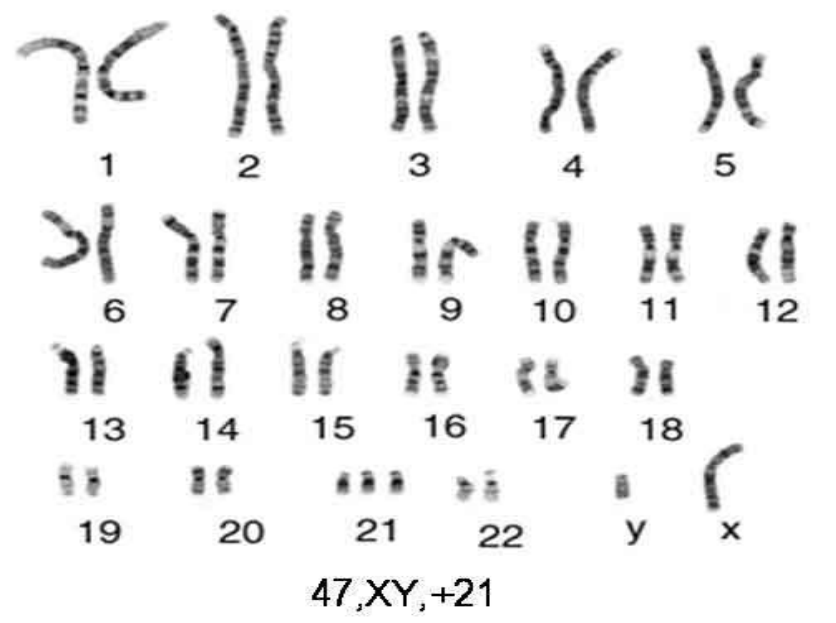
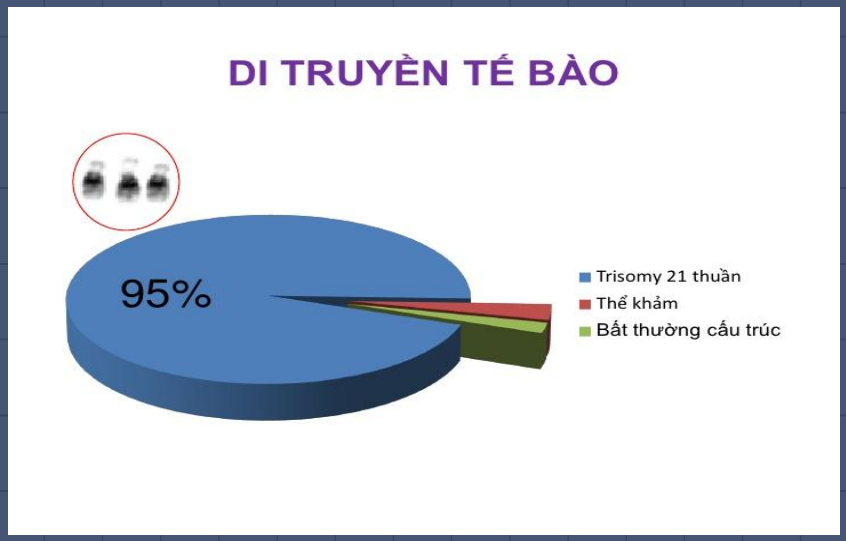
EVALUATION ON THE PRENATAL SCREENING RESULTS DETECT DOWN SYNDROME FROM CELL FREE FETAL DNA IN THE MATERNAL PLASMA

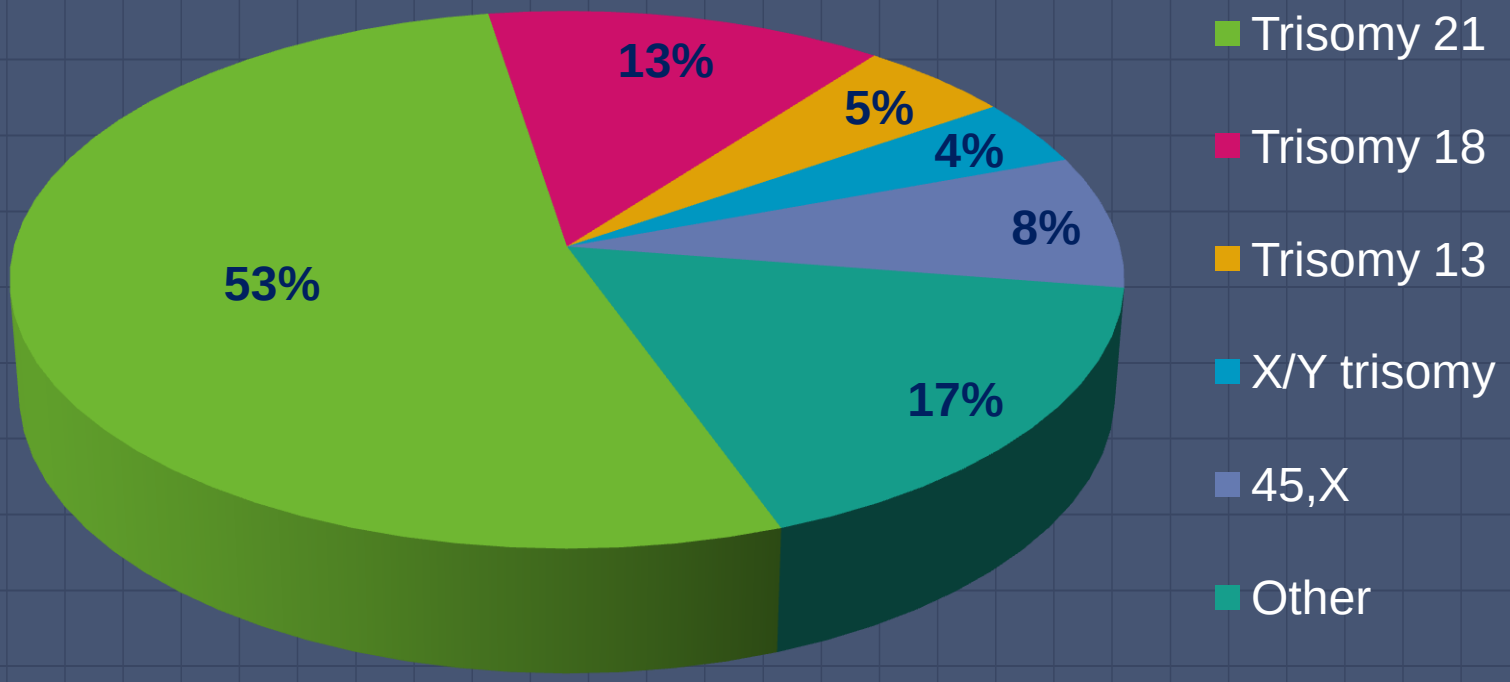
DOWN SYNDROME





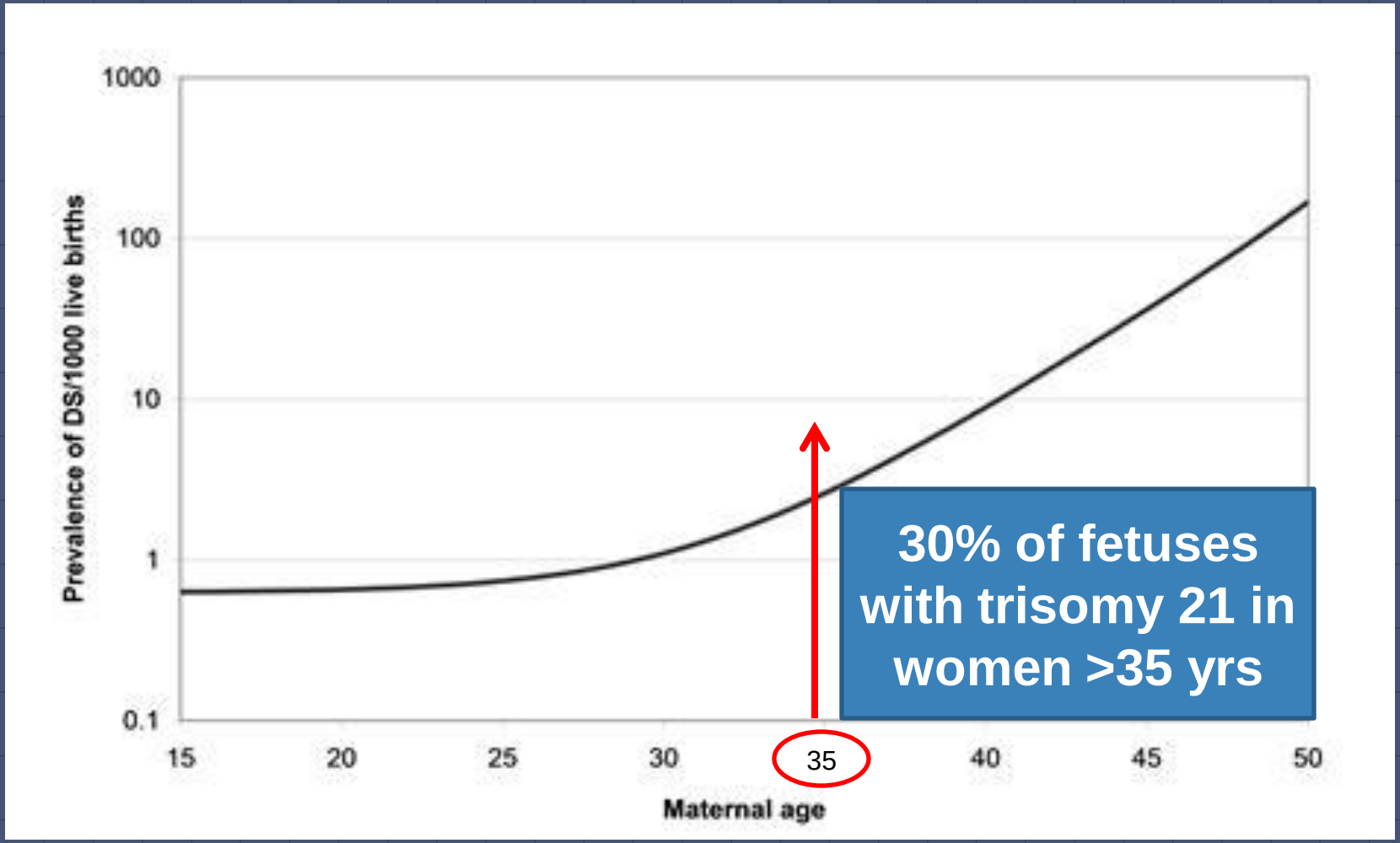
- The most common cause of prenatal chromosome abnormalities
- Frequency: 1:700

PRENATAL PREVALENCE OF CHROMOSOMAL ABNORMALITIES

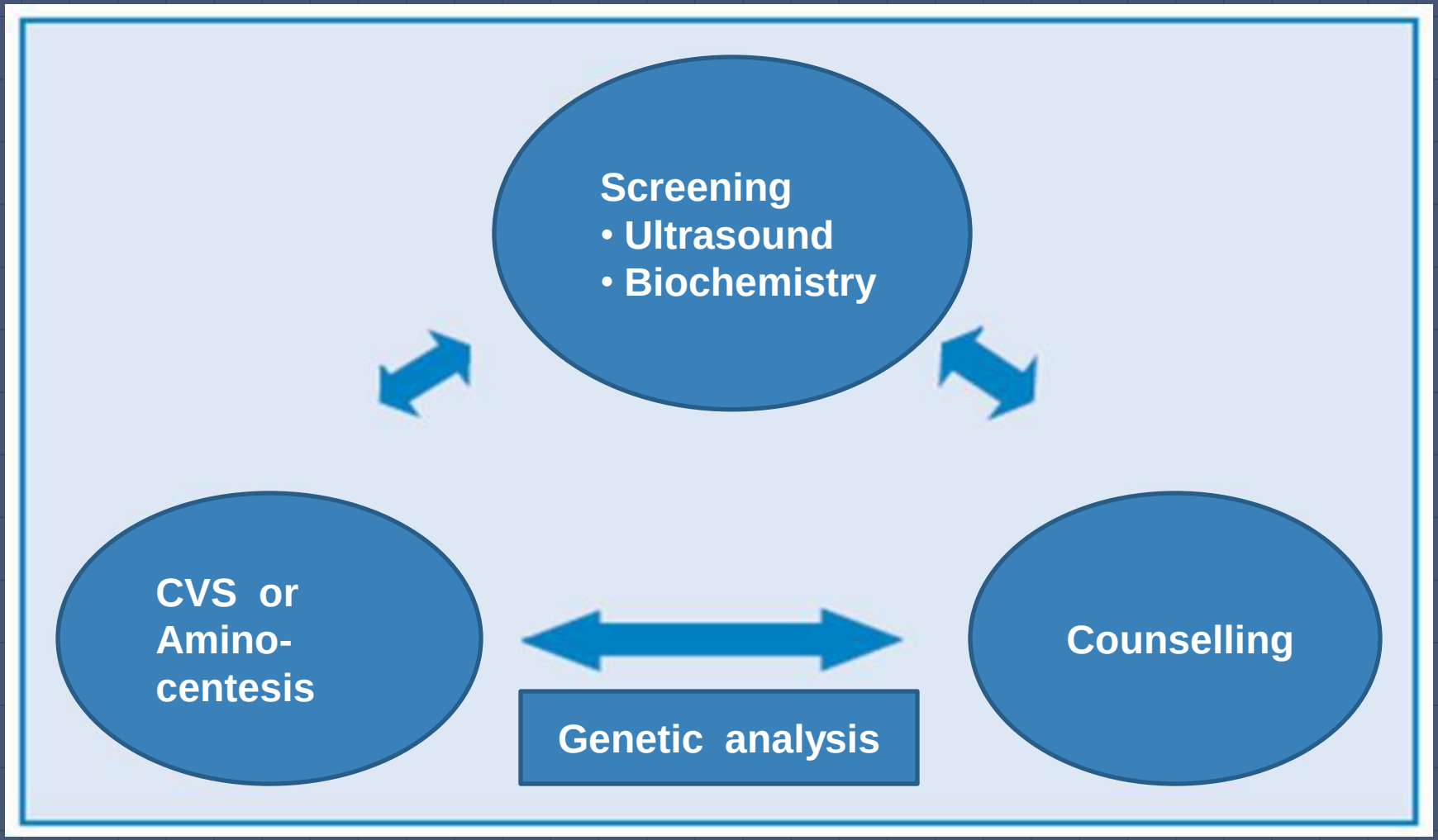


Diana Wellesley et al. Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. European Journal of Human Genetics (2012) ***20,*** *521–526*

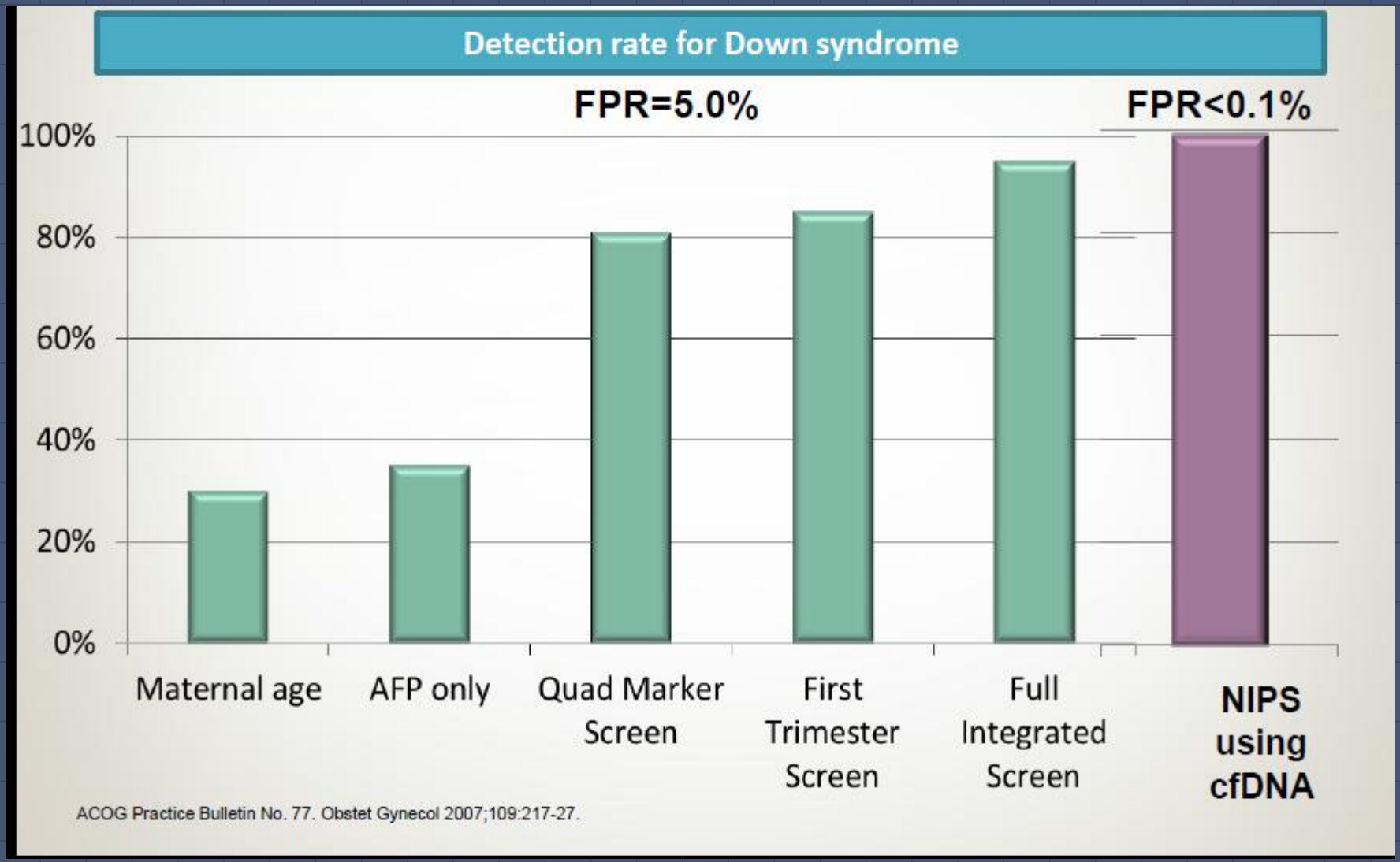
RELATIONSHIP BETWEEN MATERNAL AGE AND THE PREVALENCE OF DOWN SYNDROME



PRENATAL SCREENING FOR COMMON ANEUPLOIDIES: CURRENT PRACTICE



DETECTION RATE AND FPR BY CURRENT SCREENING PRACTICE FOR TRISOMY 21



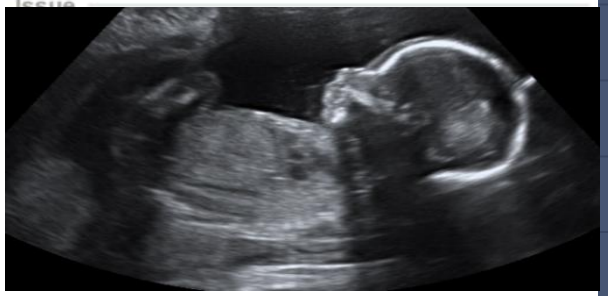
THE RISK OF FETAL LOSS ASSOCIATED WITH CVS/AC

Original Paper

The risk of fetal loss associated with invasive testing following combined first trimester risk screening for Down syndrome – a national cohort of 147 987 singleton pregnancies

Camilla Bernt Wulff[1,2], Thomas Alexander Gerds[3], Line Rode[1,4], Charlotte Kvist Ekelund[1], Olav Bjørn Petersen[5], Ann Tabor[1,2] and the Danish Fetal Medicine Study Group

DOI: 10.1002/uog.15820



Abstract

Objectives

To prospectively assess the risk of fetal loss associated with chorion villus sampling (CVS) and amniocentesis (AC) following combined first trimester screening (cFTS).

Methods

A nationwide population-based study (Danish Fetal Medicine Database, 2008-2010) included 147987 singleton pregnant women who received cFTS. Propensity score stratification was used to assess the risk of fetal loss with and without invasive test.

Analyses were performed from 3 to 21 days after cFTS for CVS and from 28 to 42 days after cFTS for AC. Results were reported as average risk differences with 95% confidence limits.

Results

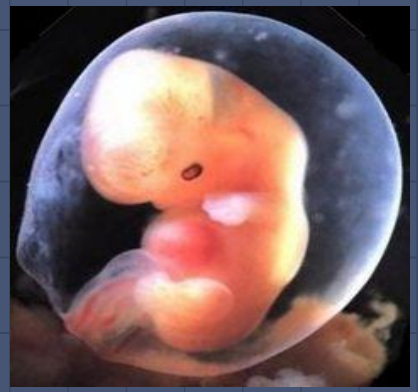
The risk of miscarriage and stillbirth was not higher in women exposed to CVS or AC compared with unexposed women independent of the analysis time point. The average effect of CVS on risk of miscarriage was -0.08% (95% CI; -0.64; 0.47) for 3 days and -0.21% (-0.58; 0.15) for 21 days, while the effect on risk of stillbirth was -0.18% (-0.50; 0.13) for 3 days and -0.27% (-0.58; 0.04) for 21 days after cFTS, respectively.

The analysis 28 days after cFTS showed a non-significant average effect of AC on risk of miscarriage of 0.56 % (-0.21; 1.33), while the effect of AC on risk of stillbirth was 0.09% (-0.39; 0.58) for 42 days after cFTS.

Conclusion

CVS or AC was not associated with increased risks of miscarriage and stillbirth. The findings of this study support that the procedure-related risk of CVS and AC is very low and correlates with the indication for the procedure.

Average rate of miscarriage: 0,86% (0,55% miscarriage & 0,31% stillbirth



Wulff CB et al. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147,987 singleton pregnancies. Ultrasound Obstet Gynecol. 2016 Jan;47(1):38-44. doi: 10.1002/uog.15820

NEXT GENERATION SEQUENCING

Sequencing of 1 – 43 billions short DNA reads (Massive Parallel Sequencing)

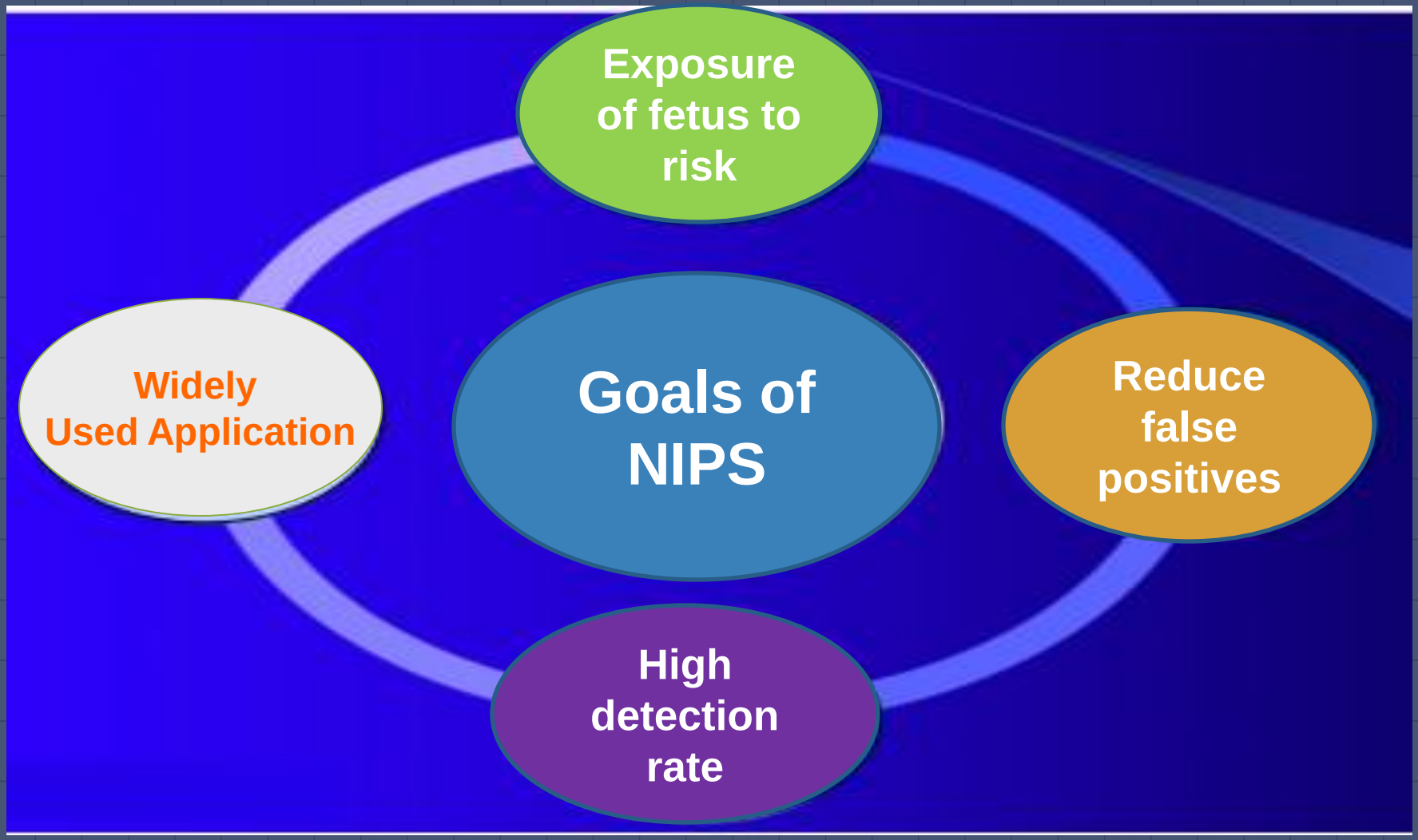
Aneuploidy Detection and Single-gene genetic disorders

2011: Introduction of NIPS

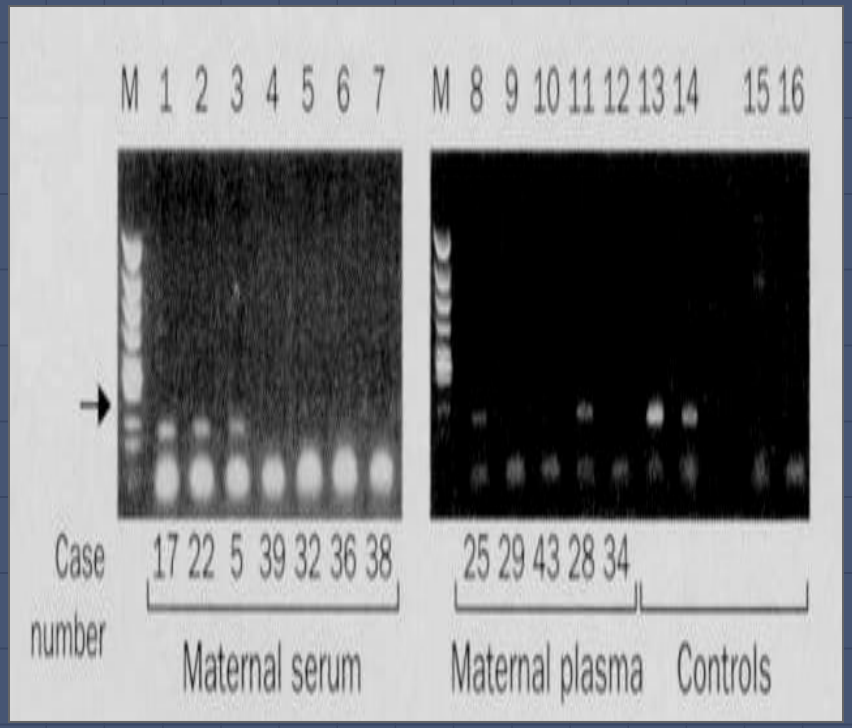
2008 detection of Trisomy 21

- **Chiu RW, Chan KC, Gao Y, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci USA 2008;105:20458-20463**
- **Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci USA 2008;105:16266-16271**

NONINVASIVE PRENATAL SCREENIG (NIPS)



NIPS: FETAL CELL FREE DNA (cffDNA)



PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Abstract

C R Seances Soc Biol Fil. 1948 Feb;142(3-4):241-3.

Les acides nucléiques du plasma sanguin chez l'homme.

[Article in Undetermined Language]

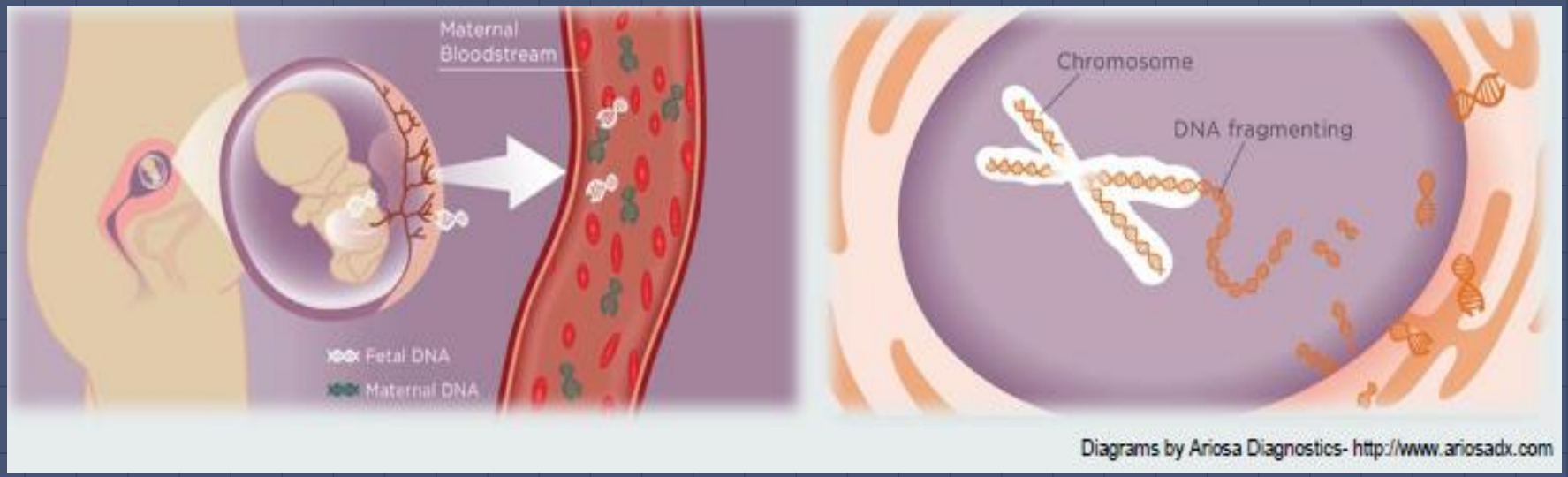
MANDEL P, METAIS P.

PMID: 18875018 [PubMed - indexed for MEDLINE]

10. Mandel P, Métais P. Les acides nucleiques du plasma sanguin chez l'homme. C R Seances Soc Biol Fil. 1948;143:241–3.

Lo et al. Lancet 1997; 350:485

FETAL CELL FREE DNA



Originates from cells of the trophoblast (placenta)

Released into bloodstream as small DNA fragments (150-200 bp)

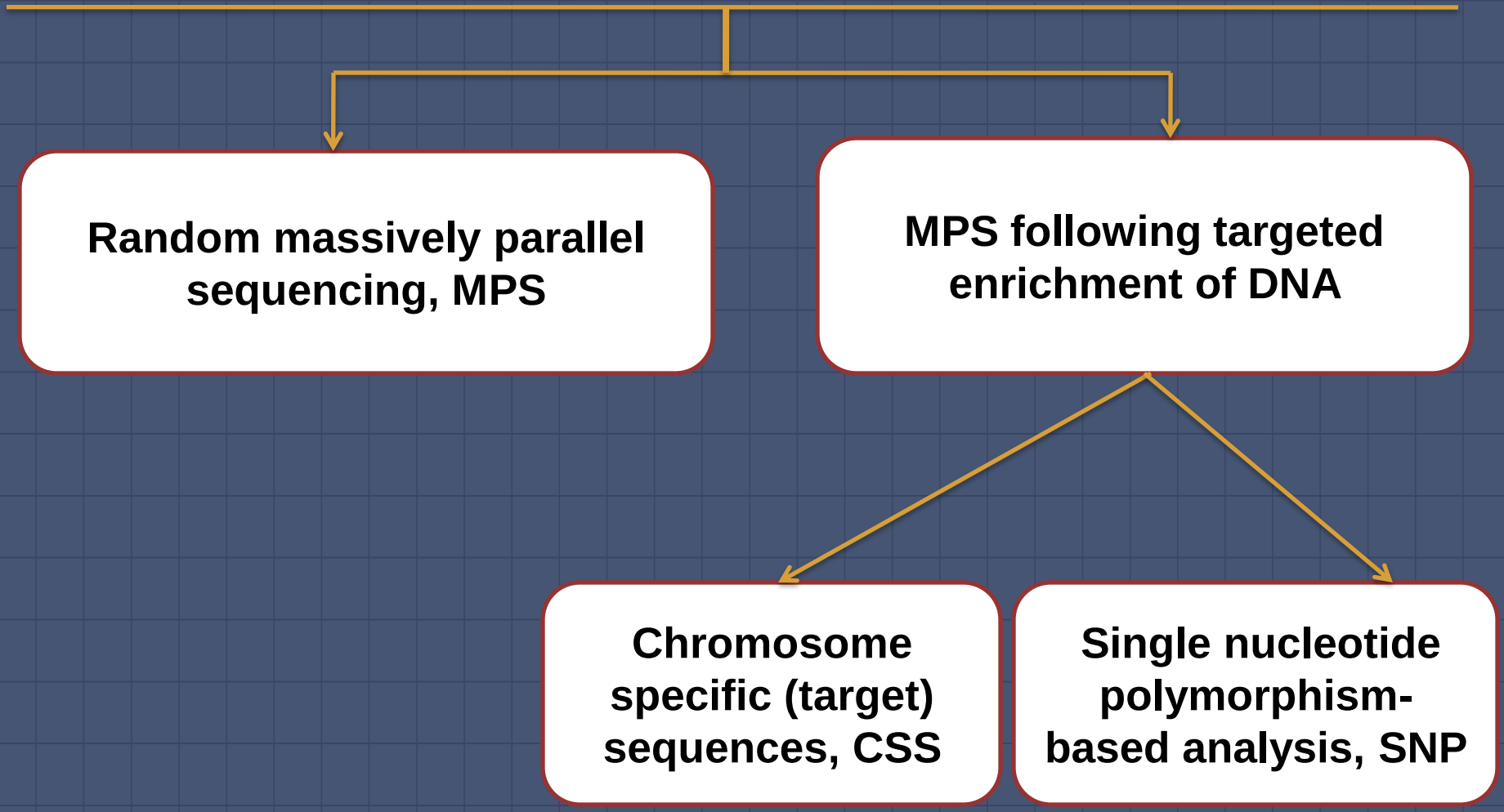
3-13% of total cell free DNA in maternal plasma

Reliably detected >9-10 weeks gestation

Short half life (16.3 min), undetectable by 2 hrs postpartum

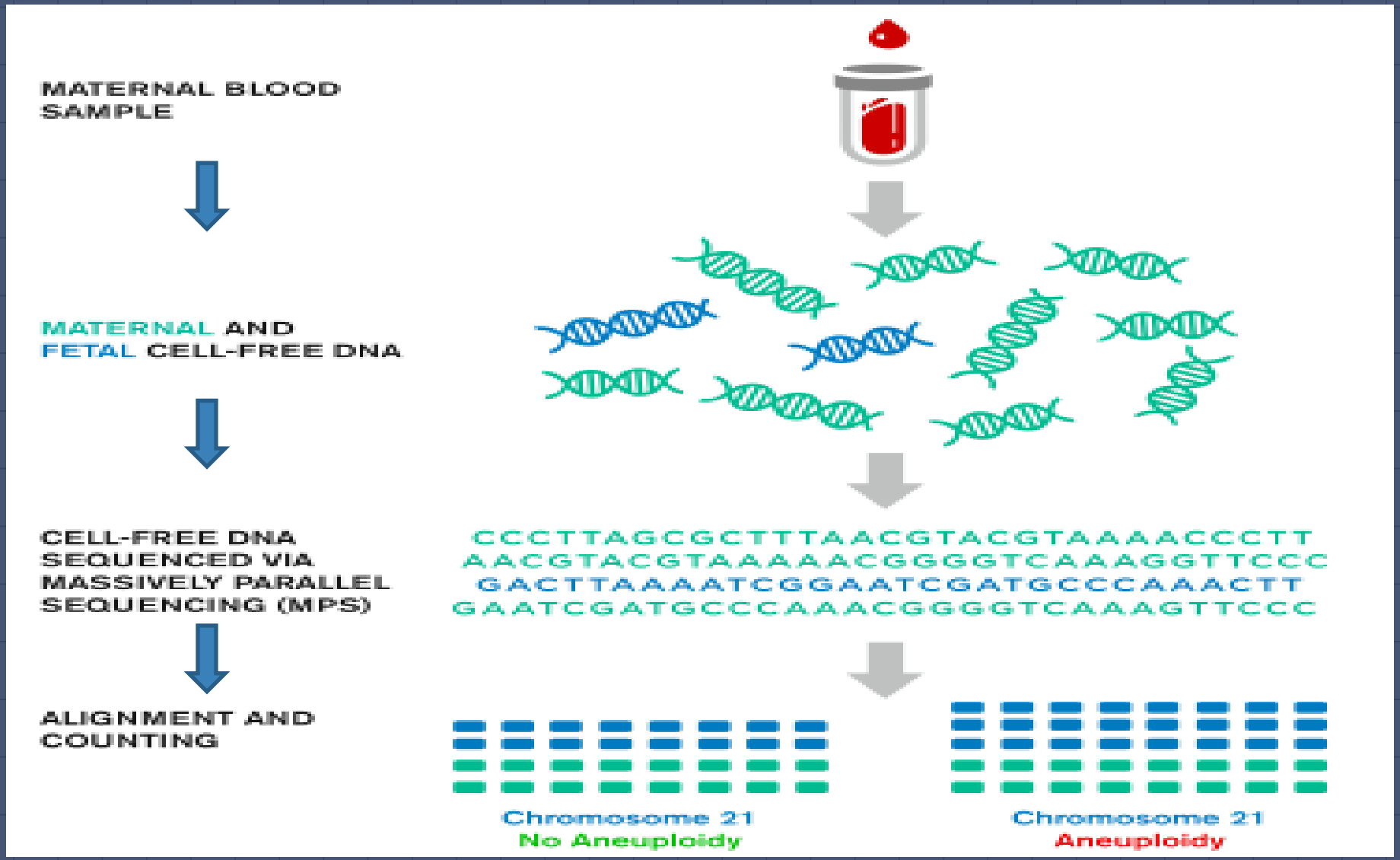
(Ehrich et al, AJOG 2011)

NIPS METHODS



Thomas Harasim et al. Current status of non-invasive prenatal testing (NIPT): genetic counseling, dominant methods and overall performance. J Lab Med 2016; 40(5): 299–306

DNA SEQUENCING USING CELL FREE DNA

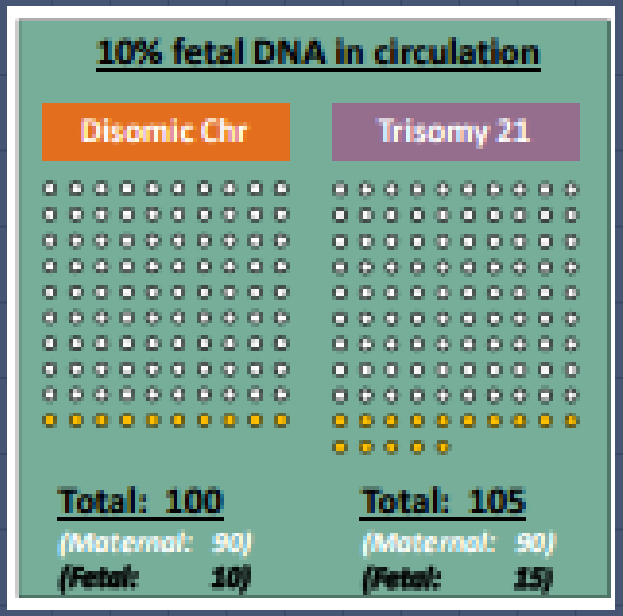


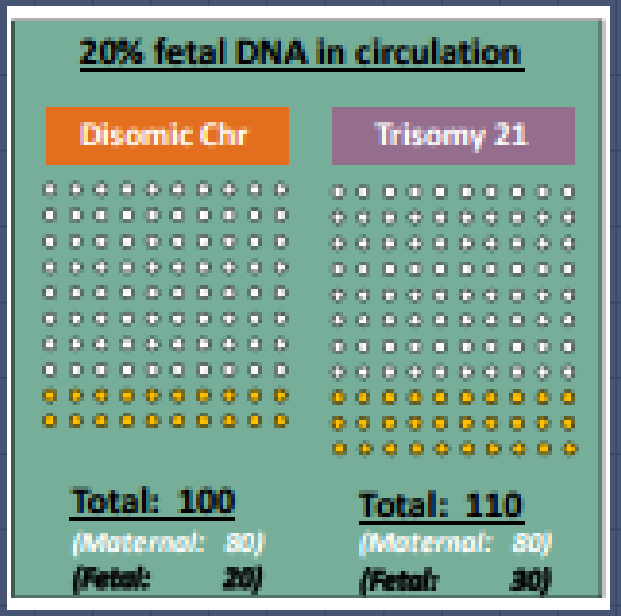
(A. Swanson, 2013)

IMPORTANCE OF cffDNA

Trisomy detection via cfDNAdepends on fraction of DNA that is fetal

The higher the fetal fraction, the easier it is to detect trisomy





EVALUATION OF NIPS (37 studies, n=21.608)

Aneuploidy	n	DR (%)	FPR (%)
Trisomy 21	1.051	99,2	0,09
Trisomy 18	389	96,3	0,13
Trisomy 13	139	91	0,13
Monosomy X	177	90,3	0,23
Other	56	93	0,14
Trisomy 21 (twin pregnancies)		93,7	0,23

Reduced risk

Gil M.M. Ultrasound Obstet Gynecol 2015

ACOG Committee Opinion on NIPT

- ***"Cell free fetal DNA appears to be the most effective screening test for aneuploidy in high risk women… is one option that can be used as a primary screening test in women at increased risk of aneuploidy"***
- ***"[NIPT] should be an informed patient choice after pretest counseling"***
- ***"[NIPT] should not be offered to low-risk women or women with multiple gestations"***
- ***"A patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results."***

Also supporting NIPT for high risk pregnancies:

ispd

National Society of Genetic Counselors

OBJECTIVES

Evalutation of NIPS: Down Syndrome Detection using NGS and cfDNA in maternal plasma

SUBJECTS

Sample collection and recruitment criteria

Singleton ≥ 10 weeks with at least one criteria:

- ❖ Maternal age ≥ 35.
- ❖ High risk biochemical screening >1/250.
- ❖ Abnormal ultrasound.
- ❖ Previous affected pregnancies: Aneuploidy, miscarriages, still births,…
- ❖ Pregnant women agreeing to participate

SUBJECTS

Not included:

- < 10 weeks pregnancies, multiple pregnancy
- Pregnant women gone through transplant or stem cell treatment
- Pregnant women gone through blood transfusion less then 30 days
- Pregnancy from egg donor, cancer
- Pregnant women with chromosomal abnormality

METHODS

Study design: Prospective Study

Patients and samples: 463 samples

Facilities:

- Department of Biochemistry, Hanoi Medical University
- Center of Prenatal Diagnosis, Hanoi Hospital O & G

Timeline *:* 5/2016 – 3/2017

Data analysis*:* SPSS 16.0, statistical analysis,...

MATERIALS

Sample:

10ml whole blood collected in Streck tube

Equipments :

Reagent and instrucments provided at center screening, prenatal diagnosis and newborn, HN O&G hospital

.

Chemicals:

cfDNA Extraction: PerkinElmer

Library Preparations and Templation: ThermoFisher

Sequencing: PI chip on Ion Proton - ThermoFisher

.

METHODS

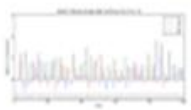
A **cfDNA Isolation**

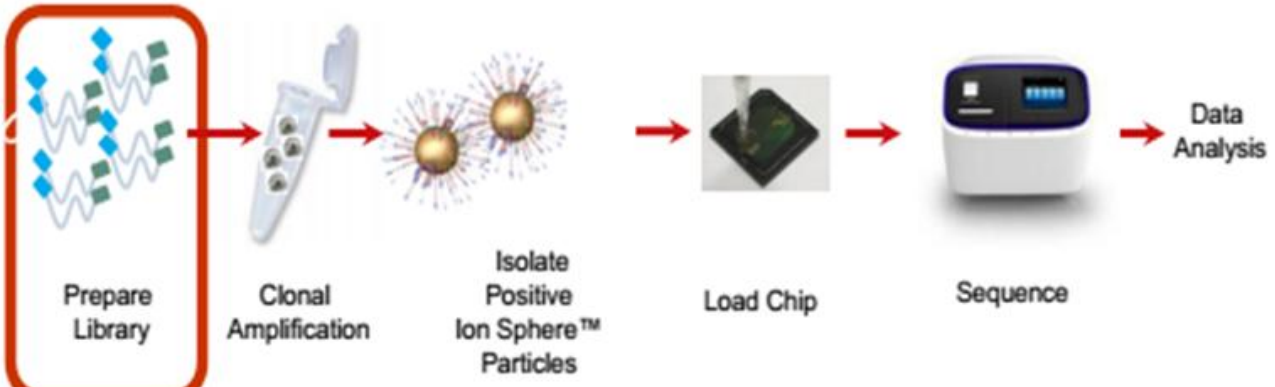
B **Library Prep and Purification**

C **Templating**

D **Sequencing**

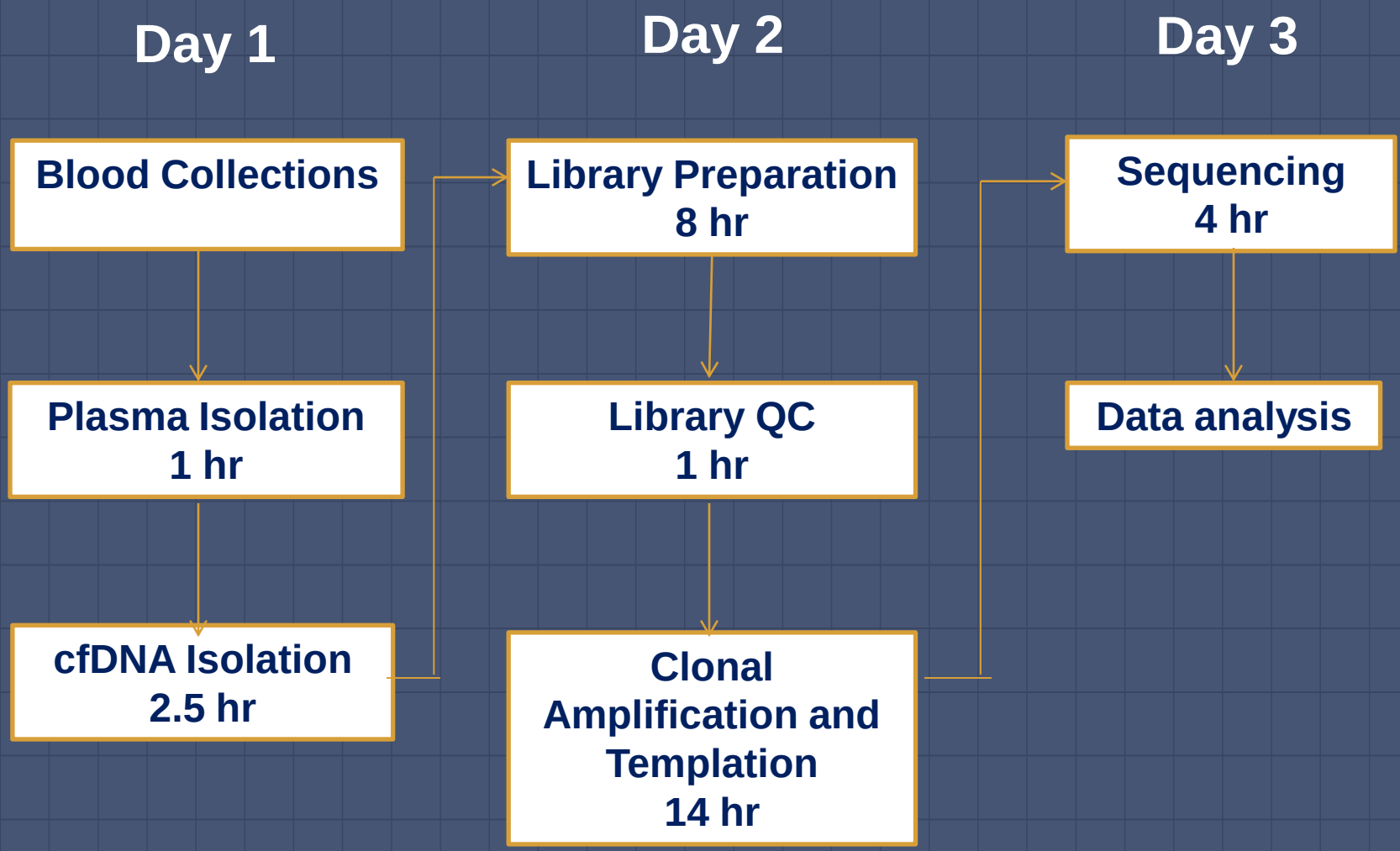
E **Data Analysis**





cfDNA fragments → **Amplification of library fragments using primers**

NGS METHOD - TIMELINE



RESULTS AND DISCUSSION

1. SUBJECTS

Maternal Age	Quantity	
	n	%
18-24	20	4.32
25-29	102	22.03
30-34	113	24.41
35-39	165	35.64
≥ 40	63	13.6
Total	463	100
X ±SD	33.6 ± 5.4	
Range	19 - 46	

Shan Dan, Wei Wang, et al (2012). *Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11105 pregnancies with mixed risk factors. Prenatal Diagnosis,*

1. SUBJECTS

Gestation	Quantity		%cffDNA ±SD
	n	%	
10 – 13 weeks 6 days	142	30.7	7.04±0.02
14 – 20 weeks 6 days	295	63.7	7.13±0.03
≥ 21 weeks	26	5.6	9.53±0.03
Total	463	100	
X ±SD	16±3.6		

Tăng dần

Yi Zhou, MD, Zhongyi Zhu, et al. (2015).

2. cffDNA

cffDNA	Quantity	
	n	%
< 3.5%	19	4.1
> 3.5%	444	95.9
Total	463	100.0

- Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH (2015). *Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol*

- Saskia Tamminga, Merel van Maarle, et al (2016). *Maternal Plasma DNA and RNA Sequencing for PrenatalTesting. Advances in Clinical Chemistry*

3. DOWN SYNDROME DETECTION

No	Maternal Age	Gestational Age	T21 Assessment	z-score	Sex	NIPS	Karyotype
1	27	17w	TT: 1/38	6.18	Male	T21	47,XY,+21
2	43	20w	TM	9.16	Male	T21	47,XY,+21
3	25	13w3d	CB: 1/13	4.87	Female	T21	47,XX,+21
4	40	16w4d	TT: 1/50	6.8	Female	T21	47,XX,+21
5	41	18w5d	CB: 1/151	10.02	Female	T21	47,XX,+21
6	34	16w	CB: 1/9; NT:3.2	16.06	Male	T21	47,XY,+21
7	36	17w3d	TM	8.97	Male	T21	47,XY,+21
8	41	10w6d	TM	4.61	Female	T21	Abortion

TM: Maternal age; TT: triple test; CB: Combined test; NT: Nuchal translucency ; T21: Trisomy 21 high risk

3. DOWN SYNDROME DETECTION

Risk	Quantity	
	n	%
High risk	8	1,73
Low risk	455	98,27
Total	463	100

- ***Shan Dan, Wei Wang, et al (2012).*** *Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11105 pregnancies with mixed risk factors. Prenatal Diagnosis,*
- *Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F (2015). Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol*

SUMMARY

Down Syndrome Detection using NGS and cfDNA in maternal plasma

- **Increased Detection Rate**
- **Decreased FPR**
- **Decreased miscarriages**

98.27% reduction of invasive procedures (CVS or amniocentesis)

Invasive testing to confirm high-risk NIPS results

THANK YOU