The Genetics Consultation in OB-GYN :

Reproductive pathologies and prenatal diagnosis

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Etiologies of malformations / genetic disorders

- *Chromosomal* (monosomies, trisomies)
- *Monogenic* (dominant, recessive, X-linked)
- *Exogenous* (pre- or postnatal environment)
- *Multifactorial* (predisposition + environment)

Genetic resolution levels

- HUMAN GENOME
  - about 30,000 genes

    (3,000,000 kilobases of DNA)
- AVERAGE CHROMOSOME
  - about 1500-2000 genes (150,000 kilobases)
- AVERAGE METAPHASE BAND
  - about 100 genes (7500 kilobases)

Prenatal diagnosis in 2001: detection of disorders of genetic etiology

- Chromosomal anomalies
  - all chromosomes studied (all but subtle structural aberrations detected); high-risk populations tested
- monogenic disorders
  - direct analysis (mutations defined)
  - indirect (gene localisation known)
- multifactorial etiology
  - diagnosis by indirect means (US; mat. serum)

Prenatal diagnosis: Limitations

- We can diagnose, but rarely treat
- We can diagnose presence of the disorder, but rarely its severity
- Test which give the most information carry risks for the pregnancy
- Results available “late” in pregnancy
- No sure means of prenatal diagnosis for the majority of malformations / syndromes

Non-invasive Prenatal diagnosis :

- Ultrasound

Types of prenatal diagnosis

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

Ultrasound markers (for Down syndrome)

- Nuccal translucency (1st trimester)
- Intestinal symptoms
- Skeletal alterations (including growth)
- Malformations

“Noninvasive” prenatal diagnosis

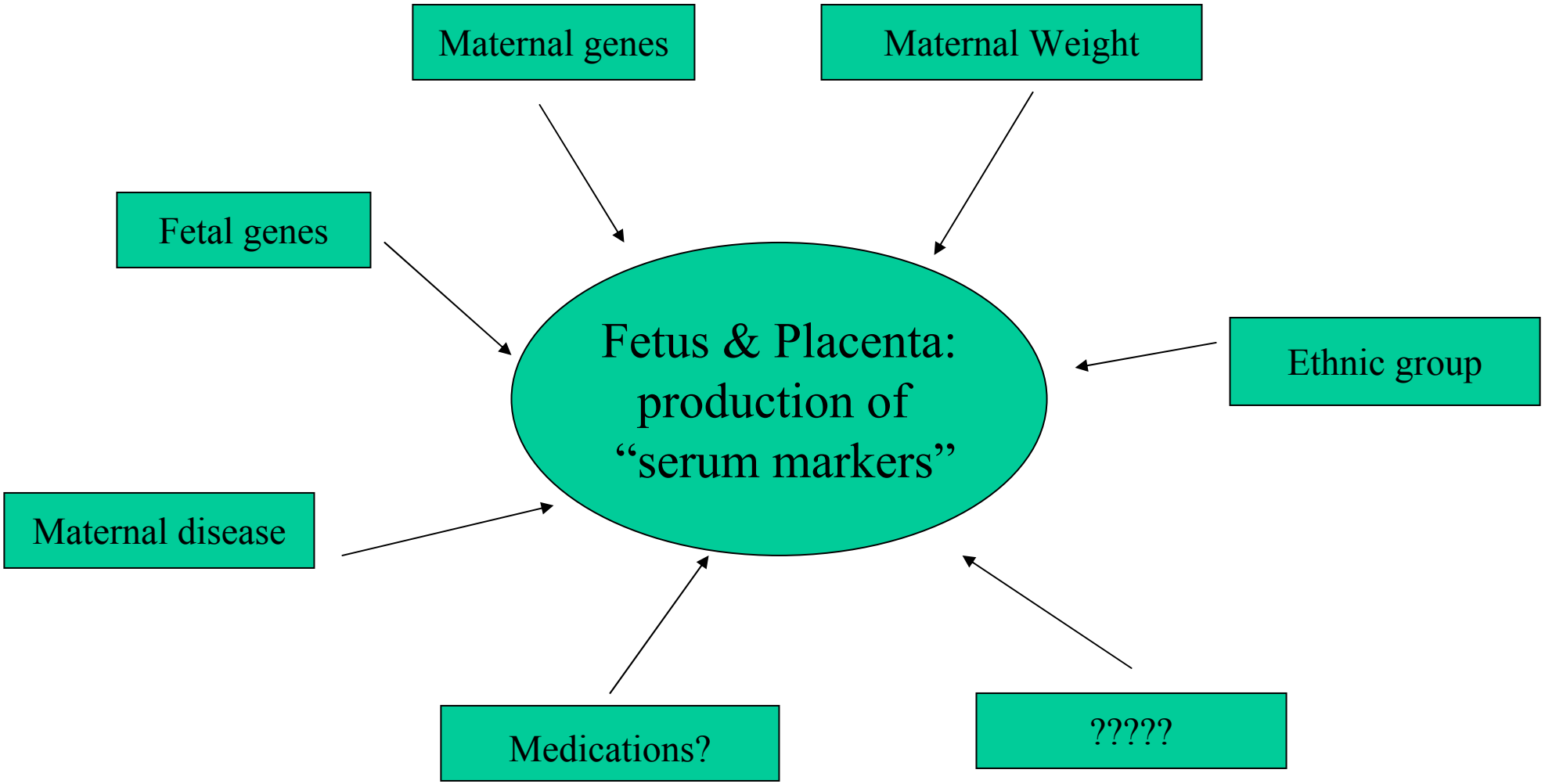
- Maternal serum screening
  - in the second trimester
  - in the first trimester
  - combined screening

Second trimester serum markers

- Alpha-fetoprotein (AFP)
- Human chorionic gonadotropin (HCG)
- Uncongugated estriol (uE3)

First trimester serum markers

- Pregnancy-associated plasma protein A (PAPP-A)
- free beta-HCG
- Others??
  - Schwanger-shafts protein 1 (SDP-1)
  - prostate-specific antigen (PSA)?


Maternal genes
Maternal Weight
Fetal genes
Fetus & Placenta: production of "serum markers"
Ethnic group
Maternal disease
Medications?
?????

Performance of Down syndrome screening at 8-14 weeks gestation

(from Kennard & Wald, 1996)

	Maternal age	with free b-HCG	and PAPP-A
risk cut-off level	detection rate (%)	False-positive rate (%)	OAPR
1 : 100	45	1.5	1 : 25
1 : 200	57	3.5	1 : 47
1 : 300	63	5.5	1 : 67
1 : 400	68	7.4	1 : 84
1 : 500	72	9.5	1 : 102

Down syndrome rates and false-positive rates

(using triple test at different ages; risk cut-off 1/250)
(from Kennard & Wald, 1995 & 1996)

Maternal age	Detection rate (%)	Positive Rate (%)
25	36	3
30	45	5
35	66	13
40	89	41
45	99	79

Maternal serum analysis

(hormones and fetal products in maternal ciruclation)

Advantages

- No risk for the pregnancy
- May reduce the number of invasive tests requested
- *Screening* test which indicates at risk pregnancies
- Can be applied on a large scale (general population)

Disadvantages

- Gives only a risk of being affected; requires additional analyses
- Second trimester screening gives late results (later amniocentesis)
- Knowledge of physiological factors affecting markers still incomplete

Counseling issues in serum screening

- Who to screen?
  - all pregnancies?
  - all but those who will have amniocentesis?
  - only women who would have amniocentesis and potential abortion?
- How to inform?
  - explained by gynecologist before test?
  - explained by gynecologist only after results?
  - by brochure or video, questions afterwards?
  - during prenatal consultation with geneticist?

Counseling difficulties linked to serum screening

- Time and timing (for counseling)
- Generation of anxiety
- False positives and false negatives
- Scientific unknowns
- Insurances and financial factors
- Disagreement within couples
- How far to go?

Analysis of fetal cells in maternal circulation

- A promising technique en theory, but:
  - technically difficult to separate fetal cells
  - genetic testing is limited by cell number
  - “misuse” of methods to be anticipated!

  - Current research concentrating on isolation of *free circulating fetal DNA*

Invasive methods

- Amniocentesis

Cytogenetics: Indications for testing

- Prenatal tests
  - advanced maternal age
  - precedent gene or chromosomal disorder
  - previous abnormal infant or pregnancy
  - positive serum screening, anxiety

  Trisomies, monosomies, and major structural anomalies detected; no routine gene analysis

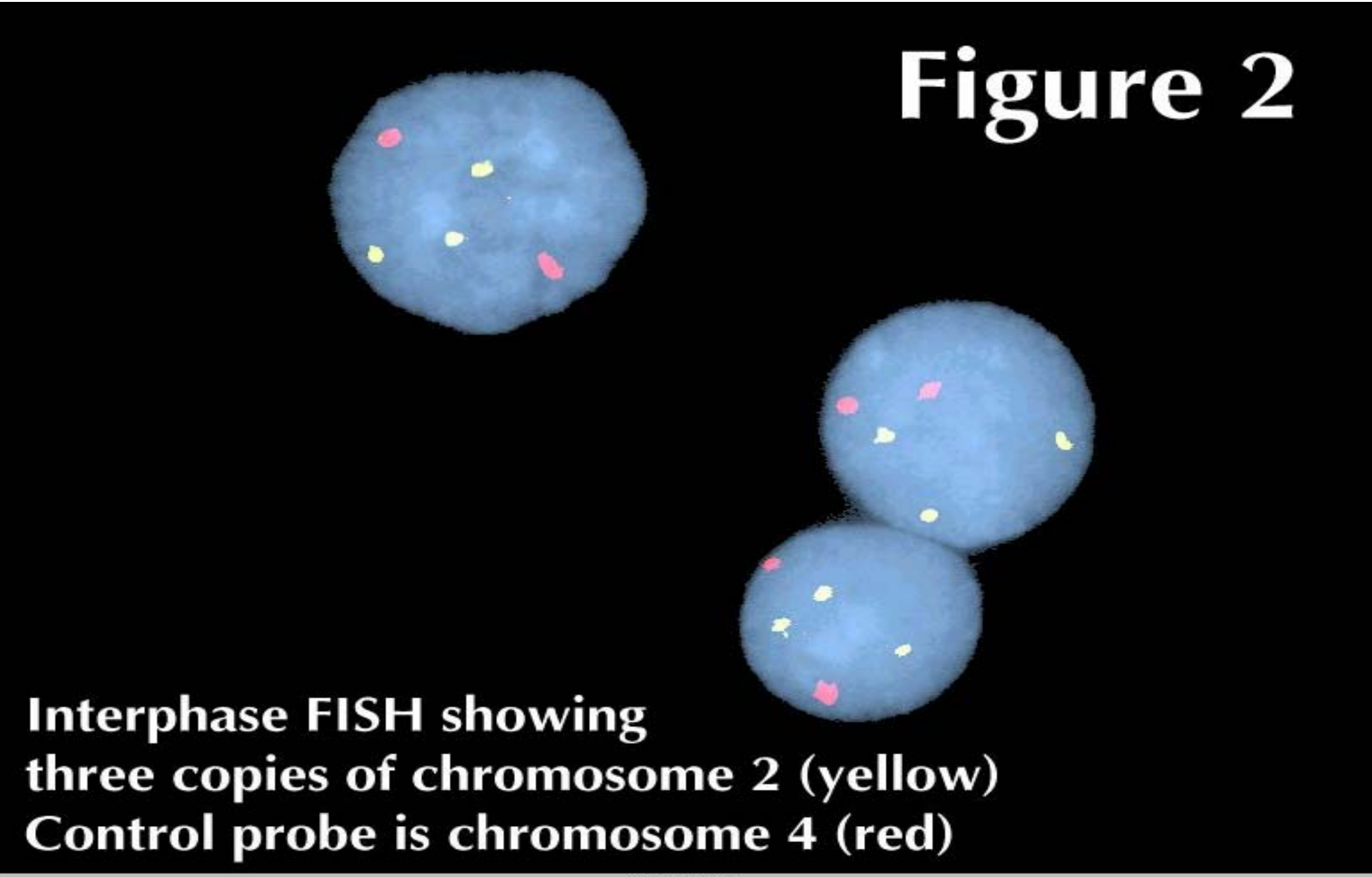
Mosaicism on amniocentesis
(from Hsu et al. PRENAT DIAGN 12:555-573)

Mosaicism

Origin of data	Cases studied	Percentage
US survey	62,279	**0.25**
European sur.	44,170	**0.10**
Canadian sur.	12,386	**0.30**
PDL data	12,000	**0.20**

FISH :
current uses in prenatal diagnosis

- family history of microdeletion/duplication
- determining chromosomal content of abnormal chromosomes
  - chromosomal paints
  - centromeric probes
- searching for mosaicism when abnormal cell(s) have been seen
- screening for common aneuploidies using uncultured cells (interphase)



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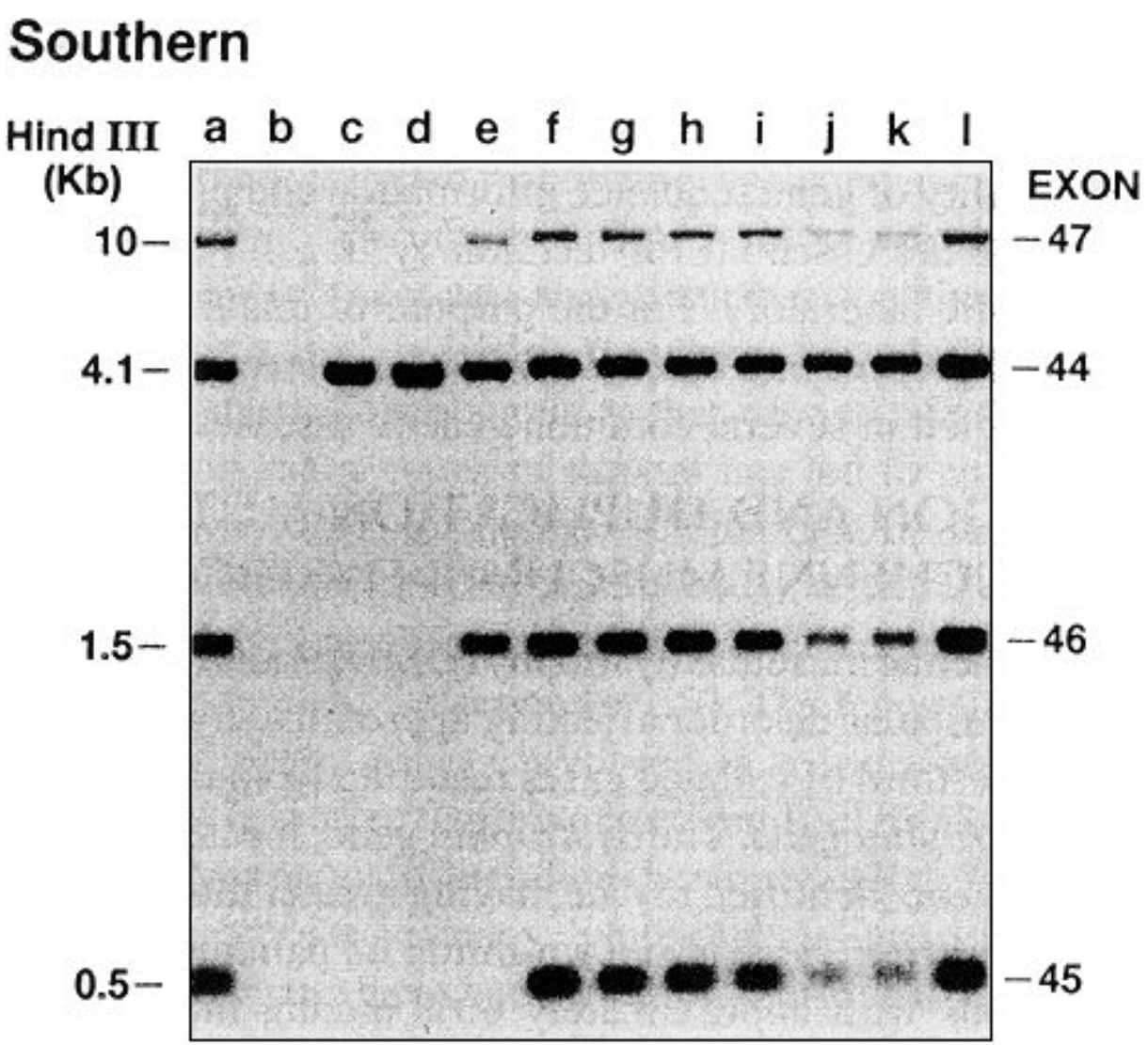
Invasive methods

- Choriocentesis

(chorionic villus sampling- CVS)

CVS is the preferred method of prenatal testing for DNA (gene) analysis!



from: Principles of Molecular Medicine J.L. Jameson, ed.
1998 Humana Press Inc. p. 86

Chromosomal mosaicism detected by Chorionic Villus Sampling

--occurs in 1-2% of CVS

-- necessitates further analyses to determine whether it is fetal or only in placenta and adnexes (further culture, amniocentesis, ultrasound)

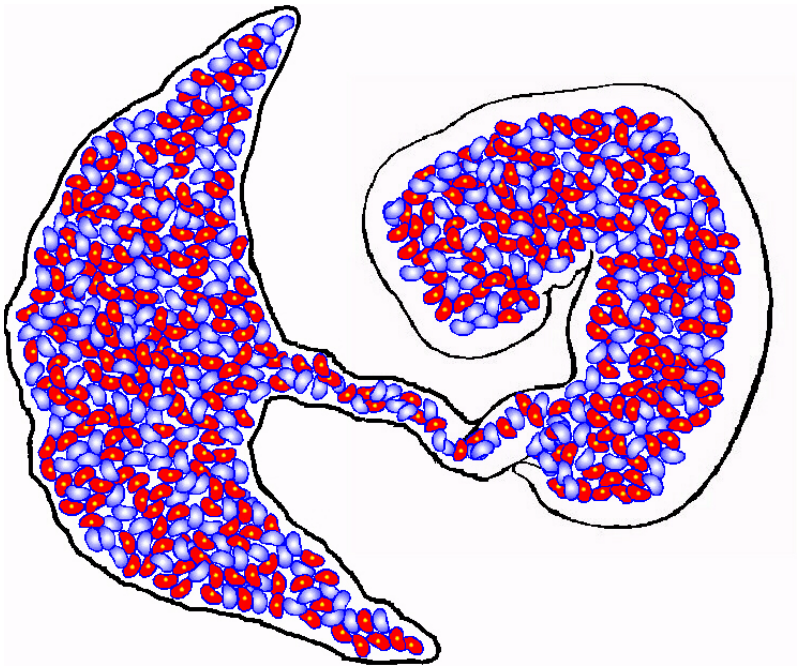
-- the majority of mosaic cases are LIMITED TO THE PLACENTA, but...

-- depending on the chromosome, may predispose to reproductive pathologies (growth retardation, etc) even if confined to placenta

True Mosaicism or CPM?

diploid

aneuploid



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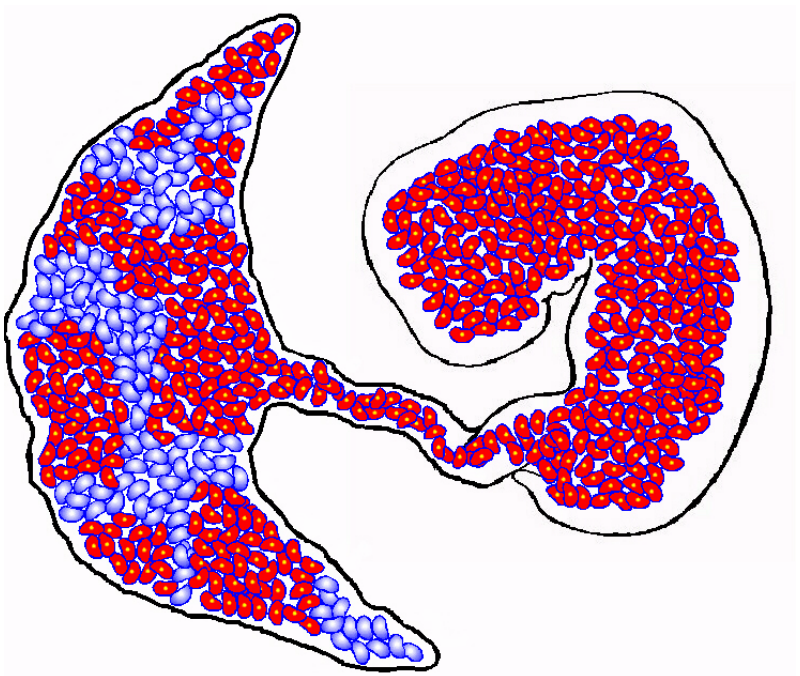

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Amniocentesis vs. Choriocentesis

Amniocentesis	Choriocentesis
higher success rate	results available earlier
superior cytogenetic image	preferred method for DNA analysis
results reflect fetal karyotype	option to verify by amniocentesis
very few false negatives or positives	problem of placental mosaicism

Invasive methods :

- Fetal blood and tissue sampling

The choice of a method of prenatal diagnosis...

- depends on what type of analyses are planned
- for cytogenetic studies:
  - amniocentesis or choriocentesis
  - each method has advantages and disadvantages
- for molecular (DNA) analysis:
  - choriocentesis is the preferred method
    - for technical reasons and early results
  - amniocentesis can also be used
    - results come late in pregnancy

Genetic prenatal diagnosis in the near future

- Increase in the number of disorders we can diagnose
  - monogenic etiologies
  - genetic predispositions
- earlier and less invasive screening tests
  - separation of fetal cells from maternal blood
  - better biochimical markers from maternal circulation
- potential for fetal therapy?

Preimplantation diagnosis

- Requires assisted reproductive technologies
- Genetic analysis done on 1-3 cells (blastomeres)
- “Healthy” embryos are chosen for implantation

The Genetics Consultation in OB-GYN :

Reproductive pathologies

Genetics and sterility (primary)

- ***sex chromosome anomalies***
  - *numerical* (ex: 47,XXX; mosaic XXY or XO)
  - *structural* (ex: deletions of X or Y)
- ***autosomal chromosome anomalies***
  - *structural* (ex: translocations in males)
- ***single gene disorders***
  - *autosomal dominant* (ex: Steinert M.D. in male)
  - *autosomal recessive* (ex: cystic fibrosis mutations)
  - *X-linked disorders* (ex: androgen resistance)

Genetics and sterility (secondary)

- ***sex chromosome anomalies***
  - *numerical* (ex: mosaic XXY or XO)
- ***autosomal chromosome anomalies***
  - *structural* (ex: translocations, inversions)
- ***single gene disorders***
  - *autosomal dominant* (ex: Steinert M.D. in female)
  - *autosomal recessive* (ex: sickle cell anemia)
  - *X-linked disorders* (ex: focal dermal hypoplasia)

Genetic testing in infertility : Goals

- Determine origin of the infertility
- Identify syndromic causes (prophylaxis for the affected individual)
- Better estimate the probability of success with ART
- Offer genetic counseling
  - concerning ART methods
  - concerning prenatal diagnosis

Medical histories

Question (proband or family)

- pulmonary or digestive symptoms?
- Masculine infertility /

  sexual ambiguity
- Neuromuscular symptoms

Disorder (genetic transmission)

- cystic fibrosis (AR)
- immotile cilia syndrome (AR)
- partial androgen resistance (XLR)
- Kennedy disease (XLR)
- Steinert myotonic dystrophy (AD)

Azoospermia

- 15% of males have a chromosomal abnormality
- 13% of males have a *de novo* deletion of the azoospermia factor (AZF) region on Yq11
- 1-2% of males have congenital bilateral absence of the vas deferens (CBAVD)

Genetic Counseling for Azoospermia - Chromosomal Etiology (numerical)

- Discuss clinical features
- Discuss karyotypic findings
- Reproductive options
  - adoption
  - donor sperm
  - MESA/ICSI/IVF/PGD
- Theoretical recurrence risks

Genetic Counseling for A/Oligospermia - Chromosomal Etiology (structural)

- Discuss karyotypic findings
- Provide empirical risks
- Discuss implications for other family members

Genetic Counseling for Azoospermia - Y Deletion

- Discuss AZF deletion versus polymorphism with no adverse phenotypic effect
- Reproductive options if de novo deletion of AZF
  - adoption
  - donor sperm
  - MESA/ICSI/IVF with/without prenatal dx

Genetic Counseling for Azoospermia - CBAVD

- Offer cystic fibrosis (CF) testing
  - 10-20% with 2 CF mutations
  - 40-60% with CF mutation
- Discuss further medical evaluations

Genetic Counseling for CBAVD Due to CF Mutation(s)

- Ascertain patient's understanding of diagnosis
- Discuss genetics and relationship of CF and CBAVD
- Offer CF carrier screening to partner
- Risk assessment

CFTR mutations in males with abnormal spermograms

(13 mutations CF)

(van de Ven et al.(1996) HUM REPROD 11:513-517)

Diagnostic	Number mutations /no.chrom.
normospermia	0 / 52 (0%)
anomalies	**18/202 (8.9%)**
azospermia	4 / 42 (9.5%)
asthenospermia	5 / 54 (9/2%)
teratospermia	1 / 8 (12.5%)
oligospermia	0 / 8 (0%)
OA	1 / 10 (10%)
OT	1 / 14 (7.1%)
OAT	5 / 32 (15.6%)
AT	1 / 34 (2.9%)

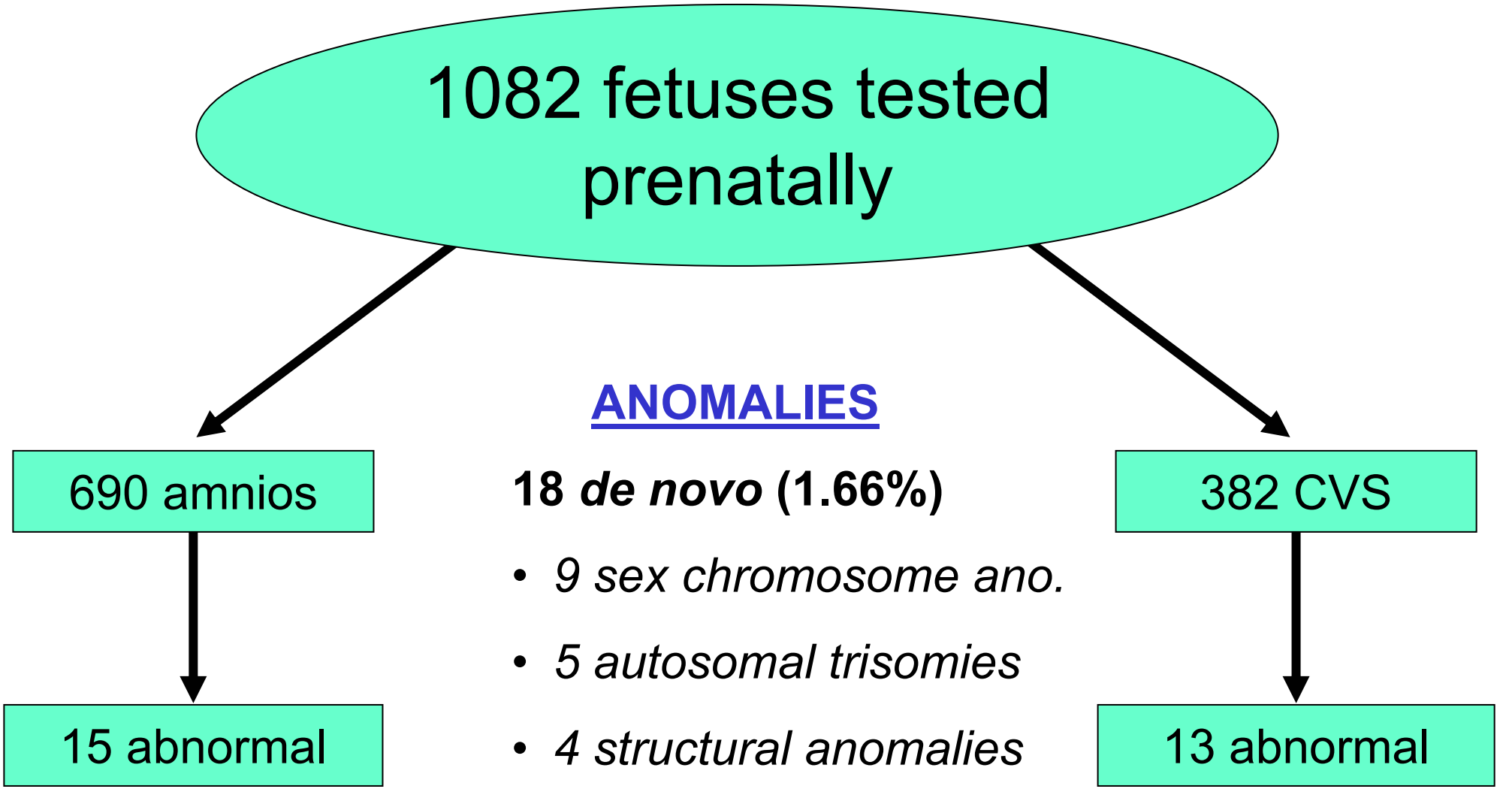
Pre-ICSI counseling
(while awaiting more definitive data…..)

Bonduelle et al., HUM REPROD 14:243-264

Before any treatment is started, patients should be informed of available data:

- risk of transmitting (parental) chromosomal aberrations
- risk of de novo, mainly sex chromosome, anomalies
- risk of transmitting fertility problems to offspring
- no increased incidence of congenital malformations

Bonduelle et al. (1999) HUM REPROD 14:243-264



Abnormal fetal karyotypes after ICSI

(Bondeulle et al., 1999, HUM REPROD 14:243-264)

- 18 cases had de novo aberrations
- not expected because of maternal age (mean of 32.5)
- value of **0.83%** of sex chromosomal anomalies is **4x higher than in newborns**
- linked to male infertility in most of these couples
- increased incidence of **de novo structural aberrations (0.36%)** as well (3-4x more than expected)

THE END

plus some image