malformations (heart, kidney, CNS,intest (CF)..)**
  - **Amniotic fluid: oligamnios, hydramnios**
  - **Fetal mouvements decreased**

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 + serum markers $\rightarrow$ risk for ongoing pregnancy
- If risk found > risk invasive PND (cut-off 1/250) $\rightarrow$ amniocentesis is offered
- Identifies $\approx$ 65% of T21

First trimester screening

- 11-14th week
- Aims 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 > risk invasive PND (cut-off) → choriocentesis is offered. 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 maternal contamination!) → result within 10-14 days**

- **In ≈1 % failure of culture or result uncertain**

- Chromosomal, FISH, DNA analysis
- **Biochemical analysis, AFP, hormones, fetal Hb..**

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**

  → Ideal for DNA analysis → rapid results ≈ type of analysis (within hours - few days).

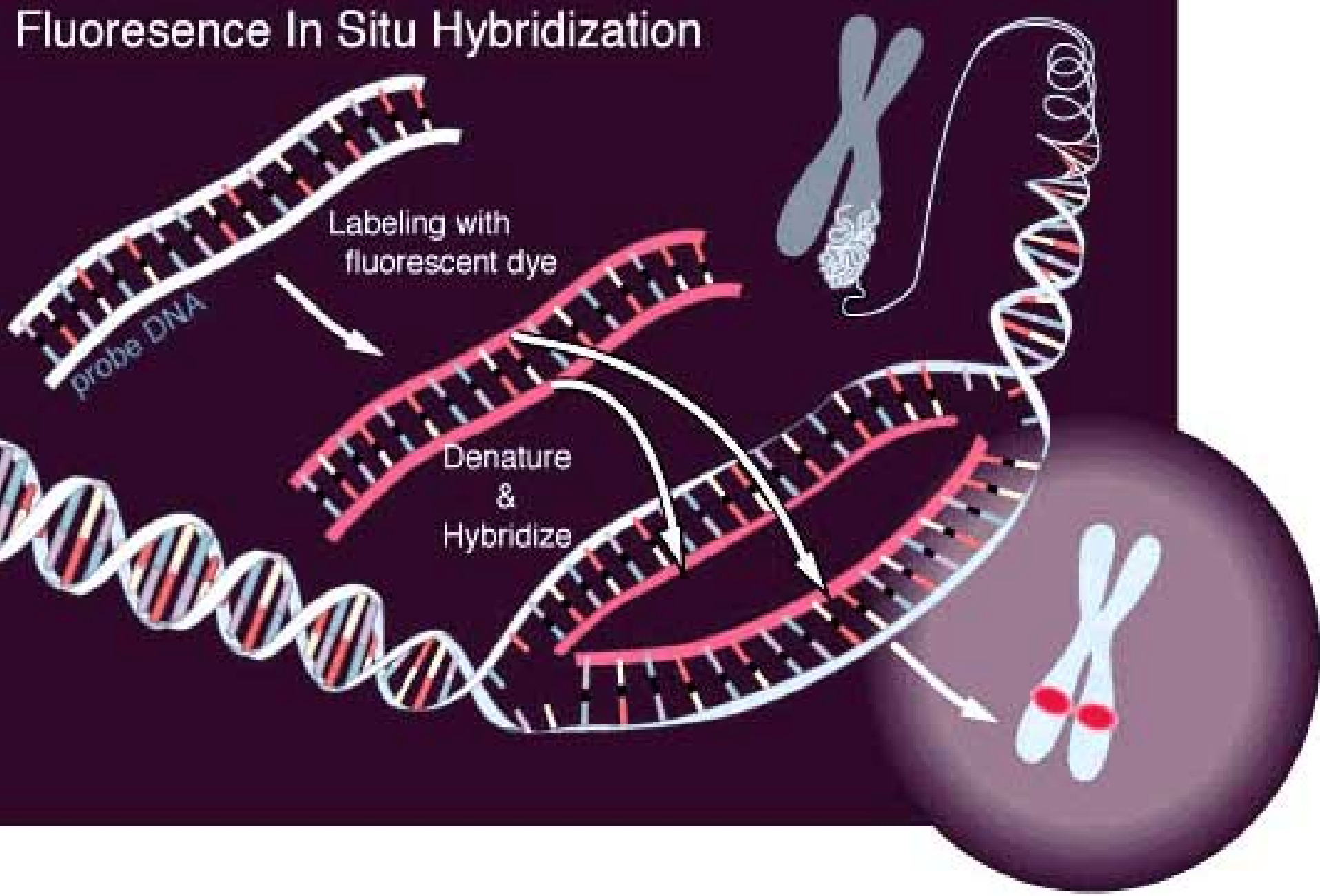
  → Chromosomal result 4-7 days (lower quality than amniocentesis) short or no culture necessary → preparation 2 days + analysis of 16-20 cells of diff. cultures (cave mat. contamination!)

  → Biochem.analysis, but no AFP

- **1-2 % Mosaicism : confined to placenta? → Amniocentesis**

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

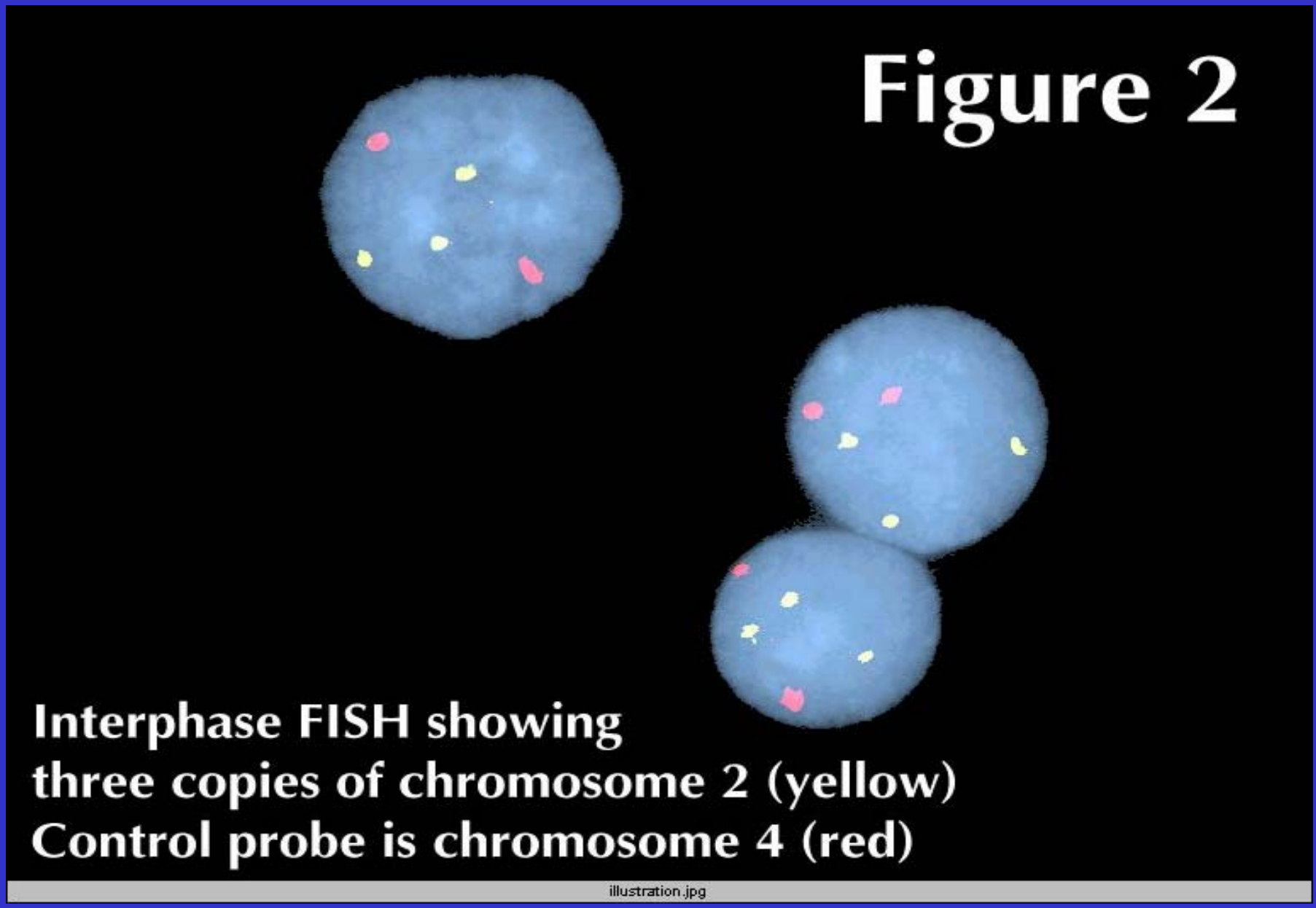


Figure 2
Interphase FISH showing
three copies of chromosome 2 (yellow)
Control probe is chromosome 4 (red)
illustration.jpg

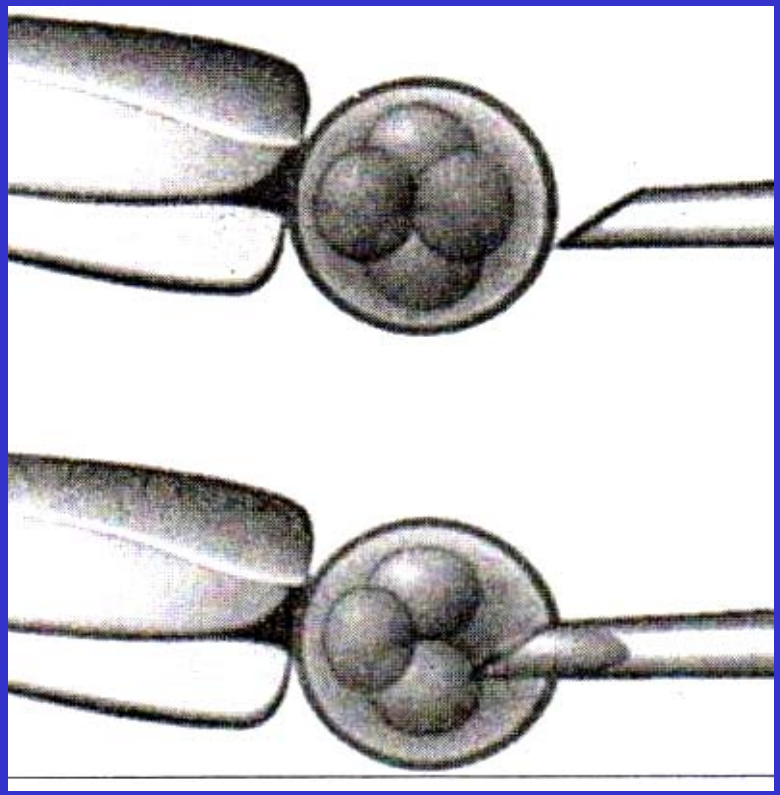
Preimplantation Diagnosis (PID)

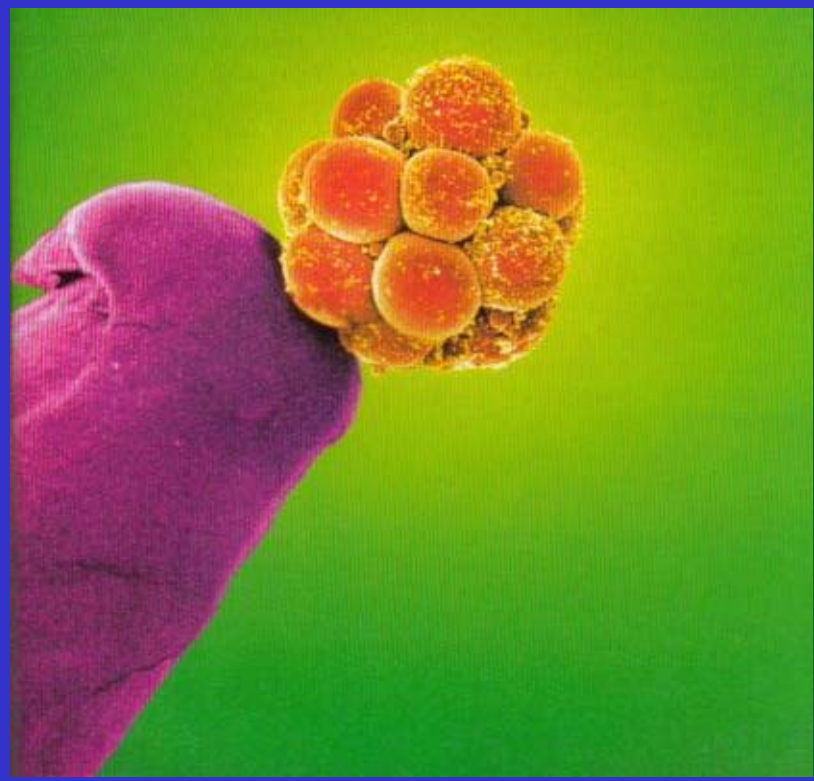
- **implies IVF (medically heavy procedure)**
- **8 cell stage (blastomere - day 3) → set apart 1-2 cells (embryo biopsy) which will be tested (FISH)**
- **Implantation of embryos with favorable test**

- **Important ethical implications, only taken into consideration for serious major pathologies.**
- **High costs, long waiting lists**

- **Different legislations around the world (illigal in our country, legal in many european countries)**

Preimplantation Diagnosis (PID)
(implies IVF)





Conclusions

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

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

Information must be neutral and complete about all available options

The ethical aspects need to be addressed thoroughly

Each case should be an individual one