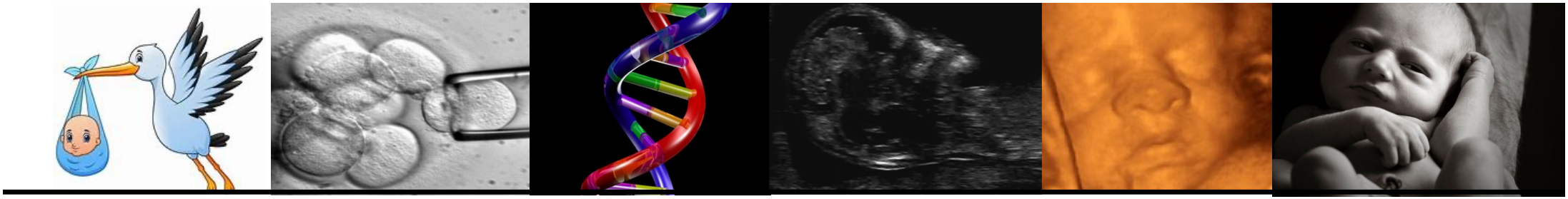


10° CORSO AOGOI - 9° TURIN IAN DONALD COURSE

TORINO 15-16 MARZO 2024

Diagnosi Prenatale Invasiva

Giovanni Monni

Direttore Genera Cagliari - *e-mail: prenatalgmonni@gmail.it*

Genera
from science to life

IVIRMA Global Research Alliance

YEAR	PRENATAL GENETIC SCREENING *AND* DIAGNOSIS TECHNOLOGY
	Where have we been? Where are we going?
1956	**Amniocentesis** first used to identify genetic disorders. Karyotyping first used to identify Trisomy 21 as a cause of Down Syndrome
1983	**Chorionic villus sampling (CVS)** first performed
2010	First use of **Chromosomal Microarray Analysis (CMA)** for analysis of samples obtained from amniocentesis or CVS
2011	**Cell-free DNA screening tests** (known as **NIPT**) first clinically available. They analyze fragments of placental and fetal DNA circulating in pregnant woman's blood to assess fetal sexand the likelihood of Trisomy 13, 18, 21, as well as microdeletions and microduplications
2013	**NIPT** available commercially
2013	The ACMG reccommends that **Whole Exome Sequency** be considered when specific genetic tests for a phenotype fail to determine a diagnosis in a fetus with multiple anomalies suggestive of a genetic disorder
2017	**Non-invasive whole genome sequencing** (WGS) is technically possible but is not yet commercially available in the prenatal context

PAST-PRESENT-FUTURE OF PRENATAL SCREENING *AND* DIAGNOSIS

Where have we been?

Where are we going?

In parallel, programs for universal parental carrier screening for autosomal recessive disorders, such as Thalassemias, Cystic Fibrosis, as well as ethnicity-based carrier screening, such as for conditions more prevalent in the Ashkenazi Jewish population, were developed to identify parents at 25% risk of having an affected child with these disorders.

Identified carrier couples can then choose Preimplantation Genetic Diagnosis (PGD) to avoid affected pregnancies, or prenatal diagnosis, allowing them to consider termination of affected pregnancies or be prepared for the birth of an affected child.

PAST-PRESENT-FUTURE OF PRENATAL SCREENING *AND* DIAGNOSIS

Where have we been?

Where are we going?

With recent technological advances in methods to identify numerical and structural chromosome abnormalities and point mutations, such as array-based copy-number analysis, also known as chromosomal microarray analysis (CMA), and next-generation sequencing (NGS), the screening for and diagnosis of genetic abnormalities in the fetus is undergoing an unprecedented rapid evolution

In parallel, CMA and NGS have also accelerated the discovery of causes of intellectual disability, birth defects, and many rare genetic and genomic disorders

This has motivated the development of expansive carrier screens for hundreds of genetic disorders at once as well as the development of non-invasive cell-free fetal DNA (cffDNA)-based screens for fetal chromosomal aneuploidy, subchromosomal abnormalities, and single-gene disorders

The availability of CMA- and NGS-based methods, such as targeted gene-panel sequencing and, recently, whole-exome sequencing (WES), has also resulted in the ability to diagnose more fetal genetic conditions from samples obtained through amniocentesis or chorionic villus sampling

PAST-PRESENT-FUTURE OF PRENATAL SCREENING *AND* DIAGNOSIS

Where have we been?

Where are we going?

All of these new tests have created new, exciting opportunities for comprehensive prenatal diagnosis and screening, but they are accompanied by important challenges.

Healthcare providers must consider the consequences of their rapid introduction into the clinic because of the still-limited knowledge about the test performance of some assays in routine clinical practice, concerns related to cost-conscious implementation of optimized screening and testing strategies, equal access, and appropriate selection of who will benefit most.

The ever-increasing amount of genetic information that can be obtained preconceptionally and prenatally also brings about ETHICAL AND GENETIC COUNSELING CHALLENGES.

PREVALENCE AND ETIOLOGY OF SERIOUS CONGENITAL ANOMALIES

~5 %

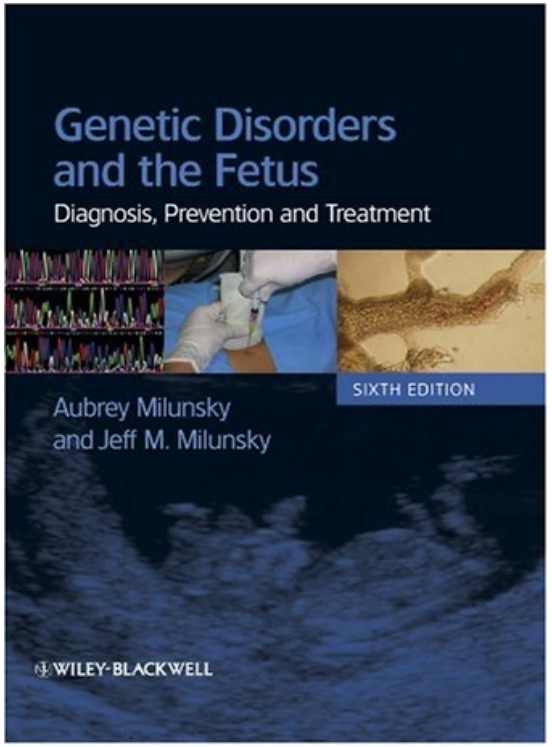
ANOMALIES	PREVALENCE %
Structural congenital anomalies* (many from de novo mutations)	~ 2.0 – 3.0
Mendelian genetic disorders	0,4
Pathogenic microdeletions and duplications	1,2
Chromosome abnormalities other than common trisomies	0,4
Common trisomies (21, 18, 13)	0,2

*Many detected by ultrasound also in the 1st trimester

Clinical Opinion, Am J Obstet Gynecol 2016

Factors that influence estimates of the incidence or prevalence in the newborn of a congenital malformation or a genetic disorder

- Maternal age
- Use of maternal serum - ultrasound screening for Down Syndrome
- Use of maternal serum screening for neural tube defects
- Frequency, inclusion, and exclusion of stillbirths, fetal deaths and TOP
- Maternal diabetes and gestational diabetes
- Previous affected child
- Availability and use of expertise in prenatal diagnostic ultrasound
- History of recurrent spontaneous abortion
- Multiple pregnancy rate
- Maternal epilepsy, lupus erythematosus, and other illnesses
- Maternal alcohol abuse, obesity and use of medication
- Family history and consanguinity
- Maternal fever or use of hot tub in the first 6 weeks of pregnancy
- Incidence and severity of prematurity
- Use of folic acid supplementation
- Economic level in developed or developing world
- Later manifestation or onset of disorder
- Previous maternal immunization/vaccination
- Frequency of certain infectious diseases
- Case selection, bias and ascertainment
- Definitions of major and minor congenital anomalies
- Use of perinatal necropsy and registry data
- Use of death certificates
- Training and expertise in examination of newborns
- In vitro fertilization and intracytoplasmic sperm injection
- Season of the year
- Paternal age



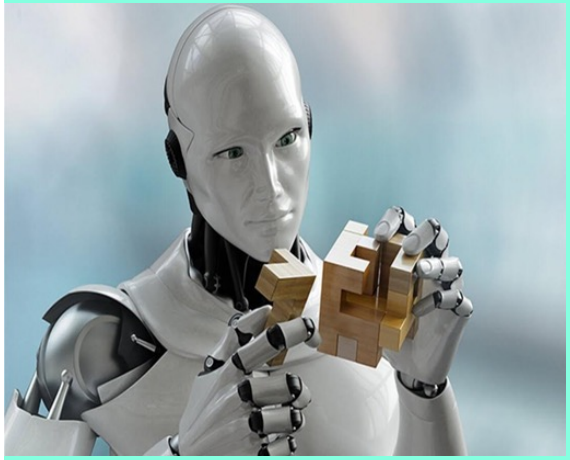
Milunsky 2010


1ST OR 2ND TRIMESTER FETAL SCREENING OR DIAGNOSIS?
cfDNA
TS
cffDNA
NIPS
Age
Nuchal translucency
Risk
TS21
ARTIFICIAL INTELLIGENCE
Invasive Testing
Down Syndrome
OMICS SCIENCES
Penta
Sequential
Contingent
CVS
TS18
Triple
Integrated
Ultrasound
Amniocentesis
Aneuploidy
Biomarkers
Quad
PGS - PGD
NIPT

ARTIFICIAL INTELLIGENCE (AI)
IN PRENATAL SCREENING AND DIAGNOSIS

The role of **AI** is founded on the power of computers to sift through and make sense of the enormous amounts of electronic data now available.

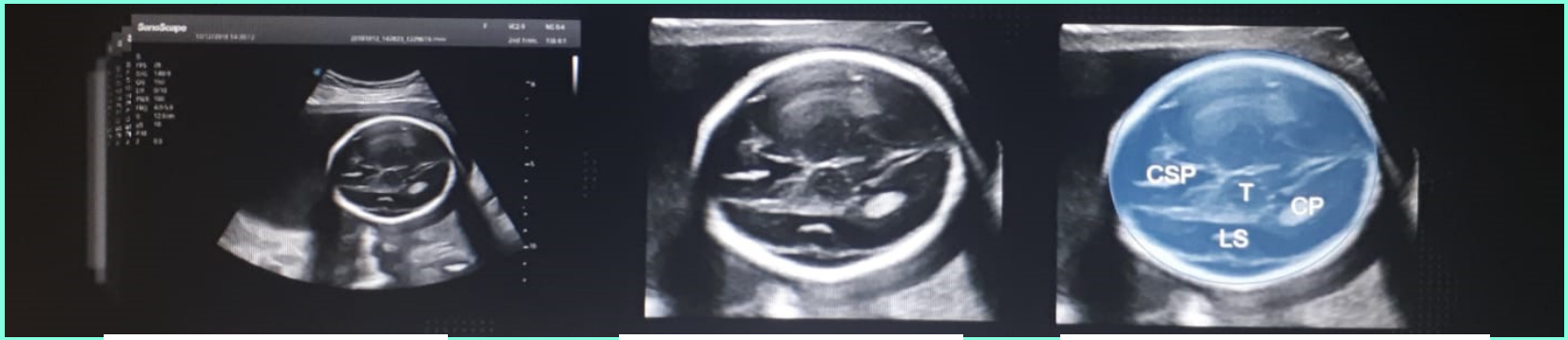
*When applied to **PRENATAL SCREENING AND DIAGNOSIS**,*



AI has the potential to **improve images acquisition and optimisation**, automated and standardizing **measurements**, **identification of outliers**, **research of DNA sequences**, **classification of diagnosis**, **choice** between different prenatal screening and invasive fetal procedures, calculating the **efficiency and fetal risk of invasive procedures**, **prediction of outcomes**, **introduction of simulators** or **model machines** for training and tutoring, **calculation of costs**, to optimize the **results** and **data for clinical research**, best **clinical trial accuracy** using communications by **telediagnosis and telecommunication channels**, providing quantitative assessment thus improving **work efficiency.**

DEEP LEARNING

- **ARTIFICIAL INTELLIGENCE:**
  Methods that allow a computer to mimic human intelligence
- **MACHINE LEARNING:**
  The ability of the computer to learn without being explicitly programmed to do so
- **DEEP LEARNING:**
  A newer subset of Machine Learning where the computer creates its own multilayered neuronal networks



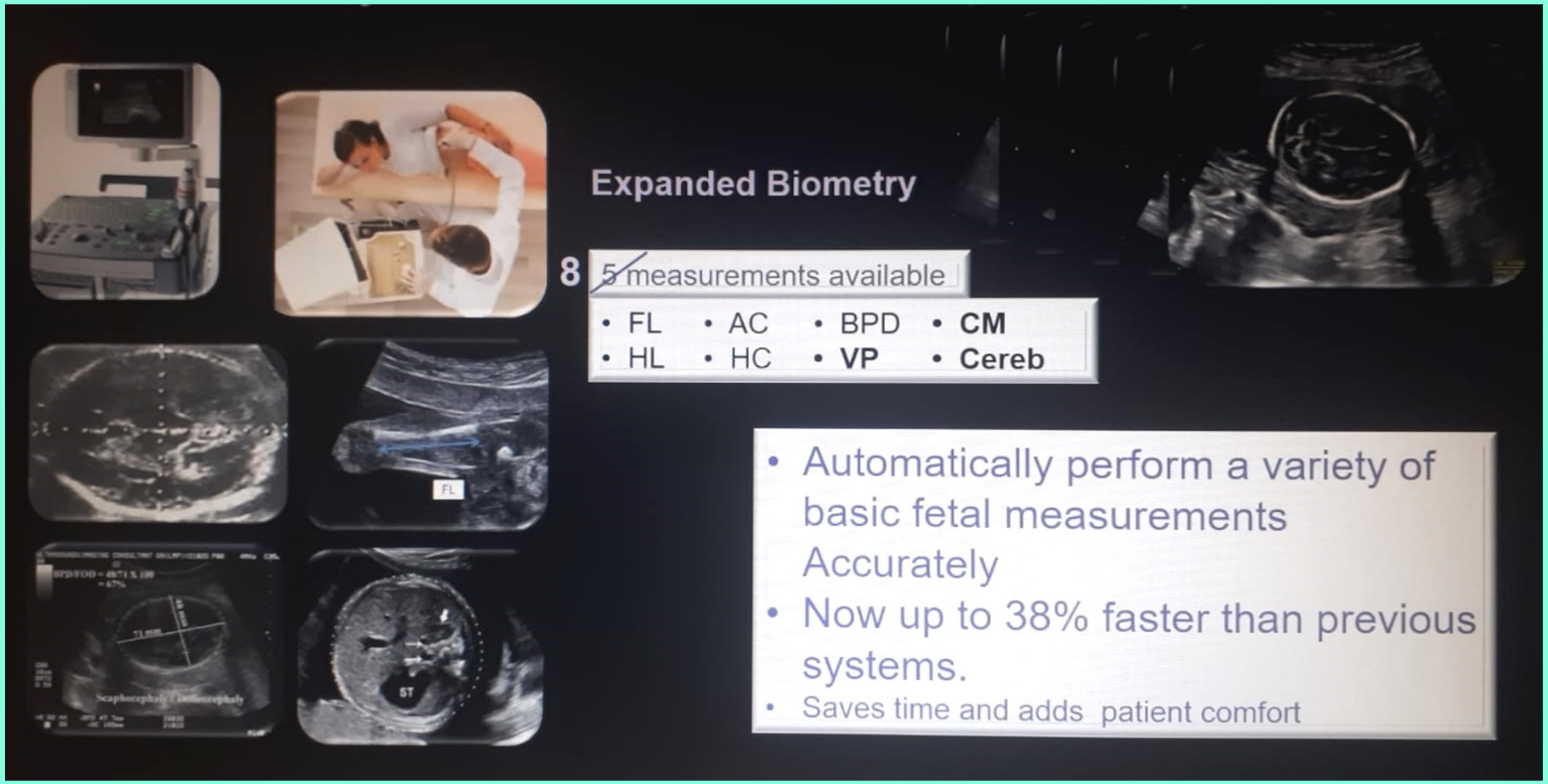
Patient Big Database | Feature Extraction | Recognition of US images

DEEP LEARNING

ARTIFICIAL INTELLIGENCE (AI) IN PRENATAL ULTRASOUND

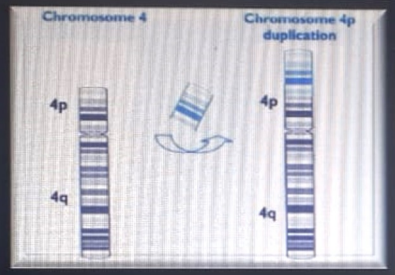
Using "REALTIME" artificial intelligence-based ultrasound scan, we can elaborate the ultrasound images on a single plane in 25 ms, 16 times superior to human abilities (400-1500 ms)

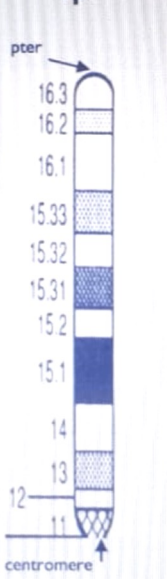
AI AND ULTRASOUND BIOMETRY



EXAMPLE OF ARTIFICIAL INTELIGENCE IN PRENATAL DIAGNOSIS

There is a single, live fetus. The fetus shows multiple anomalies. There is a microcephaly, microretrognathia, hypoplastic cerebellum & a muscular ventricular septal defect. The cerebellar transverse diameter is 23 mm: 21 weeks & 1 day ± 1 week.





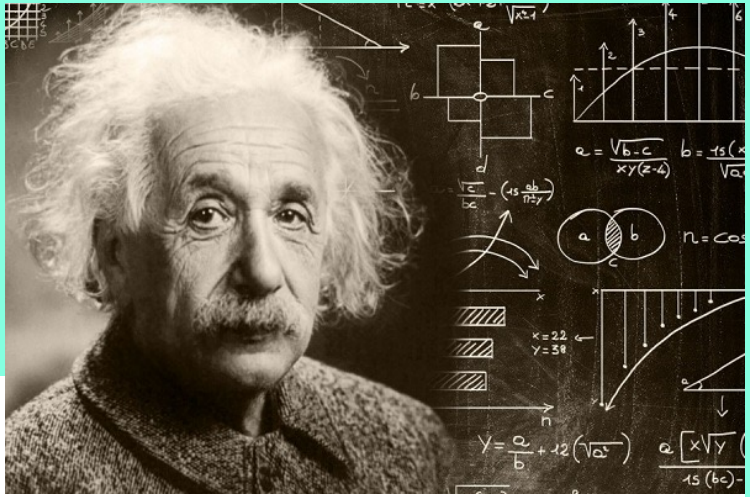
Smaller duplications

People with smaller duplications of 4p may be more mildly affected. This is a list of cases from the medical literature and others known to *Unique*, with their key symptoms.

- 4pter - p16 - behaviour difficulties, mild learning difficulties.
- 4pter - p16.1 - slight developmental delay, infantile spasms.
- 4pter - p16.3 - mild learning difficulties, tall stature.
- 4p16.3 - p16 - working semi-independently, moderate learning difficulties, delayed puberty.
- 4p16.3 - p16.1 - typical appearance for a 4p duplication, developmental delay, minor heart problem, hernia in the groin, mild ptosis (lowered upper eyelid).
- 4p16.3 - p16.1 - developmental delay.
- 4p16.3 - p15.3 - developmental delay, speech delay or absence.
- 4p16.3 - p15.3 - reads, writes, converses, excellent fine motor skills.
- 4p16.3 - p15.3 - significant developmental delay and learning disability, seizures.

A small duplication can occur with a deletion of the region in 4p16.3 that causes Wolf-Hirschhorn syndrome. The effects of the deletion can dominate the effects of the duplication. (Wyandt in Estabrooks1995; Partington 1997; Zollino 1999; Cotter 2001; Tschernigg 2002; Gerard-Blanluet 2004; Takeno 2004; U).

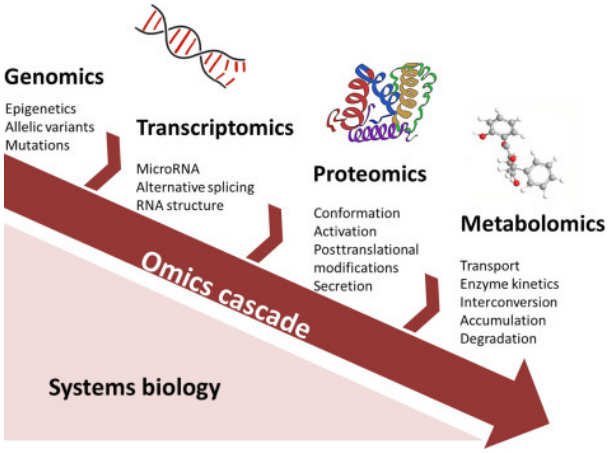
AI IN ULTRASOUND FETAL IMAGING

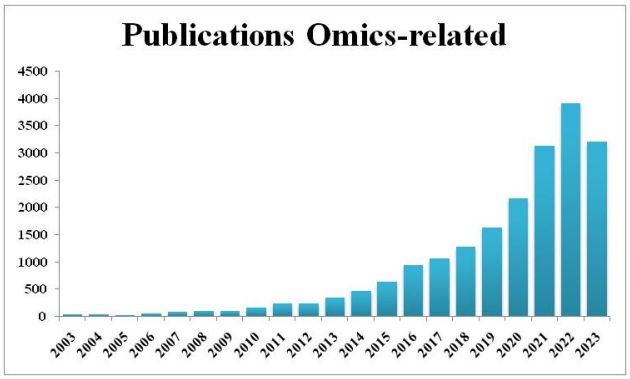


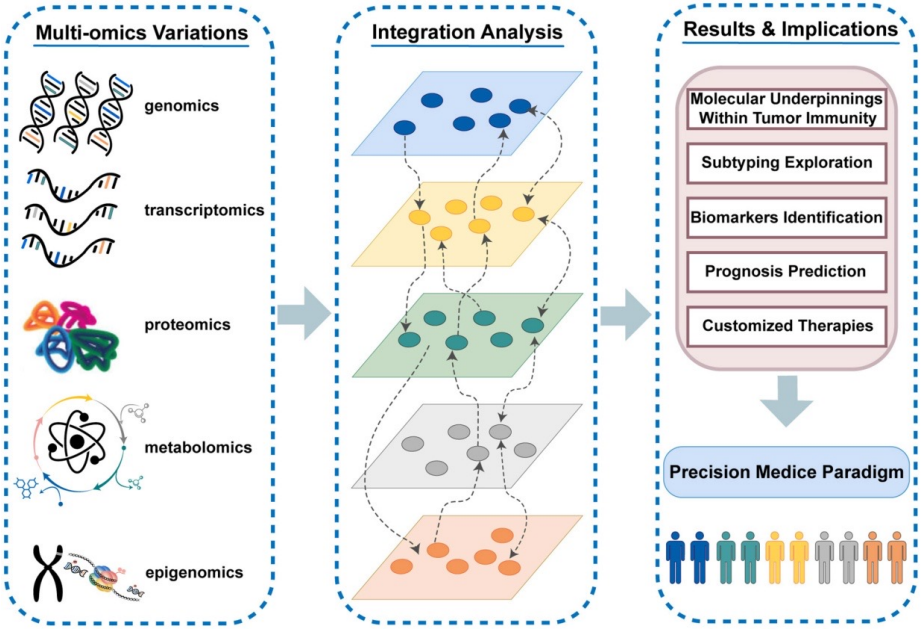
- Reduced keystrokes
- Catering to the need of medical personnel with entry and medium level skills
- Facilitating the way Maternal-Fetal Medicine is practiced by highly skilled individuals
- Not making us obsolete, but… if you do not start using AI you may become obsolete!

OMICS SCIENCES

OMICS Sciences are a set of scientific approaches that study a broad spectrum of biological information that mainly includes genomics, transcripctomics proteomics and metabolomics







Such approaches make it possible to examine the molecular mechanisms underlying the diseases and to obtain a complete and detailed overview of the interations between genes.

The information obtained from the sciences can contribute to develop of personalized therapies considering the specific molecular charateristics of each patient.

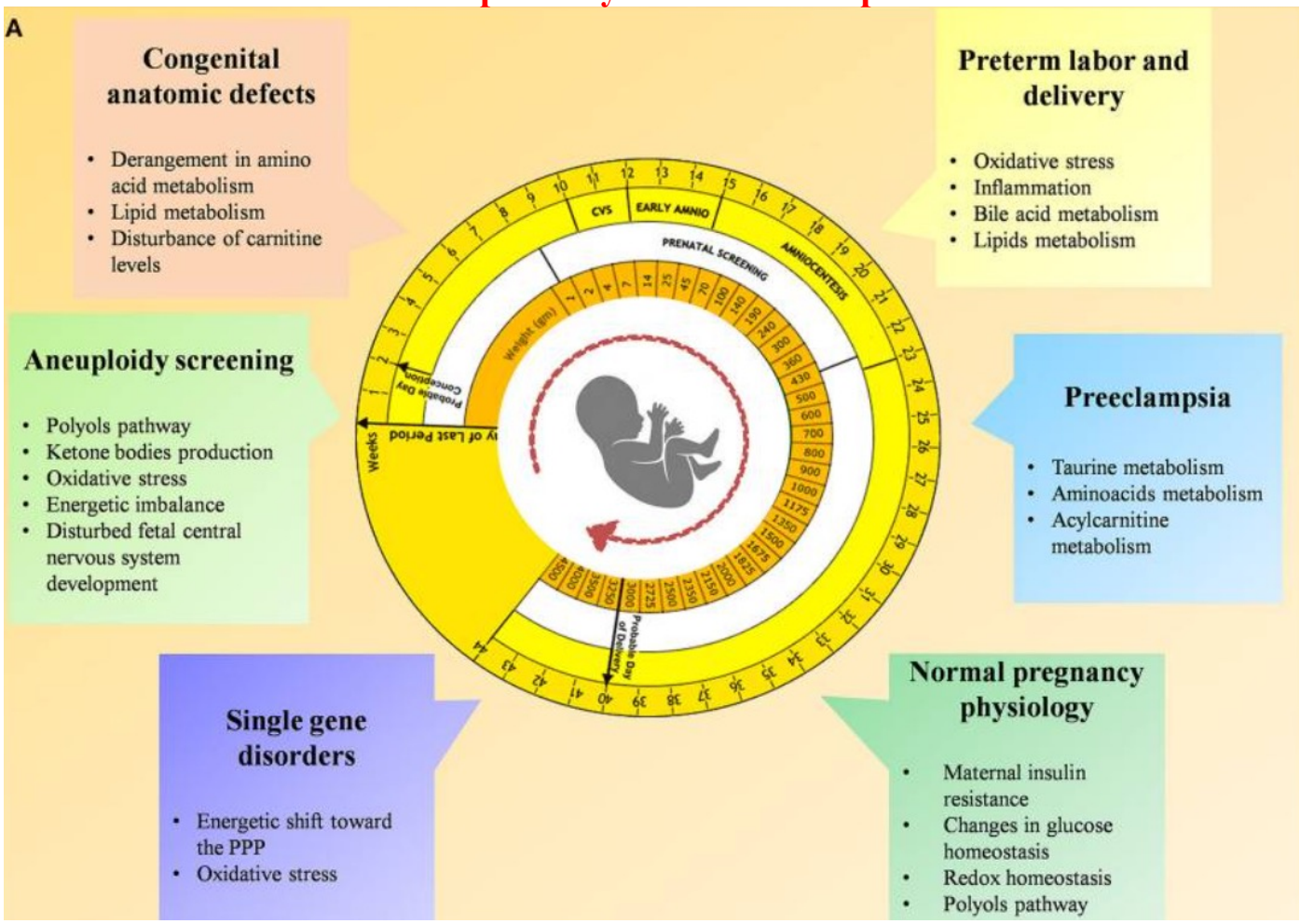
frontiers in Medicine

Metabolomics in Prenatal Medicine: A Review

Giovanni Monni[1*], Luigi Atzori[2], Valentina Corda[1], Francesca Dessolis[1], Ambra Iuculano[1], K. Joseph Hurt[3†] and Federica Murgia[1,2*†]

REVIEW
published: 25 June 2021
doi: 10.3389/fmed.2021.645118

Altered metabolic pathways associated to prenatal disorders



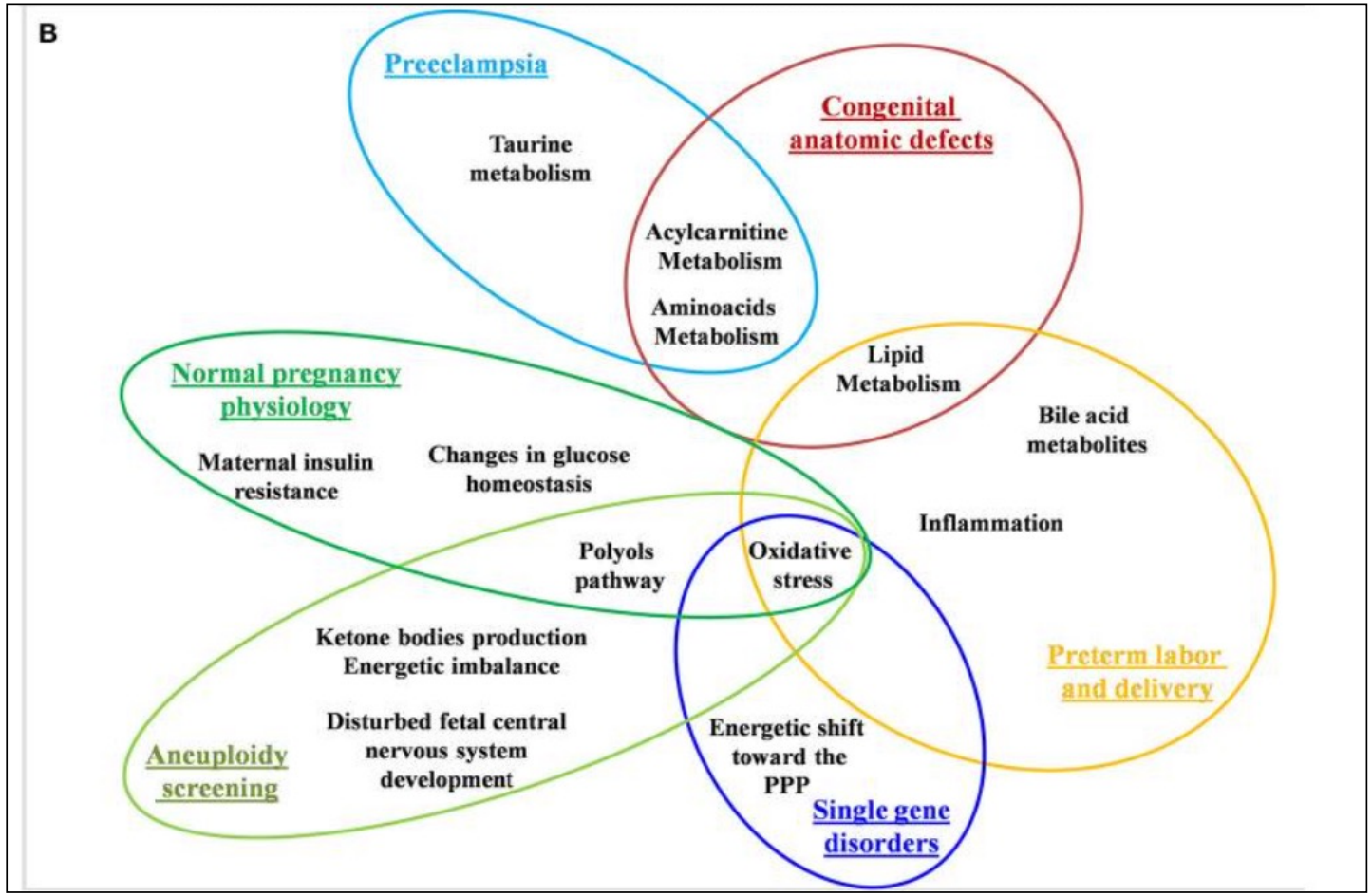
frontiers in Medicine

Metabolomics in Prenatal Medicine: A Review

Giovanni Monni[1*], Luigi Atzori[2], Valentina Corda[1], Francesca Dessolis[1], Ambra Iuculano[1], K. Joseph Hurt[3†] and Federica Murgia[1,2*†]

REVIEW
published: 25 June 2021
doi: 10.3389/fmed.2021.645118

Associated and specific altered metabolic pathways in prenatal disorders



Received: 22 February 2019 | Revised: 28 March 2019 | Accepted: 14 April 2019
DOI: 10.1002/pd.5461

ORIGINAL ARTICLE

WILEY PRENATAL DIAGNOSIS

Metabolic fingerprinting of chorionic villous samples in normal pregnancy and chromosomal disorders

Federica Murgia[1] | Ambra Iuculano[2] | Cristina Peddes[2] | Maria Laura Santoru[1] | Laura Tronci[1] | Monica Deiana[1] | Luigi Atzori[1] | Giovanni Monni[2]

Journal of *Clinical Medicine*

MDPI

Article

Metabolomic Investigation of β-Thalassemia in Chorionic Villi Samples

Giovanni Monni [1,*], Federica Murgia [2], Valentina Corda [1], Cristina Peddes [1], Ambra Iuculano [1], Laura Tronci [2], Antonella Balsamo [2] and Luigi Atzori [2]

DE GRUYTER J. Perinat. Med. 2019; aop

Ambra Iuculano, Federica Murgia, Cristina Peddes, Maria Laura Santoru, Laura Tronci, Monica Deiana, Antonella Balsamo, Anna Euser, Luigi Atzori[a] and Giovanni Monni[a,*]

Metabolic characterization of amniotic fluids of fetuses with enlarged nuchal translucency

BUT, what is today the

BEST PRENATAL TESTING in terms of

SAFETY and ACCURACY

for detecting and avoiding

FETAL BIRTH DISORDERS ?

WOMEN'S EXPECTATIONS AND CHOICES FROM PRENATAL CHROMOSOMOPATHIES AND GENETIC TESTING*

No risk for pregnancy?
Safe for the fetus

Karyotype Screening

- First trimester ultrasound screening+biochemistry
- NIPS for Trisomies 21-18-13
- Ultrasound

Get maximum information?
Safe for the mother

Karyotype and Mendelian Diseases

- PGD (to avoid TOP)
- First trimester ultrasound screening+biochemistry + CVS/Amniocentesis

*** Prenatal Testing as early as possible and to avoid TOP (Termination of Pregnancy)**

Sensitivity of Aneuploidy Screening

Screening Tests	Detection Rate %	False Positives %
2nd trimester Triple screen	70	5
2nd trimester Quad screen	81	5
1st trimester NT alone	80	5
1st trimester (NT+ Biomarkers)	90	3
1st trimester (NT+Biomarkers+US markers)	**95**	**2**
Cff DNA (Tris. 21 – 18 – 13)	**99 - 96 - 92 *** (only in patients who receive result)	1

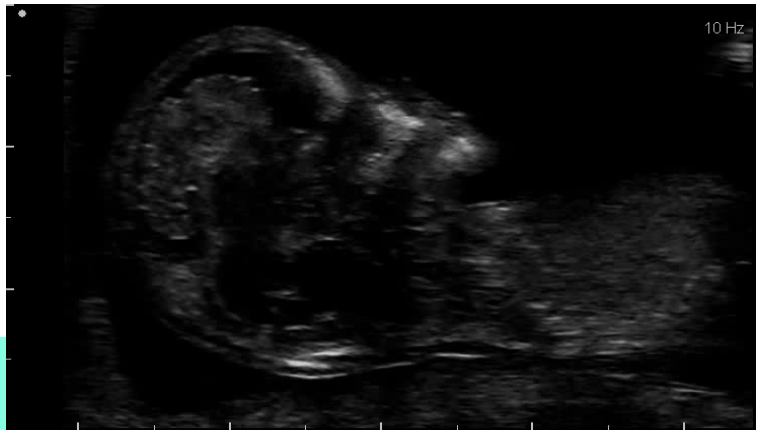
*2 – 4 % *NO RESULT; In no result the prevalence of aneuploidies ranging from* 3-16% 20 % *of Chromosomal Anomalies not detectable by cff DNA*

Pergament 2016 - Norton 2019

1st TRIMESTER ULTRASOUND SCREENING FOR ANEUPLOIDIES

- **Biometry**: CRL, HC/AC, HC/FL, HC/HL, yolk sac
- **Functional assessment**: heart rate, umbilical artery PI, pulsatile flow in the umbilical vein
- **Soft markers**: choroid plexus cysts, renal pyelectasis, unfused amnion and chorion after 14 weeks, placental edema, echogenic heart foci, hyperechogenic bowel
- **More recent 1° trimester US markers**: nasal bone, facial angle, ductal flow, tricuspid valve regurgitation, mitral gap

Nuchal Translucency !!!



1st Trimester US Detection of Major Fetal Defects

Always Detected (30%)

- Acrania
- Holoprosencephaly
- Exomphalos
- Gastroschisis
- Megacystis
- Body stalk anomaly

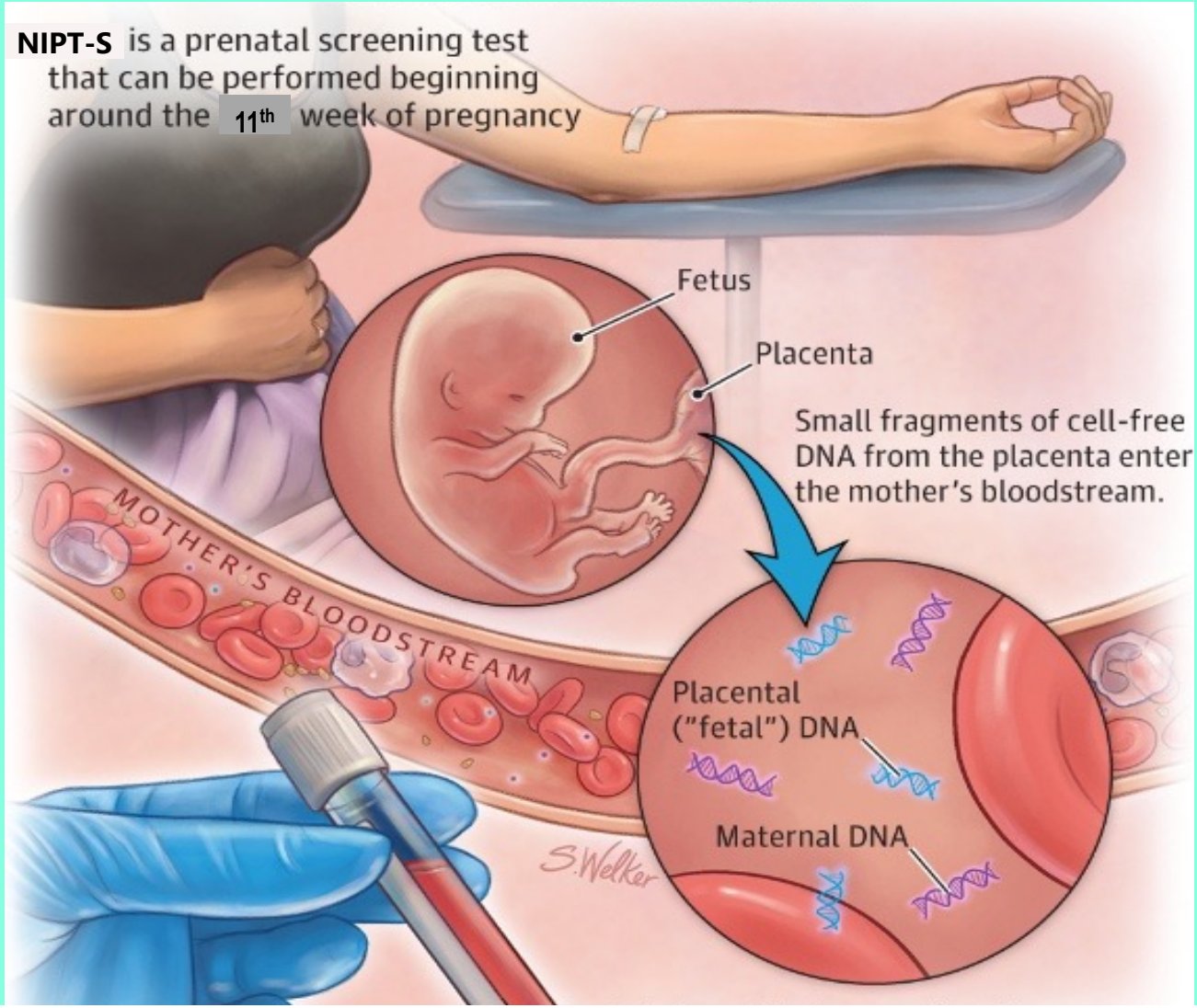
Sometimes Detected (44%)

Spina bifida	14%
Ventriculomegaly	9%
Facial cleft	5%
Major cardiac defect	33%
Diaphragmatic hernia	50%
Lethal skeletal dysplasia	50%
Absent hands/feet	60%

Undetectable (26%)

- Corpus callosum agenesis
- Cerebellum/vermis hypoplasia
- CCAM/sequestration
- Esophageal/duodenal atresia
- Bowel obstruction
- Hydronephrosis
- Talipes

NON INVASIVE PRENATAL SCREENING (NIPT/NIPS)



Estimated detection rate of cell-free DNA - NIPS for aneuploidy and positive predictive value by maternal age

	Pooled Detection Rate (%)	PPV at 25 yrs of Age* (%)	PPV at 35 yrs of Age* (%)	PPV at 45 yrs of Age* (%)
Trisomy 21	99.2	51	79	98
Trisomy 18	96.3	15	39	90
Trisomy 13	91.7	7	21	Data insufficient to calculate
Monosomy X	90.3	41	41	41

* *Predictive values calculated via the Perinatal Quality Foundation calculator. Available at perinatalquality.org; retrieved July 22, 2016*

CELL-FREE DNA ANALYSIS FOR NON INVASIVE EXAMINATION OF TRISOMY 21

Down's syndrome with the use of cell-free DNA (cfDNA) (sensitivity, 100/% {38 of 38 cases}; false positive rate, 0,06%

These promising results may be misleading because they excluded 488 patients (3% of their sample) with indeterminate cfDNA results

The prevalence of aneuploidy was higher among these patients than in the overall cohort (2,7% vs. 0,4%); thus, their exclusion may introduce bias

Smith- Bindman/ Miglioretti - N Engl Med

CELL-FREE DNA ANALYSIS FOR NON INVASIVE EXAMINATION OF TRISOMY 21

- If Included would result in a **false positive rate 3,0%** and a positive predictive value of 7,6%, much lower than the reported positive predictive value of 80,9%

- Alternatively, if indeterminate results were classified as negatives, sensitivity would be reduced to 38 of 41 cases **(93%)**

- Assuming that no patients with indeterminate results on combined screening had trisomy 21, the sensitivity of cfDNA testing and standard screening (33 of 41 cases {81%}); **would not be significantly different** (P=0,22 by McNemar's test)

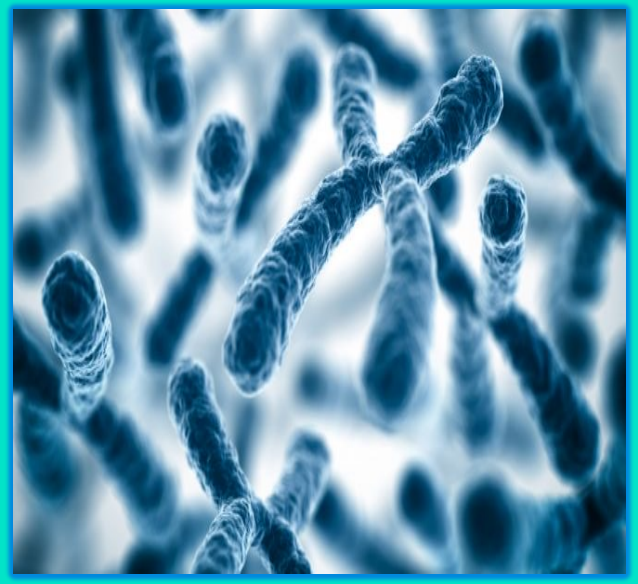
Smith- Bindman/ Migliorretti - N Engl Med

Chromosomal Abnormalities detectable by NIPT

~ 80 %

ANEUPLOIDY

- Trisomy 21 (Down Syndrome)
- Trisomy 18 (Edwards Syndrome)
- Trisomy 13 (Patau Syndrome)
- Monosomy X (Turner Syndrome)
- XXX (Trisomy X)
- XXY (Klinefelter Syndrome)
- XYY (Jacobs Syndrome)



Not detectable ***~ 20%*** *of other chromosomal anomalies*

FALSE NEGATIVE NIPT IS INFLUENCED BY:

- Vanishing twin
- Maternal age, weight and parity
- Vitamin B 12 deficiency
- Active autoimmune diseases
- Maternal cancer
- Immunotherapy and blood transfusion
- Bone marrow or organ transplantation
- Donor stem cell therapy
- Maternal chromosome anomalies
- Maternal balanced translocation mosaicism

-Fetal fraction vital to sample quality and statistical control and confidence (< ff 4%)

NIPT FALSE POSITIVE and CPM

CONFINED PLACENTAL MOSAICISM (CPM)

is the main source of **False Positive results**

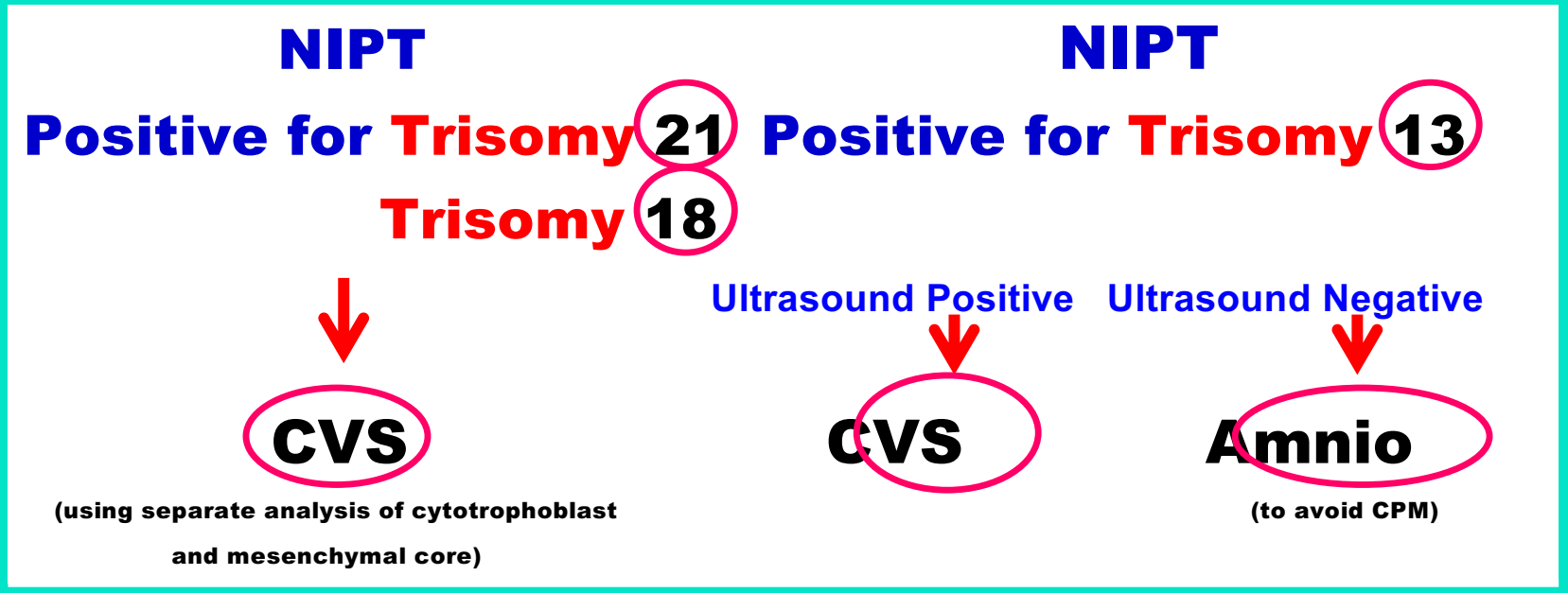
CPM is the type of chromosomal mosaicism in which the chromosome abnormality

is present in the **cytotrophoblast** of the **placenta (chorionic villi)**

but **not in the fetus** (in **~ 1% - 2%** in high-risk pregnancies)

Royal College

CVS OR AMNIO *AFTER* POSITIVE NIPT*



In women who have already done previous 1st trimester combined fetal screening

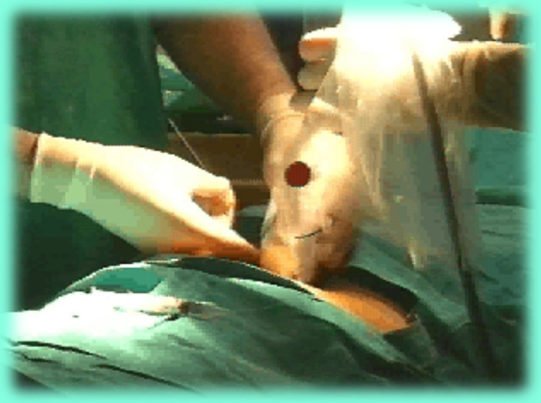
ROLE OF INVASIVE PRENATAL PROCEDURES

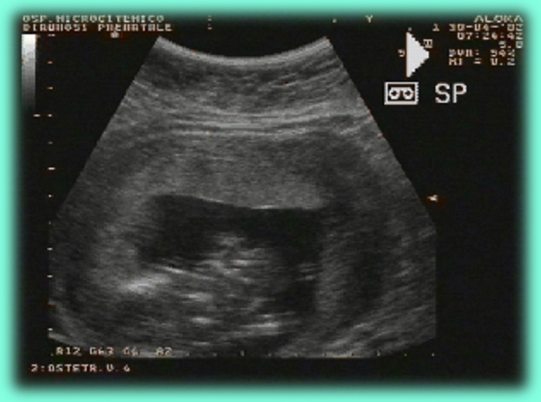
POSITIVE PRENATAL COMBINED SCREENING

***and* POSITIVE CELL-FREE DNA (NIPT-NIPS)**

must be* CONFIRMED *and* DIAGNOSED *by

CHORIONIC VILLOUS SAMPLING *or* AMNIOCENTESIS

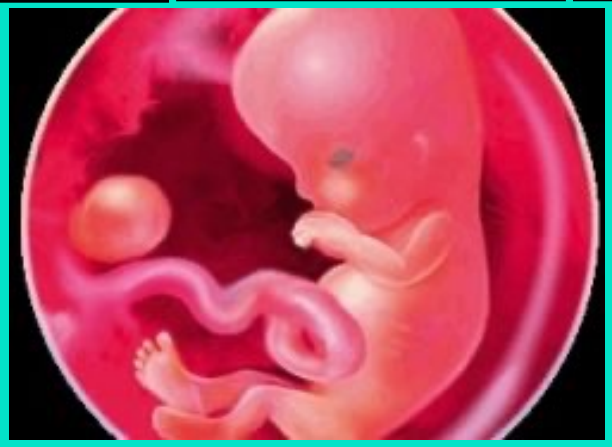




INVASIVE PRENATAL PROCEDURES

1970 - 1990

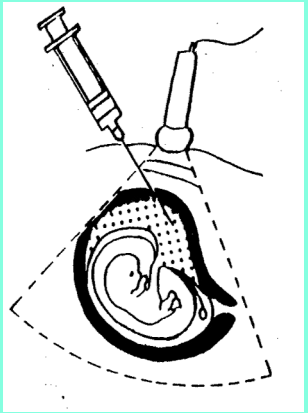
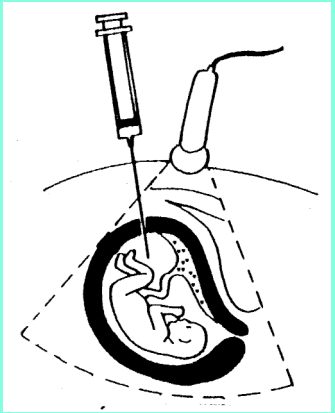
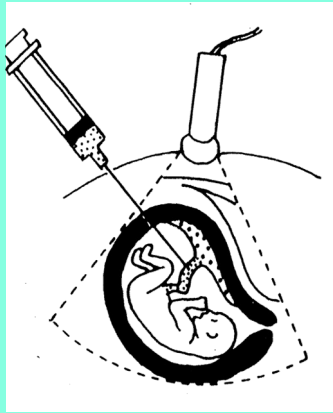
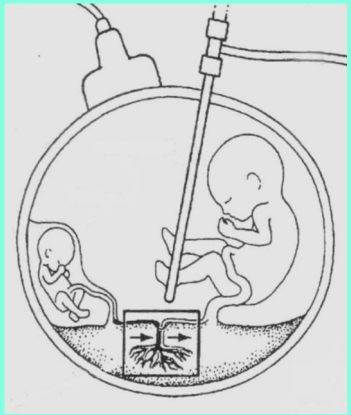
Placentacentesis Fetoscopy Cordocentesis Intrahepatic Vein Puncture	Amniocentesis Coelocentesis	Transcervical CVS Transabdominal CVS



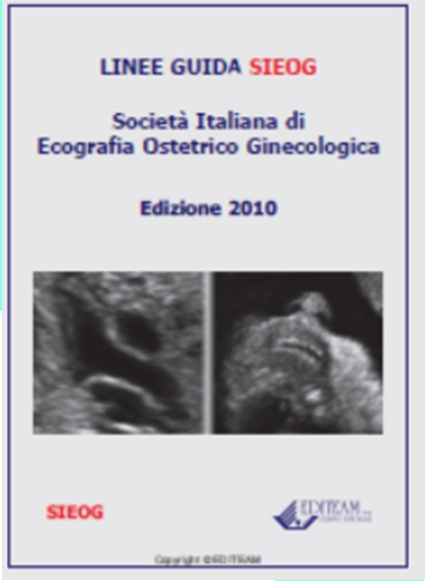
1990 - 2024

Chorionic Villous Sampling Amniocentesis Cordocentesis	Preimplantation Genetic Diagnosis Fetal Therapy

PREIMPLANTATION, PRENATAL GENETIC DIAGNOSIS AND FETAL THERAPY

In Vitro	In pregnancy under ultrasound monitoring			
  PGD	 CVS (>10 weeks) FETAL LOSS 0,11%	 AMNIOCENTESIS (>15 weeks) FETAL LOSS 0,12%	 CORDOCENTESIS (>18 weeks) FETAL LOSS 1-2%	 FETAL THERAPY

COUNSELLING BEFORE SCREENING AND INVASIVE PROCEDURES



INFORMATION ALL THE WOMEN ON

a) The amount of genetic risk of which they are carriers

b) The possibilities of screening, diagnosis, prognosis and treatment of congenital defects

c) **The risks related to the invasive diagnoses, on their diagnostic limitations and the time necessary for receiving the diagnosis**

d) The modes of execution of the diagnostic procedures

e) The possibility for diagnostic clarification in uncertain cases

f) The options of what to do after the diagnosis so as to give the woman the possibility to decide considering risks and benefits

BUT, what is the

BEST PRENATAL DIAGNOSIS PROCEDURE

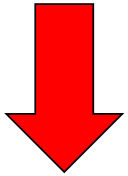
(CVS OR AMNIOCENTESIS)

in terms of **SAFETY** and **ACCURACY ?**

CHORIONIC VILLOUS SAMPLING

and

AMNIOCENTESIS:



CURRENT MISCARRIAGE RISK

PRENATAL INVASIVE PROCEDURES

FOR MANY YEARS THE FETAL LOSS RISK AFTER INVASIVE PRENATAL PROCEDURES WAS ESTIMATED TO BE 1% (Tabor Lancet 1986).

BUT TODAY,

IS THE TERRORISTIC TERM **"INVASIVE"**

OBSOLETE AND STILL APPROPRIATE

FOR PRENATAL DIAGNOSTIC PROCEDURES IN THE MODERN MATERNAL-FETAL MEDICINE ?

INVASIVE AND NON INVASIVE

These terms have been used for

agressive marketing strategies by companies

whilst conveying the misleading message

that NIPT is a safe alternative to

chorionic villus sampling and amniocentesis

OLD DATA ON FETAL LOSS AMNIO-CVS

AMNIO	1%	Tabor 1986 Lancet
AMNIO-CVS	0,06%	Faster Study 2007 Obstet Gynecol
AMNIO-CVS	0,13%	Odibo 2008 Obstet Gynecol

RECENT FETAL LOSS META-ANALYSIS
CVS-AMNIO

CVS	0,22 % - 1:500
AMNIO	0,11 % - 1:900

Akolekar R, Ultrasound Obstet Gynecol 2015

MOST RECENT DATA ON RISK OF MISCARRIAGE FOLLOWING AMNIOCENTESIS OR CVS:

SYSTEMATIC REVIEW FROM 2943 CITATIONS IN LITERATURE AND UPDATED META-ANALYSIS

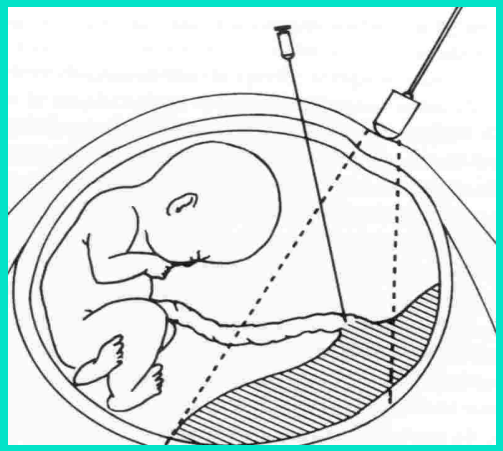
AMNIO	**0,12 %**
CVS	**0,11 %**

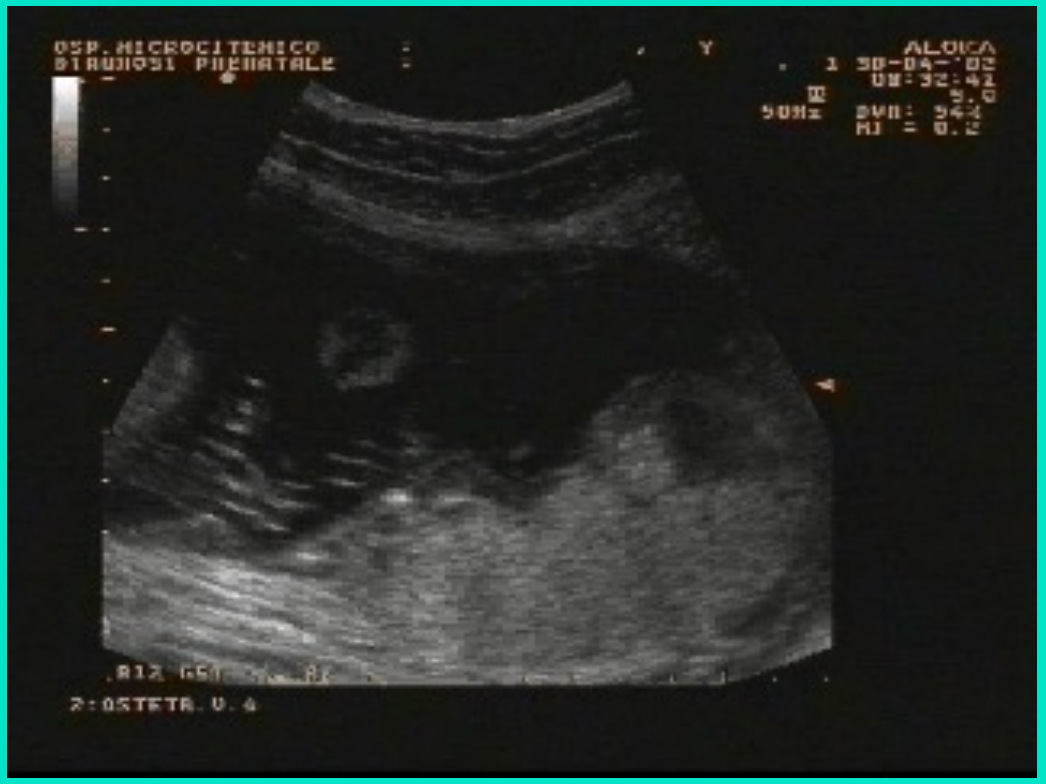
The procedure-related risks of miscarriage following Amniocentesis and CVS **are lower** than currently quoted to women. The risk appears to be negligible when these interventions were compared to control groups of the same risk profile.

There is no evidence that CVS is less safe than Amniocentesis

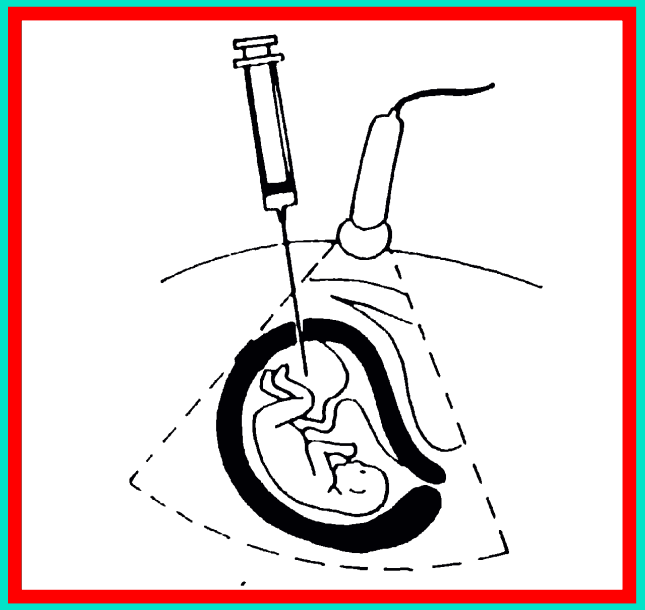
Salomon L.J. et al, UOG September 2019

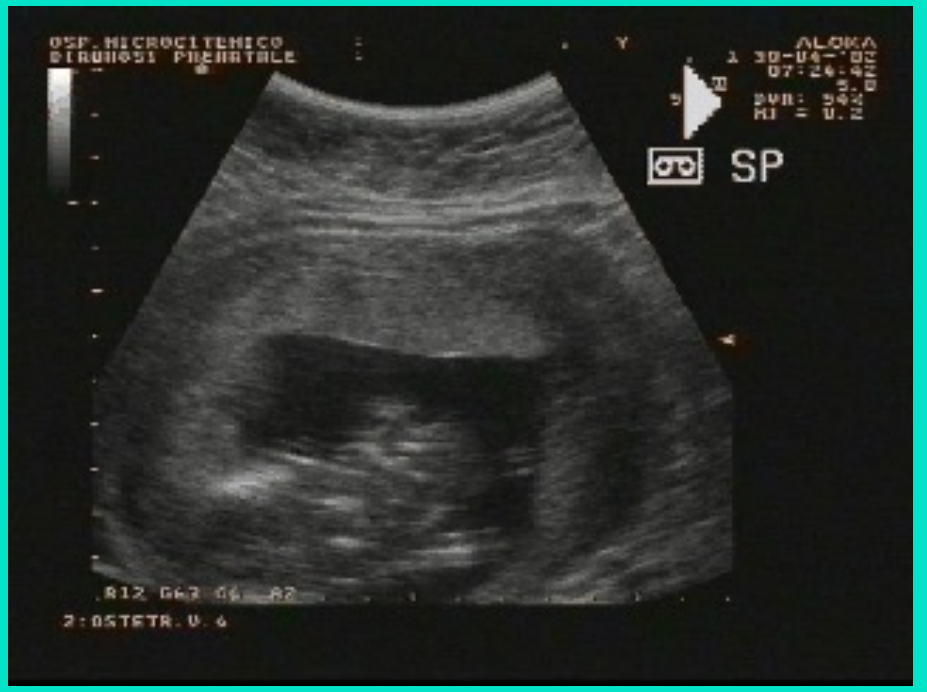
FETAL BLOOD SAMPLING



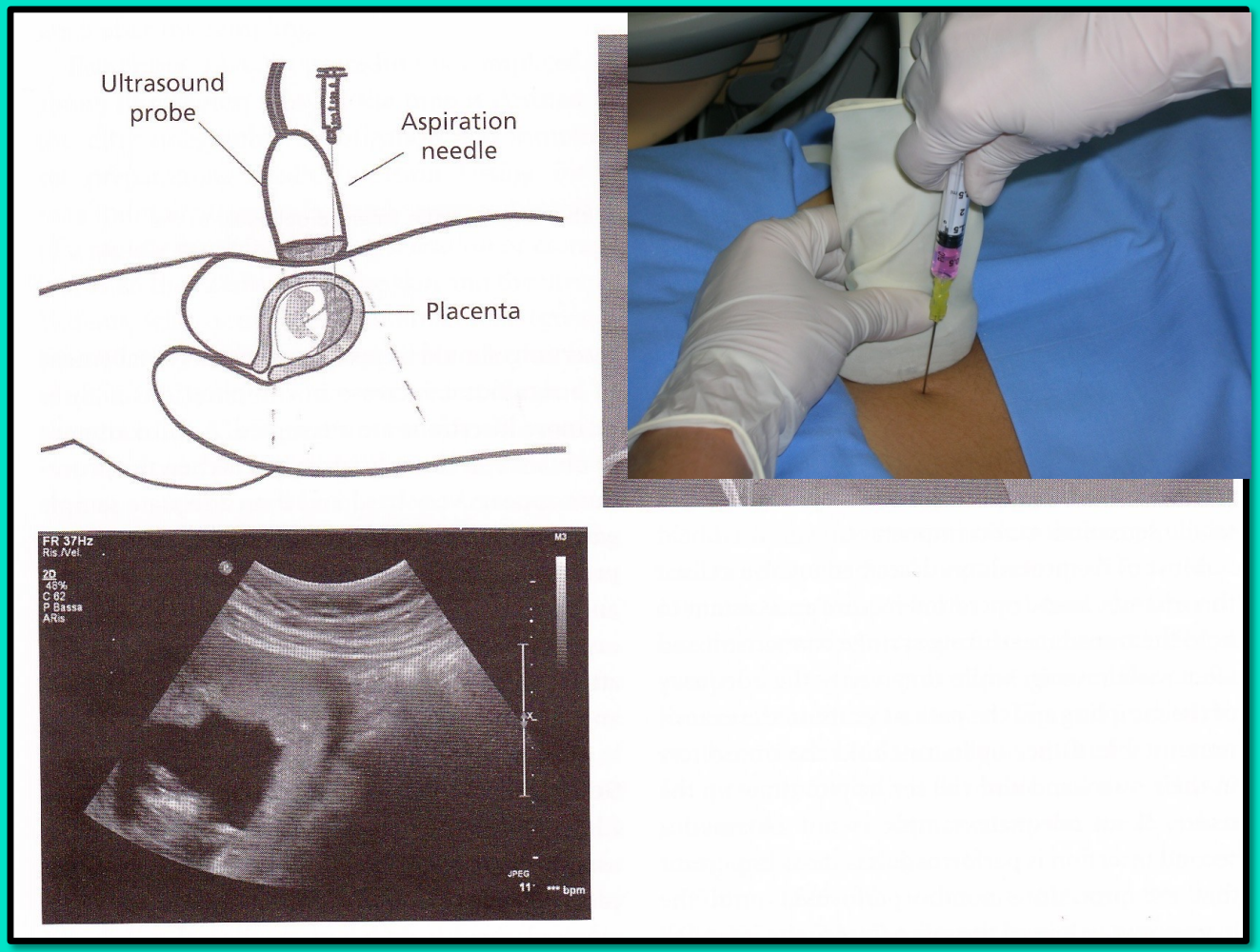


AMNIOCENTESIS



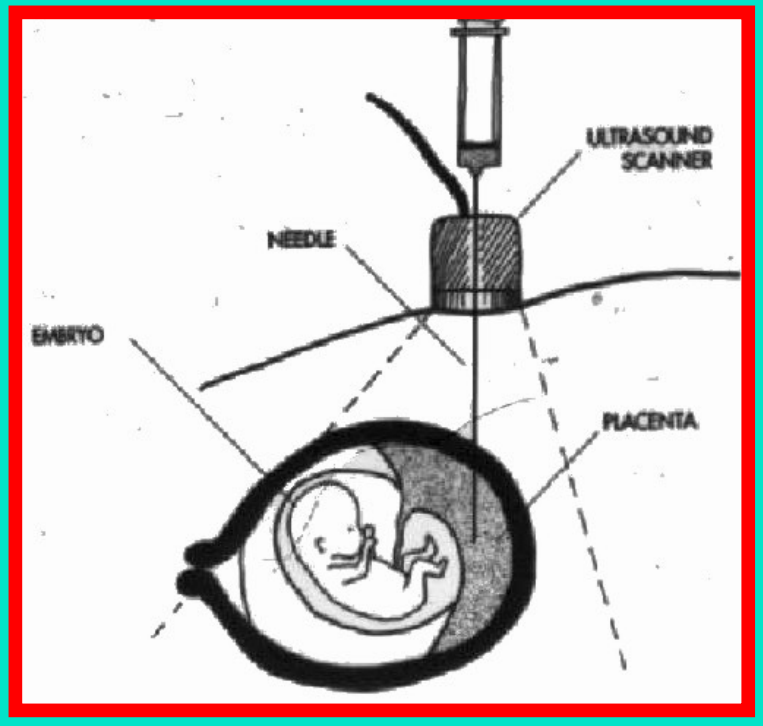


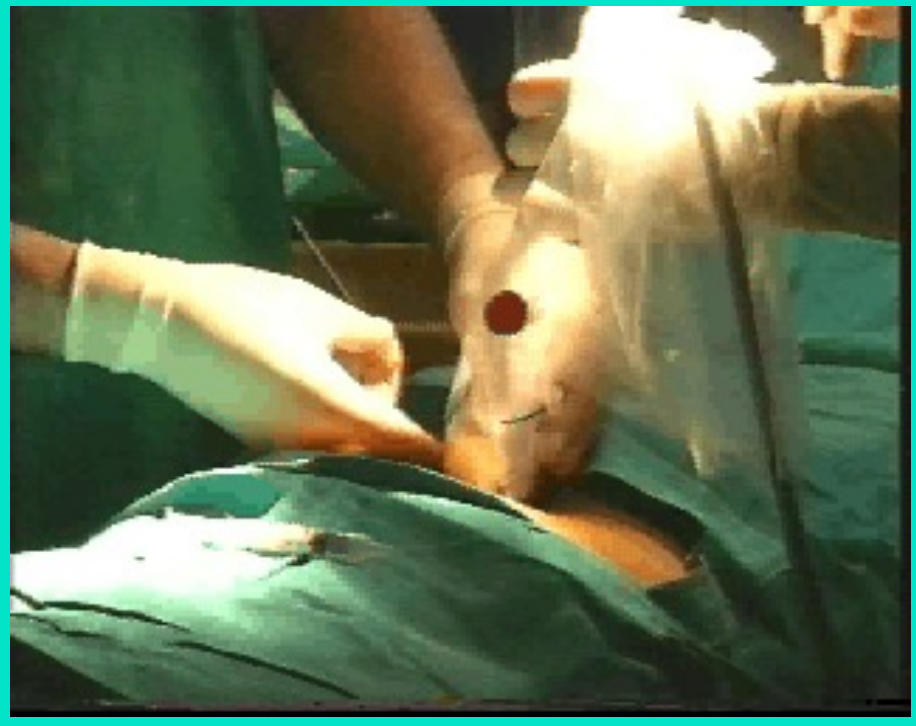
TRANSABDOMINAL CHORIONIC VILLUS SAMPLING (TA - CVS) BY FREEHAND PERPENDICULAR NEEDLE INSERTION



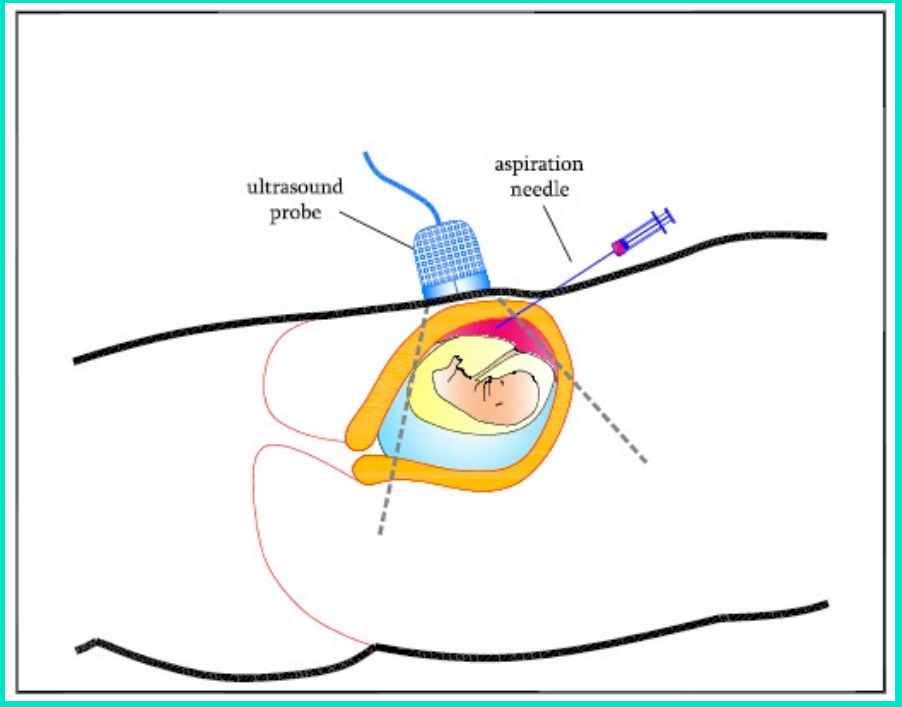
Monni 2010 from 6th Milunsky ed.

TRANSABDOMINAL CHORIONIC VILLUS SAMPLING (TA - CVS) BY FREEHAND PERPENDICULAR NEEDLE INSERTION





TA-CVS OBLIQUE NEEDLE INSERTION BY FREEHAND TECHNIQUE



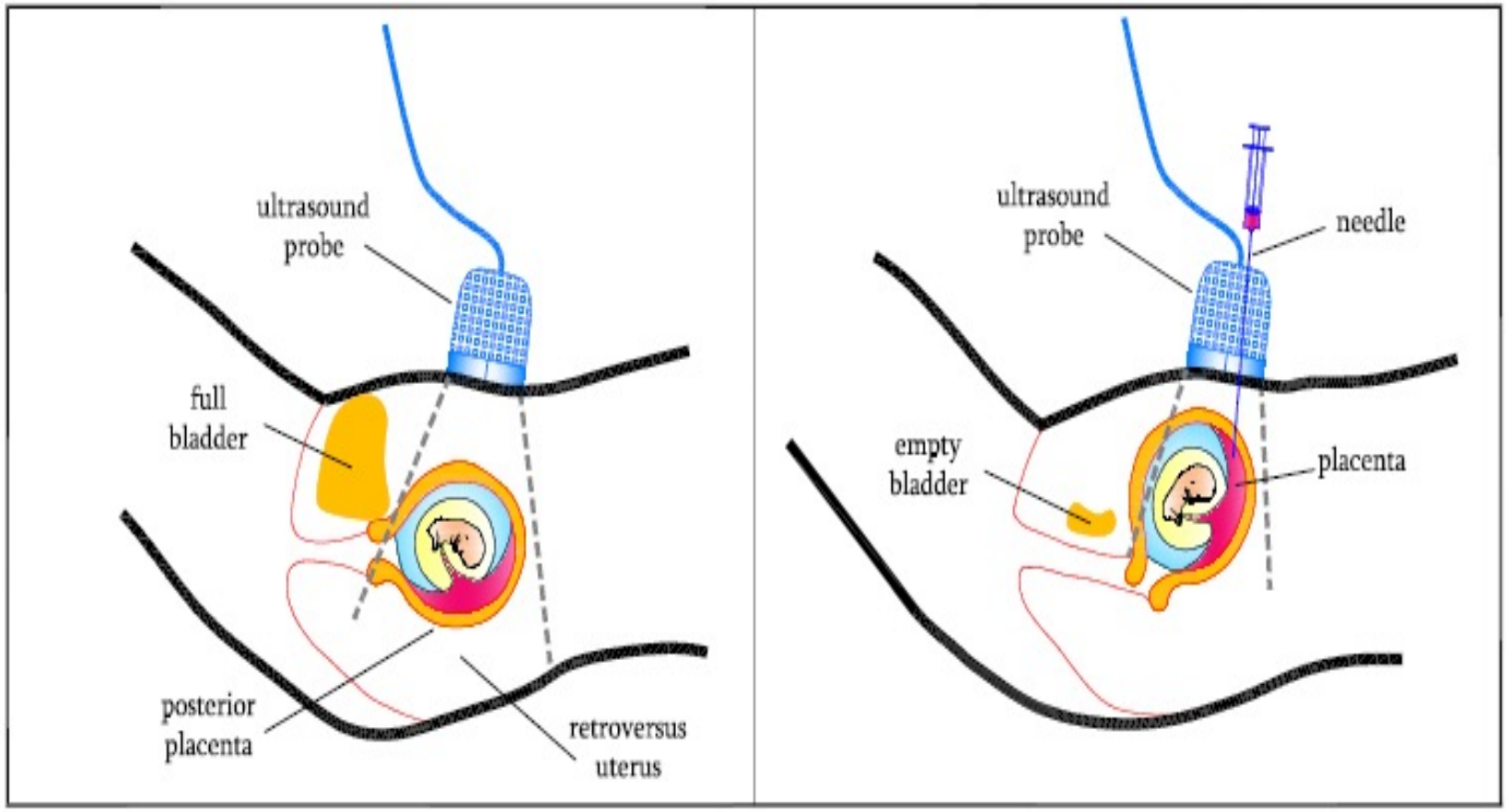
PERPENDICULAR OR OBLIQUE NEEDLE INSERTION?

An oblique insertion (at the extremity of the probe) allows full visualization of the needle throughout sampling but it implies a longer course, which means there are more possibilities to prompt a uterine wall contraction. The procedure is therefore more painful and so local anaesthesia should be used.

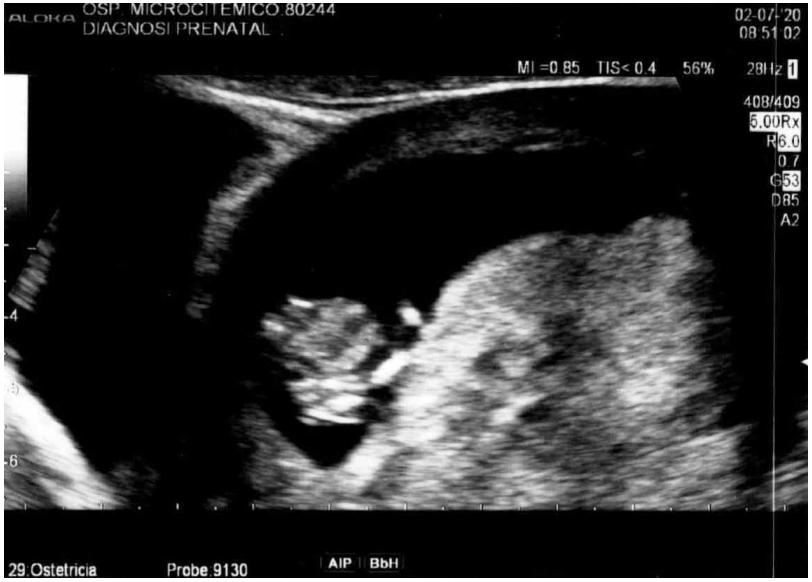
In contrast, puncture through a perpendicular insertion orientation makes it much easier to slightly correct the trajectory of the needle when necessary, and is easily feasible with new needles that have a special external echogenic coating so as to enhance tip placement. This, along with the fact that is much less painful for the patient, makes perpendicular approach the one of choice in our institution.

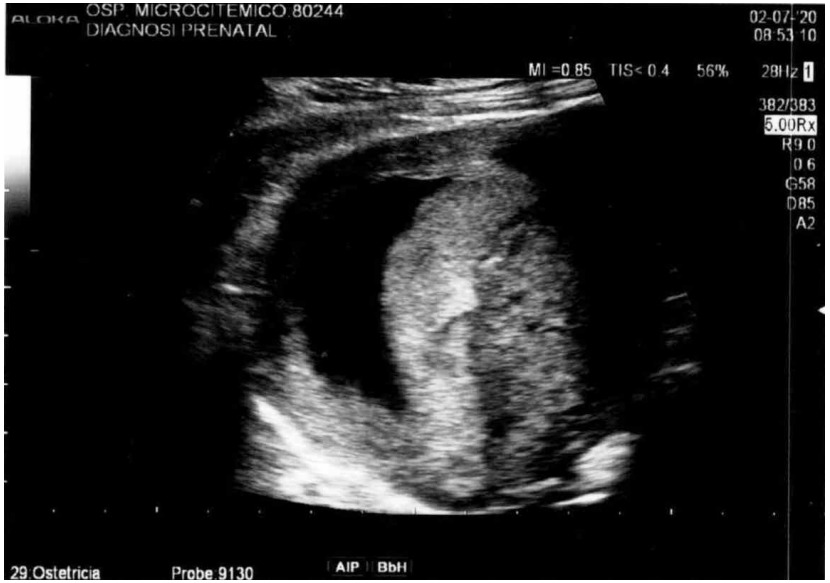
Monni 2012, Clin Mat Fet Med, Winn, Chervenak, Romero Ed.

FULL AND EMPTY BLADDER DURING TA-CVS

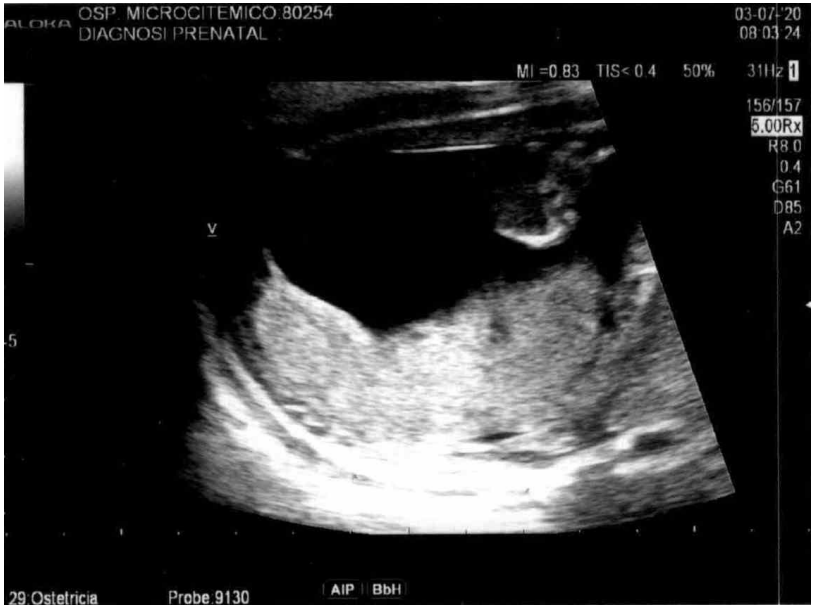


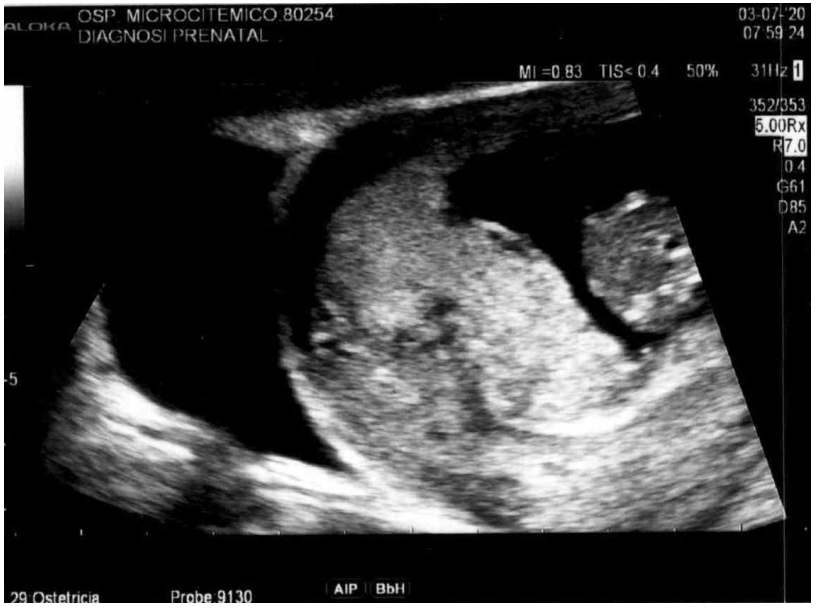
FULL AND EMPTY BLADDER IN POSTERIOR PLACENTA



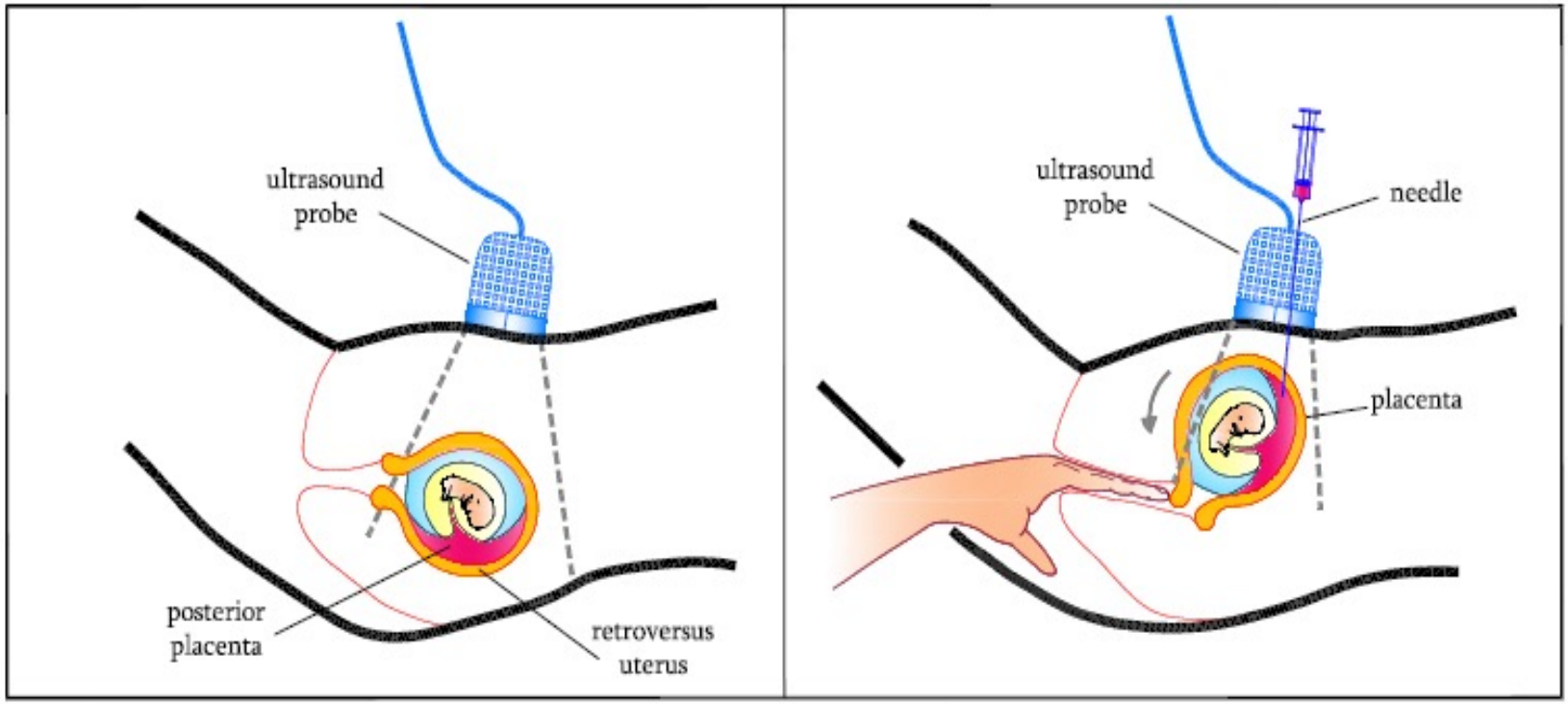


FULL AND EMPTY BLADDER IN ANTERIOR PLACENTA





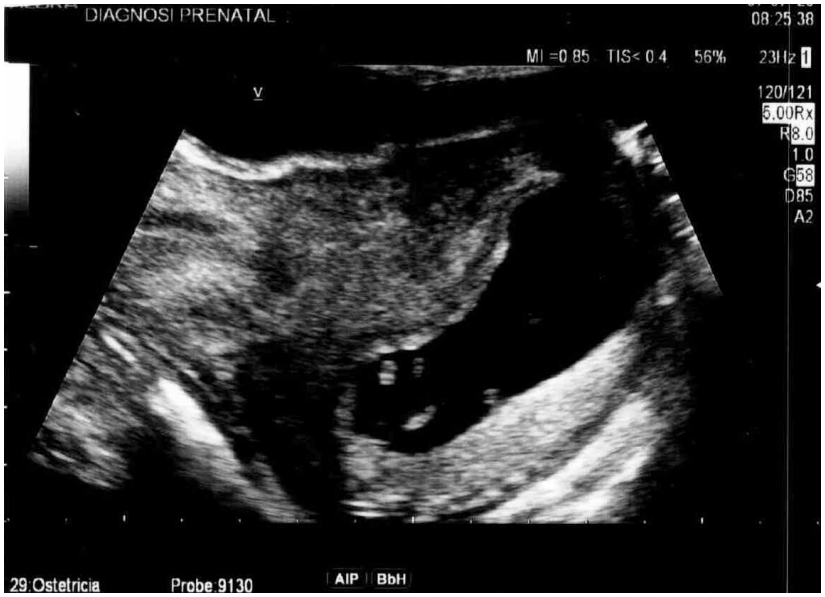
TA-CVS IN COMPLETE POSTERIOR PLACENTA AND RETROVERTED UTERUS BY MANUAL VAGINAL MANIPULATION

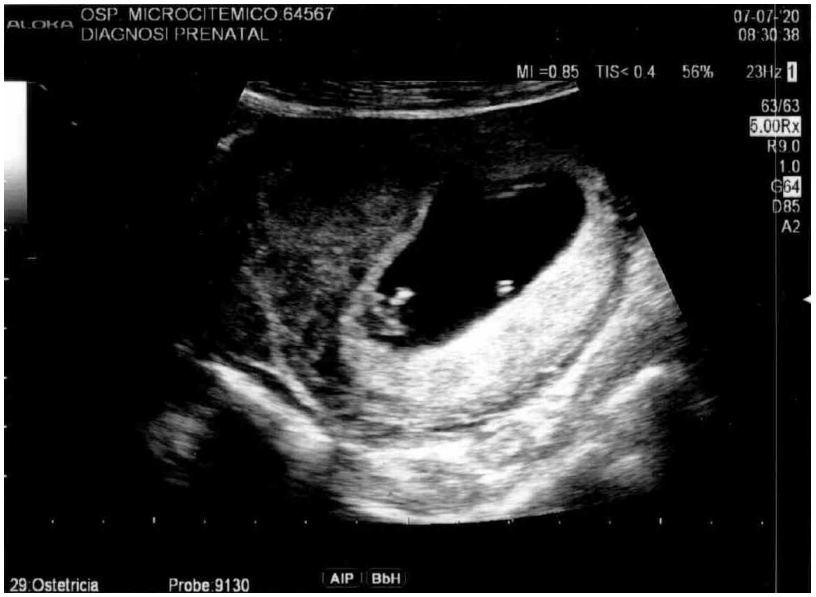


Monni 2010 from 6th Milunsky ed.

POSTERIOR PLACENTA IN RETROVERED UTERUS

and MANUAL VAGINAL MANIPULATION





THE JOURNAL OF
MATERNAL-FETAL
& NEONATAL
MEDICINE

http://informahealthcare.com/jmf
ISSN: 1476-7058 (print), 1476-4954 (electronic)

J Matern Fetal Neonatal Med, Early Online: 1–7
 DOI: 10.3109/14767058.2015.1051959

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

How to perform transabdominal chorionic villus sampling: a practical guideline

Giovanni Monni, Giorgio Pagani, Valentina Stagnati, Ambra Iuculano, and Rosa Maria Ibba

Department of Prenatal Genetic Diagnosis and Fetal Therapy, Ospedale Microcitemico, Cagliari, Italy

Abstract

The spread of both first trimester screening for chromosomal abnormalities and the possibility to check for single gene disorders at DNA-analysis has increased the request for chorionic villus sampling (CVS) in the first trimester. In order to perform placental biopsy, two routes are possible: the transcervical (TC) and the transabdominal (TA). In early days, the trancervical technique was the most diffused, but since its introduction into clinical practice, the TA technique has become the approach of choice in detriment of the TC technique. In our institution, we have a 30-year experience in TA-CVS with more than 26000 procedures performed. Considering the expertise and the volume of procedures undertaken at our unit, we suggest a practical guideline for novel operators in TA-CVS.

Keywords

Chorionic villus sampling, genetic testing, invasive procedure, practical guide, prenatal diagnosis

History

Received 8 May 2015
Accepted 13 May 2015
Published online 10 September 2015

Ultrasound Obstet Gynecol 2016; 48: 256–268
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/uog.15945

isuog.org

GUIDELINES

ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis

Ghi, Ultrasound Obstet Gynecol, August 2016

REPLY

Re: ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis

Monni, Ultrasound Obstet Gynecol, March 2017

CONSEQUENCES OF FIRST TRIMESTER COMBINED SCREENING

- **REDUCTION IN THE FETAL NUMBER OF INVASIVE PROCEDURES PERFORMED FOR PRENATAL KARYOTYPE**
- **REDISTRIBUTION OF THE PROPORTION OF PROCEDURES PERFORMED BY AMNIO AND CVS**
- **PREVALENCE OF ANEUPLOIDIES DIAGNOSED BY CVS**

- **Denmark (BMJ 2008)**: in 2006 CVS in 66%

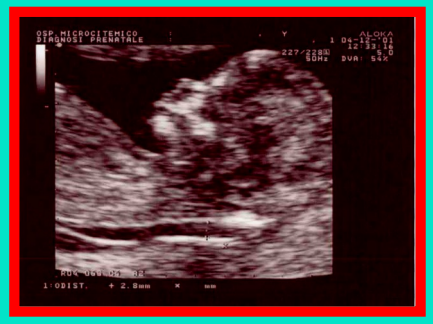
- **UK (UOG 2013)**: in 2003 Amnio/CVS 3:1
  in 2011 Amnio/CVS 1:1

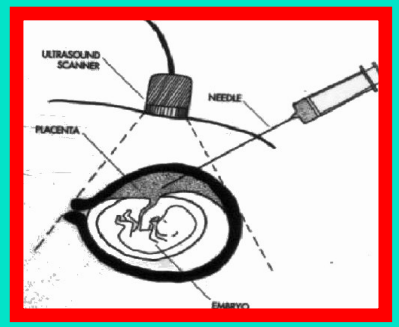
Monni, Ultrasound Obstet Gynecol 2013: Opinion

NUCHAL TRANSLUCENCY TEST IN WOMEN AGED 35 AND OLDER

- Could Decrease the Demand for Invasive Prenatal Diagnosis
- Could Lead to an Earlier Invasive Diagnosis of Chromosomopathies by CVS

Zoppi, Monni, Obstet Gynecol 2001





DE GRUYTER J. Perinat. Med. 2020; 48(4): 307–312

Review

Giovanni Monni*, Valentina Corda, Ambra Iuculano and Yalda Afshar

The decline of amniocentesis and the increase of chorionic villus sampling in modern perinatal medicine

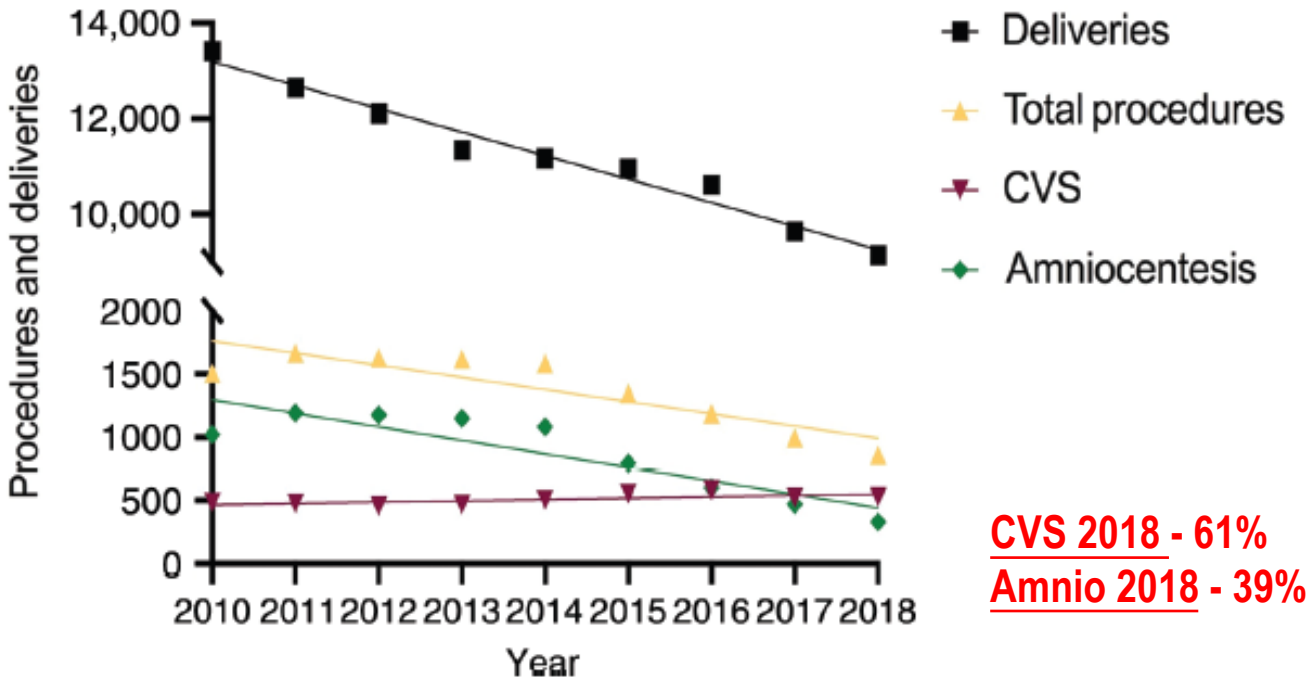
PRENATAL INVASIVE PROCEDURES FOR KARYOTYPE BY TA-CVS & AMNIO, CAGLIARI 2010-2018

Years	Deliveries	Total Prenatal Procedures		TA-CVS			Amniocentesis		
		No	Procedures/ Deliveries	No	Procedures ratio	Deliveries ratio	No	Procedures ratio	Deliveries ratio
2010	13413	1506	11,23%	486	32,27%	3,62%	1020	67,73%	7,60%
2011	12650	1667	13.18%	477	28,61%	3,77%	1190	71,39%	9,41%
2012	12107	1630	13,46%	456	27,98%	3,77%	1174	72,02%	9,70%
2013	11347	1620	14,28%	470	29,01%	4,14%	1150	70,99%	10,13%
2014	11168	1585	14,19%	502	31,67%	4,49%	1083	68,33%	9,70%
2015	10947	1349	12,32%	554	41,07%	5,08%	795	58,93%	7,26%
2016	10610	1185	11,17%	583	49,41%	5,70%	602	50,59%	5,67%
2017	9640	995	10,32%	523	52,56%	5,43%	472	47,44%	4,90%
2018	9143	858	9,38%	523	61,0%	5,70%	335	39,0%	3,60

In the era of combined screening: 2010 - 2013

In the era of combined screening + NIPS: 2014 - 2018

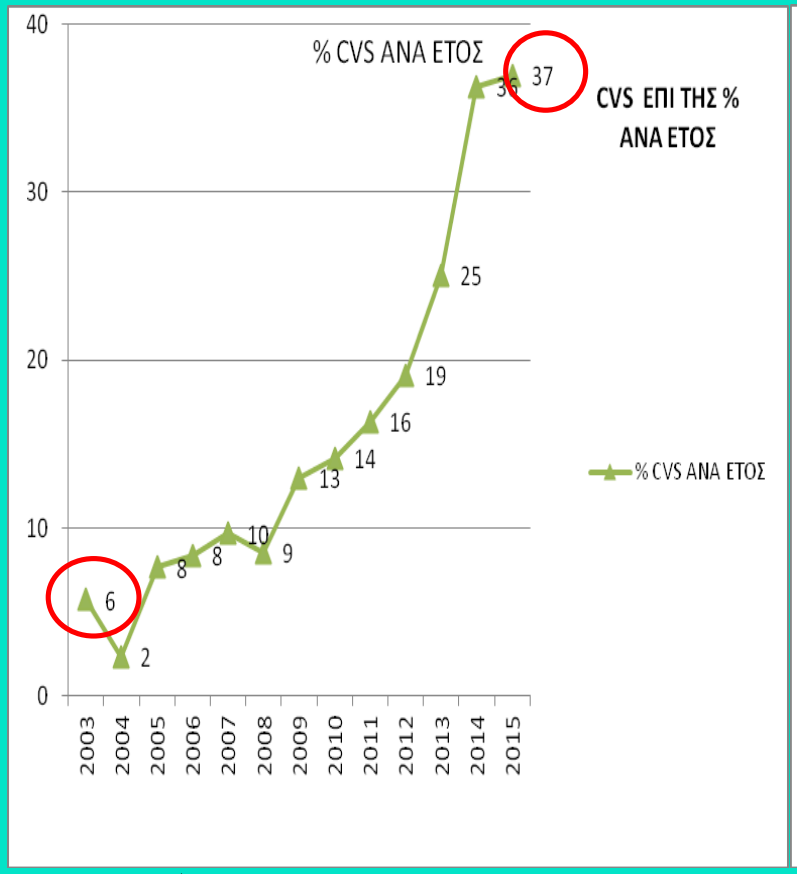
TRENDS IN DELIVERY VOLUME AND INVASIVE PRENATAL PROCEDURES IN SARDINIA, 2010-2018



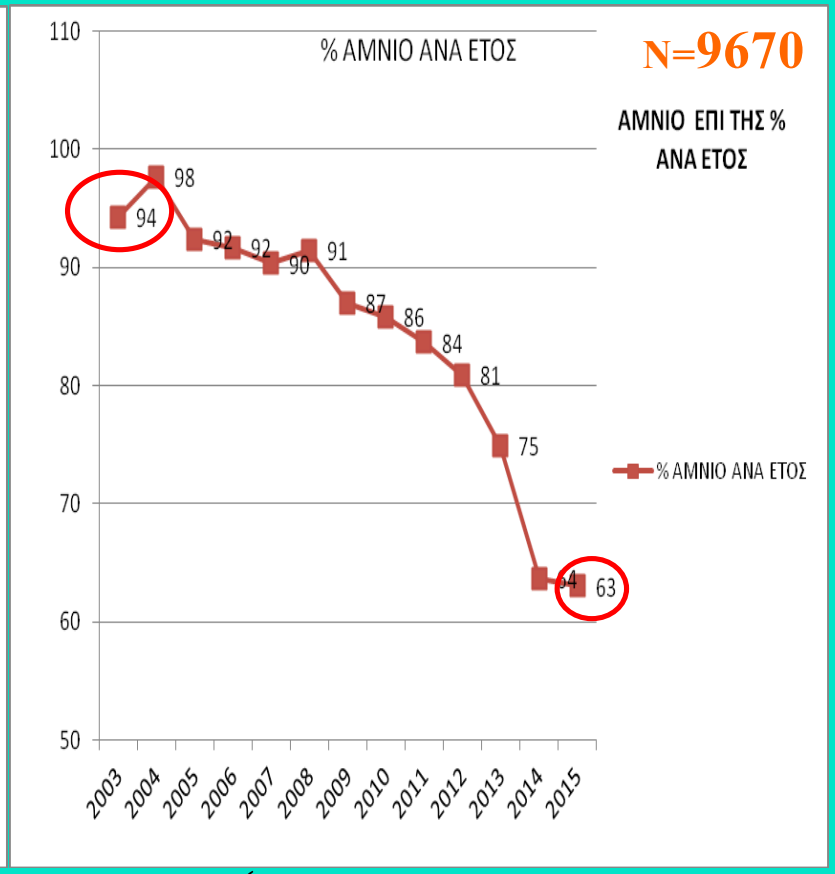
CVS 2018 - 61%
Amnio 2018 - 39%

Monni, J Perinat Med, Feb 2020

Percentage of CVS & amniocentesis according all invasive procedures / year



✓ Increase 616%



✓ Decrease 67,02%

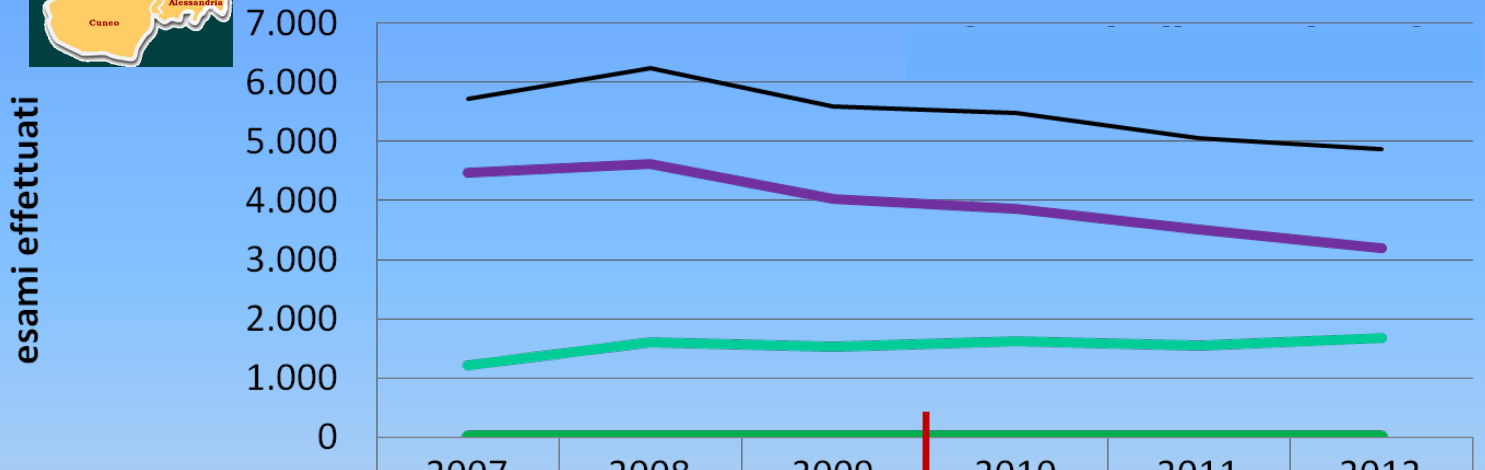
Athanasiadis, Thessaloniki

REDUCTION IN PRENATAL DIAGNOSIS PROCEDURES

INVASIVE PROCEDURE:			
Fiscal year	**Amniocentesis**	**CVS**	**Total**
2003/4	28,700	8,268	36,968
2004/5	24,349	7,980	32,329
2005/6	22,625	7,819	30,444
2006/7	14,733	4,781	19,514
2007/8	12,932	4,681	17,613
2008/9	8317	3129	11,446
2009/10	6795	3669	10,464
2010/11	6353	4195	10,548
2011/12	5171	5044	10,215
2012/13	4049	4423	8499
2013/14	4034	3826	7612
2014/15	3175	3226	6146

Pandya, London

PRENATAL INVASIVE DIAGNOSIS in PIEMONTE



	2007	2008	2009	2010	2011	2012
Villocentesi	1.216	1.596	1.524	1.625	1.548	1.674
Amniocentesi	4.480	4.622	4.031	3.851	3.497	3.185
Funicolocentesi	9	8	7	4	3	9
Totale	5705	6226	5589	5480	5048	4868

17.520

16.2% of 107.808

15.396

14.3% of 107.256

CAUSES OF DECREASE IN CVS/AMNIO FOR KARYOTYPE ANALYSIS

- Birth-rate decrease (denatality)
- First Trimester Combined Screening
- NIPT
- Less possibilities for tutoring

No decrease of CVS for Mendelian Diseases
No decrease of Amniocentesis for Congenital Infections

CONSEQUENCES OF REDUCTIONS OF CVS/AMNIO

- Fewer procedures to maintain expertise
- Centralization of prenatal procedures
- Fewer opportunities to train in vivo
- Need for simulation

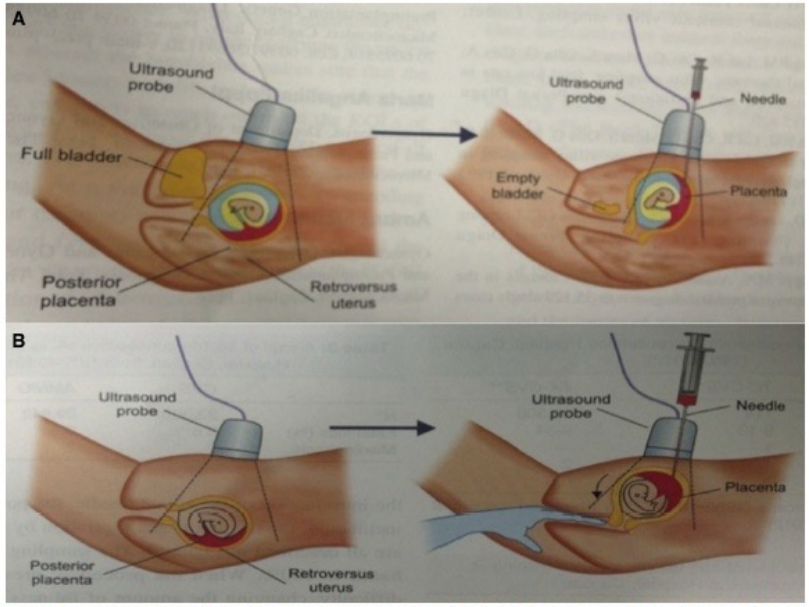
Training for transabdominal villous sampling is feasible and safe

OBJECTIVE: In the near future, the spread of noninvasive prenatal testing will drastically decrease the number of invasive diagnostic procedures currently performed to diagnose aneuploidy.[1-3] Thus, adequate training will be a challenge with a decreased number of available procedures.[4] The demand for chorionic villous sampling (CVS) has increased as this is the method of choice for DNA analysis and prenatal diagnosis of inborn errors of metabolism.[5] Among the available techniques, transabdominal (TA) CVS offers advantages over the transcervical approach.[4,5]

STUDY DESIGN: Training for TA-CVS remains an important clinical need. However, there is not a well-structured training method to achieve proficiency with this procedure.[4] The aim of this study was to determine the frequency of procedure success and the fetal loss after TA-CVS performed by trainees of a senior physician expert with CVS. This was a retrospective cohort study of procedure and pregnancy outcomes of TA-CVS cases performed by individuals trained under our direction from January 1986 through December 2014. Each fellow underwent a 2-week period of training by a single senior physician (G.M.). The program consisted of observation of CVS, followed by the performance of amniocentesis and then mentor experience with TA-CVS. Firstly, fellows assisted several procedures; then performed tutored amniocentesis; finally, fellows

FIGURE

Needle insertion tangential to probe and perpendicular to placenta



Changes according to **A**, bladder's filling grade, and **B**, vaginal manipulation to allow perpendicular needle insertion in case of complete posterior placenta and retroverted uterus.

Monni G, Am J Obstet Gynecol. 2015

REASONS FOR INCREASING CVS

Increased risk for aneuploidy for maternal age

Amnio late analysis 16-20 wks following positive combined screening and positive NIPT

After US Fetal Abnormalities +CGH-array

Anxiety for attending late diagnosis (16-20 wks) in women at high risk

High genetic risk (≥ 25 %) Thalassemia, Cystic Fibrosis, Duchenne, Mental Genetic diseases, etc.

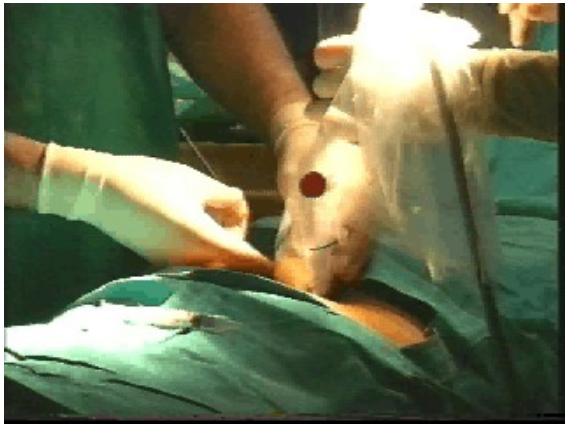
Error of metabolism

Selective Embryoreduction in multiple pregnancies

Twins and multiples more than 3 fetuses

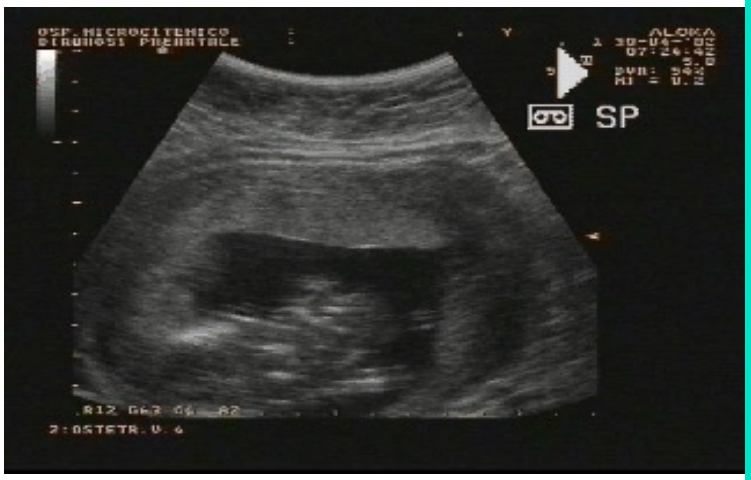
Legal aspects

Women's ansiety and women's choices



AMNIOCENTESIS IS STILL BETTER TO CONTINUE TO USE IN CASE OF:

- **Operators inexperienced in CVS**
- **Maternal transmittable infectious diseases:** ***toxoplasmosis, cytomegalovirus, rubeolla***
- **Mosaicism for aneuploidy**
- **Amniotic α-feto protein for NTD**
- **High order multiple pregnancies (> 3 fetuses)**



- **Following second trimester biochemistry screening:** ***triple, quadruple test***

CVS/AMNIO ARE STILL MANDATORY FOR

- Most accurate prenatal diagnosis
- Rapid karyotype analysis
- Microdeletion syndromes
- First trimester fetal risk assessment
- Positive or failure NIPS
- Second trimester positive triple-quadruple test
- First and second trimester fetal ultrasound abnormalities
- Placenta fetal confined mosaicism and fetal mosaicism
- Congenital infectious diseases
- Amniotic alfafetoprotein for NTD
- Multiple pregnancies
- Maternal chromosomal abnormalities
- Vanishing twin
- X-linked diseases
- Mendelian genetic diseases
- Metabolic genetic diseases
- Autosomal dominant diseases of maternal origin

An international chorionic villus sampling training program in ongoing-pregnancies with demonstrable outcomes: a survey study

REGIONE AUTÒNOMA DE SARDIGNA
REGIONE AUTONOMA DELLA SARDEGNA

David Geffen School of Medicine

Giovanni Monni, MD[1]; Yalda Afshar, MD, PhD[2]; Jeffrey Sperling, MD[3], Cristina Peddes, MD[1]; Valentina Corda, MD[1], Ambra Iuculano, MD[1]
[1]Department of Prenatal Genetic Diagnosis and Fetal Therapy; Ospedale Microcitemico; Cagliari, Italy; [2]Division of Maternal Fetal Medicine Department of Obstetrics and Gynecology; University of California Los Angeles; Los Angeles, California; USA; [3]Kaiser Permanente Northern California; Modesto, California; USA

Background

- Chorionic villus sampling (CVS) remains the sole method for first-trimester prenatal diagnosis.
- Most physicians are unable to provide this service because of a lack of training.

Objective: To identify the impact of a well-established international training program in invasive diagnosis on provider confidence and practice pattern among those trained through the hands-on program.

Study Design

- 20-question online survey was sent to all MDs who had completed training at the host institution.
- Included questions about the trainees educational and procedural experiences, estimates of the number, type of procedures performed, and self-evaluation of competence.
- Descriptive statistics were performed and the Student t-test was used as indicated. Multiple logistic regression was used to adjust for covariates.

Results

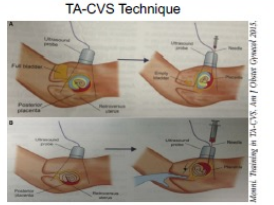
- 72 surveys sent, 47 (65.3%) were returned. 63.8% of respondents were female. All trainees were OBs/MFMs except one radiologist who had completed an average of 8.7 (SD: 21.8) CVS and 115.8 (SD: 222.8) amniocentesis before the training program.
- Rotators were 25 (51%) were faculty and 22 (47%) were residents/fellows. 24 (51.1%) work in a private practice, 18 (38.3%) in academics, and 5 (10.6%) in public hospitals. The mean length of rotation was 2.7 weeks. Attendees came from 13 countries with 11(23.4%) from the U.S..
- Comfort with CVS (3.26 to 4.70, p<0.001) and amniocentesis (Likert 1.86 to 3.93, p<0.001) improved significantly after the training Following training, 34.7% are currently teaching or mentoring CVS following training.
- There was no association with pre- vs post-training comfort in CVS or amniocentesis following training, when adjusted for age, gender, or experience (p>0.05).

Conclusion: **As comprehensive training in invasive fetal procedures dwindles, a program able to improve confidence and skill in these procedures is critical. The critical components of training remain challenging.**

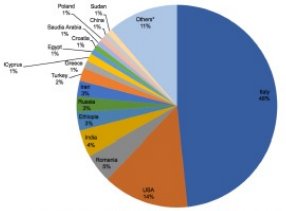
We have established a successful international training program in trans-abdominal **chorionic villus sampling in ongoing pregnancies**.

Questions?
Take a picture of this QR code to access the poster
or email Dr. Giovanni Monni at **prenatalgmonni@gmail.com**

TA-CVS Technique

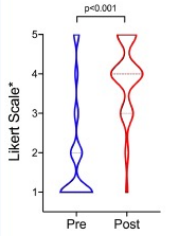


Country of Origin of Trainees Rotating in International TA-CVS Program n=236

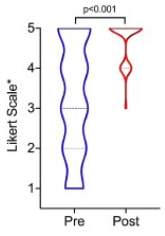


*Others: n=1 from Argentina, Azerbaijan, Bosnia, Czech Republic, Canada, Georgia, Japan, France, Germany, Kosovo, Lebanon, Mongolia, Morocco, Nigeria, Netherlands, Pakistan, Portugal, Qatar, Slovenia, Spain, Sudan, Emirates, Venezuela

Comfort with CVS



Comfort with Amniocentesis



*1=not comfortable, 5=very comfortable

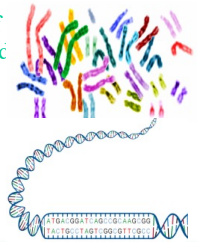
*1=not comfortable, 5=very comfortable

Dallas, USA, 40°SMFM Annual Meeting, February 2020

NEW MOLECULAR APPROACHES

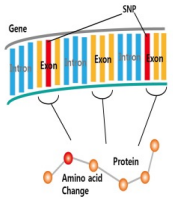
WHOLE GENOME SEQUENCING (WGS)

DNA sequencing of the entire coding and non-coding regions of the genome



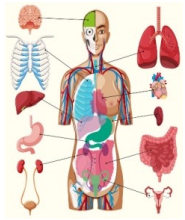
WHOLE EXOME SEQUENCING (WES)

DNA sequencing all of the protein-coding regions (1-2% of the genome)



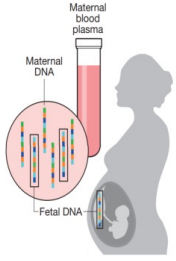
TARGETED GENE PANELS

Targeted DNA strategy enabling sequencing of coding and and noncoding regions of specific genes

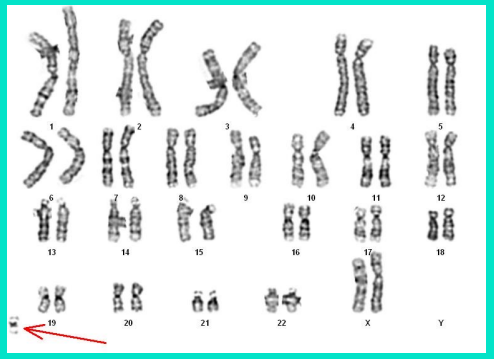


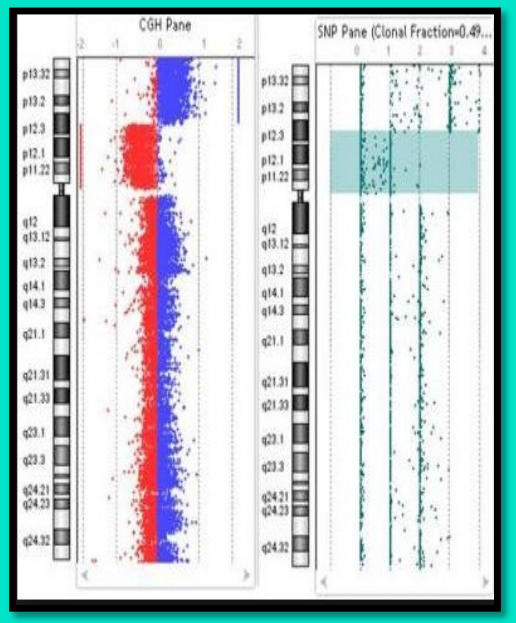
NON-INVASIVE PRENATAL TESTING

Cell-free fetal DNA analysis for aneuploidy and genomic diseases



Cytogenetic or Molecular Cytogenomics





- Karyotype
- Chromosome microarray
- Target mutation testing
- NGS panel
- Whole genome sequencing
- Viral DNA research

Procedures	Sample	Tests
CVS	Chorionic villi	Karyotype, Microarray, DNA Testing
AMNIOCENTESIS	Amniotic fluid	Karyotype, Microarray, DNA Testing, Enzyme Testing, Fetal AFP, Viral PCR
CORDOCENTESIS	Fetal blood	Karyotype, Microarray, DNA Testing, Viral PCR, Bllod Typing

ACCEPTANCE RATE OF PRENATAL DIAGNOSIS OF ß- THALASSEMIA IN SARDINIA ACCORDING TO THE INVASIVE GENETIC PROCEDURE

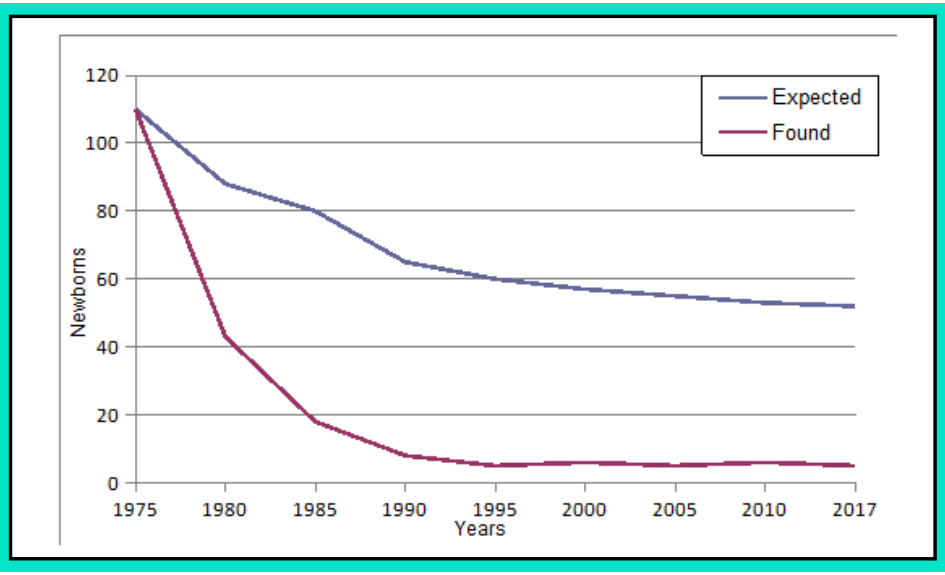
TECHNIQUE	ACCEPTANCE (%)
Fetal Blood Sampling	93.2
Amniocentesis	96.4
Chorion Villus Sampling	99,3

Cao, Monni , Prenat Diagn 1987

CONSEQUENCES OF PRENATAL GENETIC DIAGNOSIS IN SARDINIA

Fall in the birth rate of homozygous β-thalassemia in Sardinia. The top line represents affected children born in absence of prenatal diagnosis and the bottom line the affected children born with prenatal testing

Monni, J Clin Med 2018



Although taking in consideration the decreasing birth rate in Sardinia, when antenatal screening and diagnosis programs were still not available in 1977, the thalassemic newborns were 120 whereas in the last ten years the number has gradually diminished to 3-4 newborns per year.

Monni, J Perinat Med 2021

PAST-PRESENT-FUTURE OF PRENATAL SCREENING *AND* DIAGNOSIS

Where have we been?

Where are we going?

- **The advances of Genomic Medicine are impacting prenatal diagnosis, just like any other medical field.**

- **While these offer exciting new opportunities and can empower families with increased knowledge about their reproductive risks and with decision-making autonomy, they have to be carefully introduced in an evidence-based and ethically responsible manner and monitored after implementation.**

- **Considering that many of these innovations are driven by for-profit companies, professional societies will play an increasingly important role in providing objective guidance to patients and providers.**

INFORMATION TO PREVENT CONGENITAL FETAL ANOMALIES