Andrea Orús Alcalde
Grau en Biologia
andreaorus@gmail.com

UAB
Universitat Autònoma de Barcelona

Cytogenetic analysis in prenatal diagnosis

INTRODUCTION

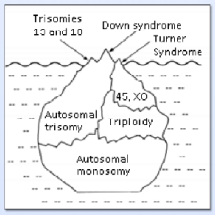

FIGURE 1. The iceberg of miscarriage due to chromosomal abnormalities.
(Adaptation of: Gardner, 2012)

PARENTS' CONCERN → PRENATAL DIAGNOSIS → GENETIC COUNSELING

- Syndrome detection
- ↓ Syndrome prevalence
- Parents' decision

- Psychological healing
- Procedure explanation

The aim of this study is to review the main procedures used in invasive prenatal diagnosis, as well as focusing on the clinical procedures, both sampling and cytogenetic analysis, and on genetic counseling.

INDICATIONS

- **ADVANCED MATERNAL AGE**
  - >35 years old

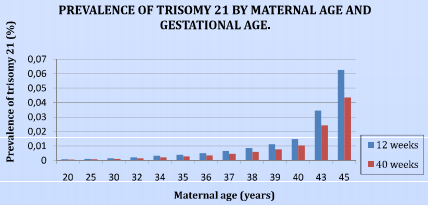

FIGURE 2. Prevalence of trisomy 21 by maternal age and gestational age (12 and 40 weeks). (Adaptation from: Shiefa, 2013)

- **SCREENING TEST RESULTS**
  - Probability higher than 1/250

Biochemical markers and Ultrasound Screening

	First trimester detection				Second trimester detection		
	NT	PAPP-A	β-hCG	uE_3	AFP	β-hCG	Inhibin A
Trisomy 21	>1	<1	>1	<1	<1	>1	>1
Trisomy 18	>1	<1	<1	<1	<1	<1	-
Trisomy 13	>1	<1	<1	<1	<1	<1	>1

TABLE 1. Increased and decreased biochemical and ultrasound parameters for trisomies 21, 18 and 13. This values are expressed in MoMs (Multiple of Media), being 1 MoM the standard value for normal pregnancies. (Shiefa, 2013; Turnpenny, 2007)

- Screening detection rates are low when screening tests are used separately → This is higher when several tests are combined and when maternal age is considered.
- First trimester screening tests > Second trimester screening tests (Zournatzi et al., 2008).

- **FAMILY HISTORY OF GENETIC DISORDERS**

SAMPLING

CHORIONIC VILLUS SAMPLING

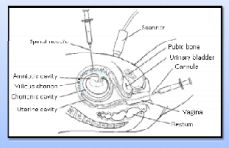

FIGURE 3. Chorionic villus sampling procedure. *(Nussbaum, 2007)*

- 11 – 12 weeks of pregnancy
- US before the procedure
- Direct analysis or after culture
- Risk of miscarriage: 1%

AMNIOCENTESIS

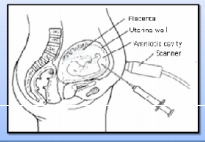

FIGURE 4. Amniocentesis procedure. *(Nussbaum, 2007)*

- 14 – 20 weeks of pregnancy
- US before the procedure
- Results available in 2 – 3 weeks
- Risk of miscarriage: 0'5 – 1%

CYTOGENETIC ANALYSIS

G-BANDING

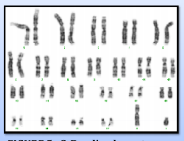

FIGURE 5. G-Banding karyotype

Description	Chromosomes are stained with Giemsa, giving a characteristic pattern for each chromosome.
Detection	Aneuploidies, new centromere formation, translocations, inversions, deletions and duplications (> 3-10 Mb) *(Crotwell, 2012)*.
Detection rate	Depends on the resolution → ≈ 35%. *(Lichtenbelt, 2011)*
Limitations	Impossible to detect small chromosome abnormalities.

FISH (FLUORESCENCE IN SITU HYBRIDIZATION)

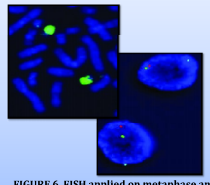

FIGURE 6. FISH applied on metaphase and on interphase. (Adaptation of Riegel, 2014)

Description	Hybridization of probes labelled with fluorescent dyes within chromosomes (either in metaphase or in interphase) immobilized ion microscope slides.
Detection	Aneuploidies, deletions, duplications, translocations and fetal sex.
Advantages	Rapid diagnosis: Results are available in 24-48 h
Limitations	Only few targets can be selected → Only few syndromes can be searched in one test.

ARRAY CGH (COMPARATIVE GENOMIC HYBRIDIZATION)

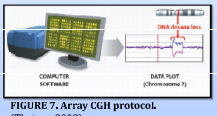

FIGURE 7. Array CGH protocol. (Theisen, 2008)

Description	Cohybridization of DNA patient sample and DNA control sample onto a microchip that contains single-stranded nucleotides
Detection	Aneuploidies, large segmental aneuploidies, insertions, duplications and deletions.
Detection rate	Depends on the platform array applied, the probe location and the probe coverage.
Advantages	Higher resolution and wider coverage than conventional karyotyping and FISH
Limitations	Cannot detect triploidies, balanced rearrangement and low level mosaicism

GENETIC COUNSELING

Genetic counseling is performed throughout the prenatal diagnosis process.

PRE-TEST COUNSELLING

- Focus on Down syndrome → Counselors must explain that there are other syndromes
- Background risk and individual increased risks
- Limitations of PD and risks associated with the procedures

STRESS → Clinicians should be sensitive about their psychological issues.

POST-TEST COUNSELLING

- Explanatory process about the test and its limitations
- Description of the syndrome: Physical, Cognitive and Social implications
- Discussion about the parents' ability and willingness to have a child affected by a syndrome

MAKING A DECISION → Counselors must explain all the possible options in a non-directive way
- Pregnancy termination
- Continuation the pregnancy
- Adoption

COUNSELLORS MUST RESPECT PARENTS' DECISION and be ensured that no one is pressuring them in the decision-making process.

PARENTS' SHOCK makes it very complicated to assimilate information → PUT THE INFORMATION ON PAPER

CONCLUSIONS

- IPD is not performed on all pregnant women → In cases where the probability of having an affected fetus is >1/250. All pregnant women must undergo to screening tests.
- CVS is more advisable than amniocentesis → CVS is performed earlier.
- Array CGH has higher resolution and wider coverage than G-banding and FISH, respectively. Results obtained by array CGH must be confirmed using other methods. FISH only can be used to analyze few syndromes in each essay → Applied to seek the main syndromes or when a syndrome is suspected.
- Genetic counseling → Explain the tests, the syndrome's implications and to provide psychological healing.

REFERENCES

- Crotwell, P. L., & Hoyme H. E. (2012). Advances in Whole-Genome genetic testing: From chromosomes to microarrays. *Current problems in pediatric and adolescent health care, 42*, 47 – 73.
- Gardner R. J. M., Sutherland G. R. & Shaffer L. G. (2012). *Chromosome abnormalities and genetic counseling.* 4th Edition, Oxford, Oxford University Press.
- Lichtenbelt K. D., Knoers N. V. a M. & Schuring-Blom G. H. (2011). From karyotyping to array-CGH in prenatal diagnosis. *Cytogenetic and genome research, 135*(3-4), 241–50.
- Nussbaum, R. L., Maclnnes, R. R., Willard, H. F., & Hamosh, A. (2007). *Thompson and Thompson Genetics in Medicine.* 7th Edition. Philadelphia. *Saunders Elsevier*
- Riegel, M. (2014). Human molecular cytogenetics: From cells to nucleotides. *Genetics and molecular biology, 37*, 194–209.
- Shiefa S., Amargandhi M., Bhupendra J., Moulali S. & Kristine T. (2013). First Trimester Maternal Serum Screening Using Biochemical Markers PAPP-A and Free β-hCG for Down Syndrome, Patau Syndrome and Edward Syndrome. *Indian journal of clinical biochemistry : IJCB, 28*(1), 3–12.
- Theisen, A. (2008) Microarray-based comparative genomic hybridization (aCGH). *Nature Education* 1(1),45 – 48.
- Turnpenny, P. D., & Ellard, S. (2007). *Emery's elements of medical genetics,* 13th Edition, Edinburgh, Elsevier/Churchill Livingstone
- Zournatzi V., Daniilidis A., Karidas C., Tantanasis T., Loufopoulos A. & Tzafettas J. (2008). A prospective two years study of first trimester screening for Down Syndrome. *Hippokratia, 12*, 28–32.