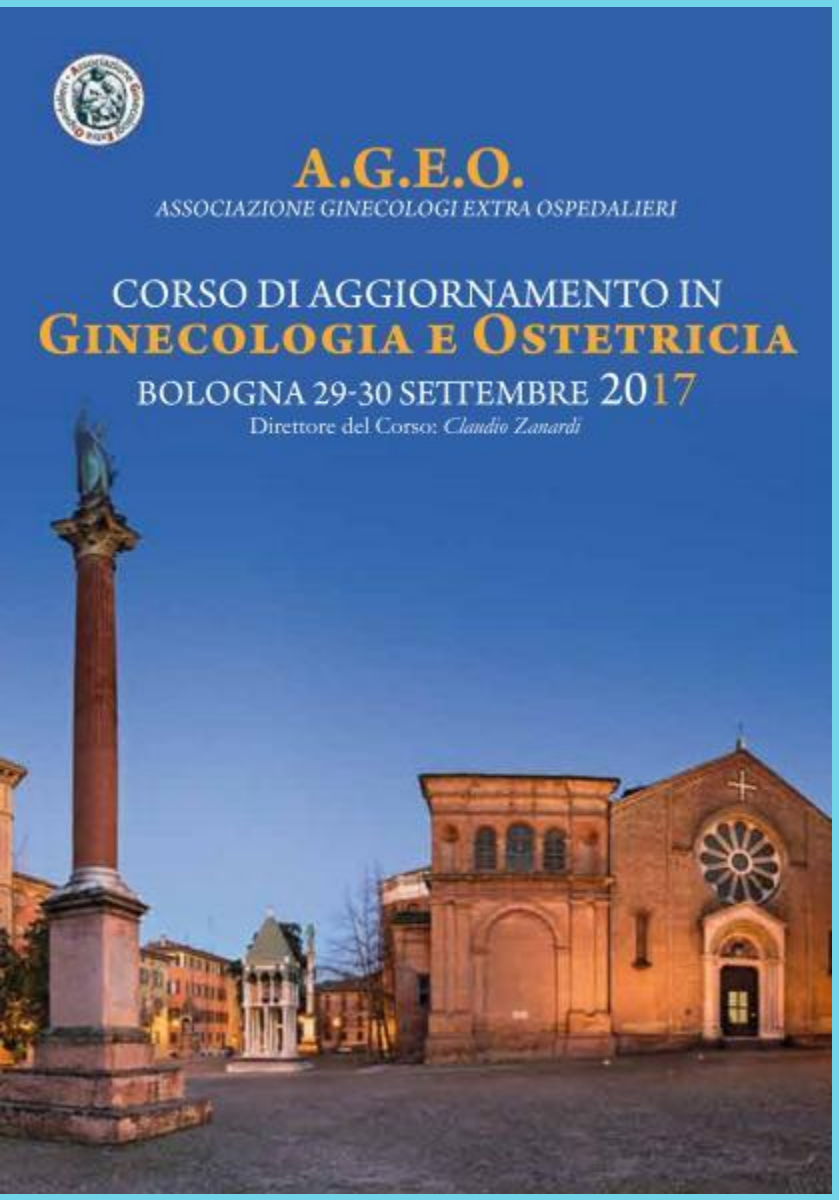


Studio prenatale della patologia cromosomica

Luigi Filippo Orsini

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lforsini@studiofilippoorsini.it

Diagnosi o Screening?

Quale tecnica?

Rischi, costi, affidabilità

Motivi personali o sociali?



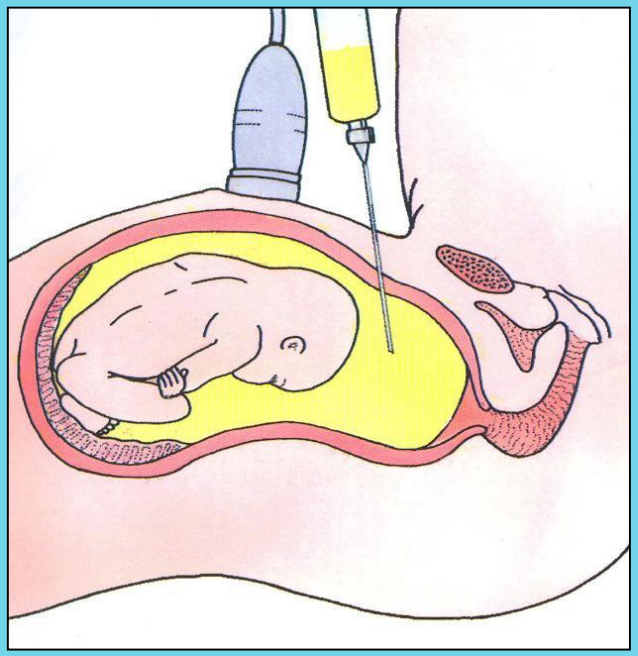
Aspetti morali e religiosi

Ostetrico o Genetista?

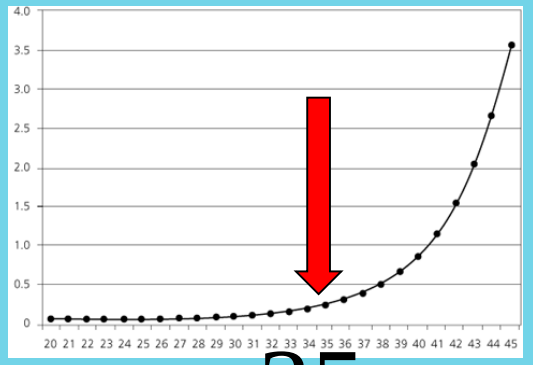
Citogenetica o Molecolare?

Aspetti legislativi

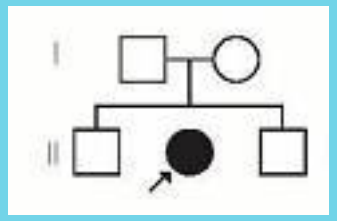
C'era una volta…

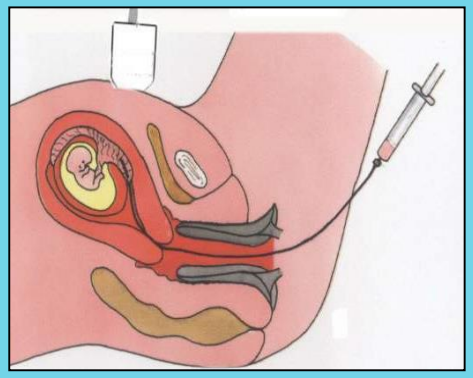


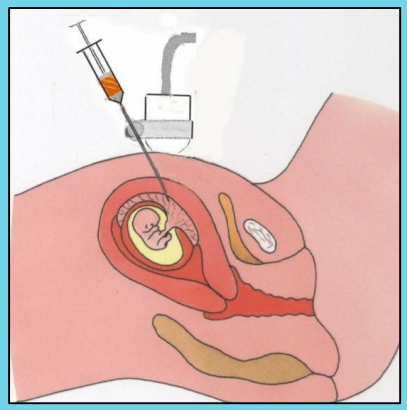


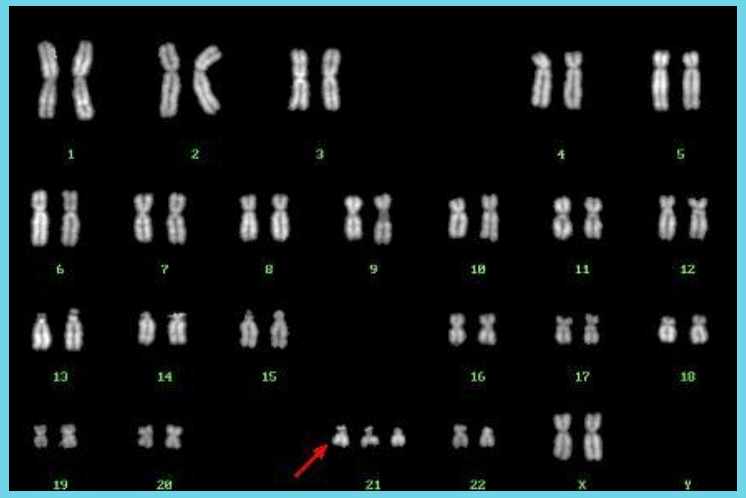


35

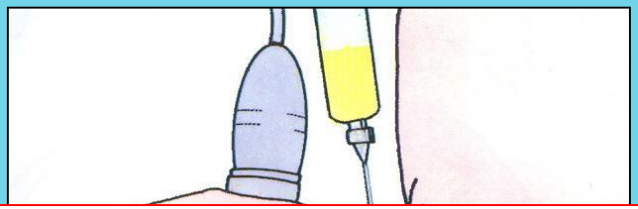






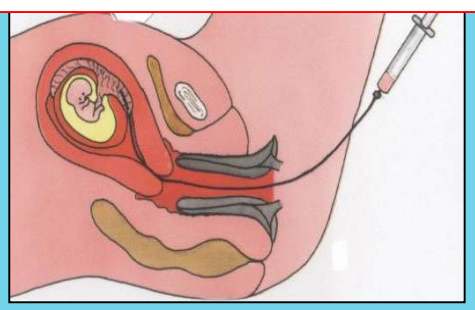


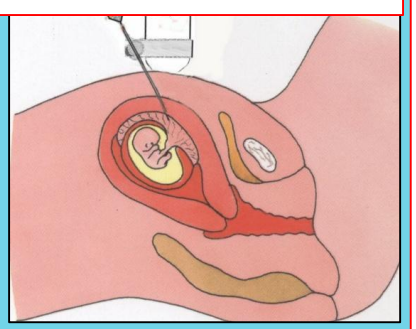
Il presente…



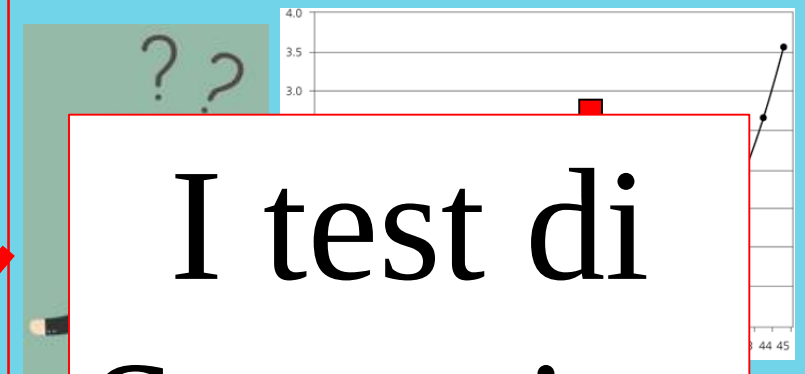
Amnio

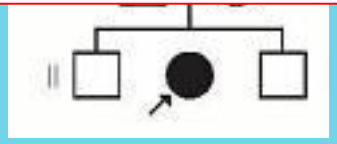
Villocentesi





I test di Screening





Il DNA fetale

enetica vs Molecolare

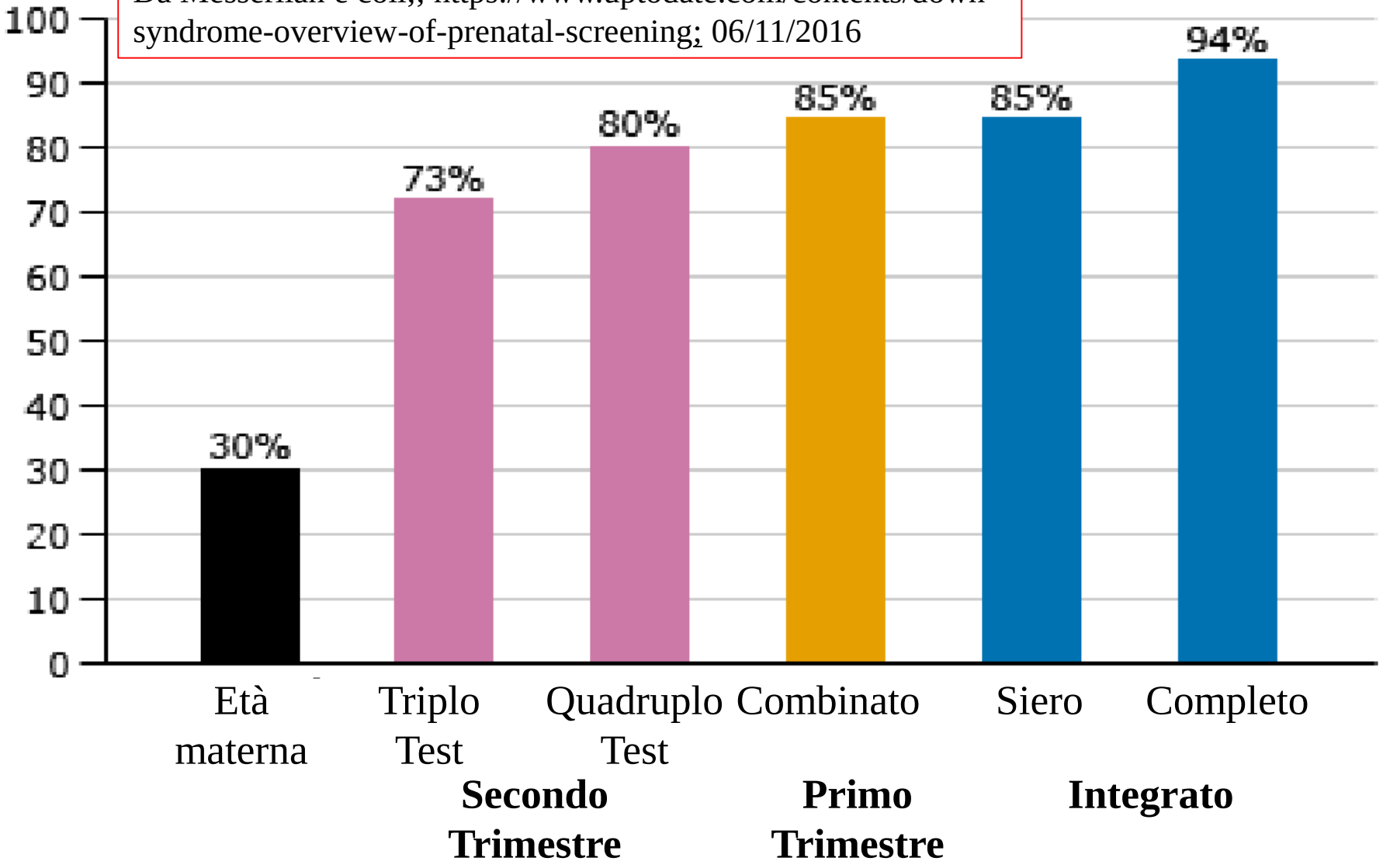
Principali test di screening della trisomia 21

Test	Metodica	Sett	Vantaggi / Svantaggi
Combinato	PAPP-A + hCG + NT	**10–13**	**Precoce-Buona DR (anche in base al cut off)-Screening altre patologie / Ecografia specializzata**
Triplo-Quadruplo	H CG, AFP, uE3 + Inibina A	**15-22**	**No ecografia–Screening DTN / Tardivo-Bassa DR**
Integrato solo siero	PAPP-A + Quadruplo Test	**10-13 15-22**	**No ecografia–Screening DTN Buona DR/ Esito tardivo–2 prelievi**
Integrato completo	NT+PAPP-A + Quadruplo Test	**10-13 15-22**	**Alta DR-Screening DTN / Esito tardivo–2 prelievi**
Sequenziale	Combinato + Quadruplo Test	**10-13 15-22**	**Alta DR-Esito noto nel 1° trimestre ma definitivo nel 2° trimestre**
Contingente (in base a rischio basso,medio,alto)	Combinato +/- Quadruplo Test o Diagnosi Diretta	**10-13 (15-22)**	**Alta DR-Esito noto nel 1° trimestre ma definitivo nel 2° trimestre**

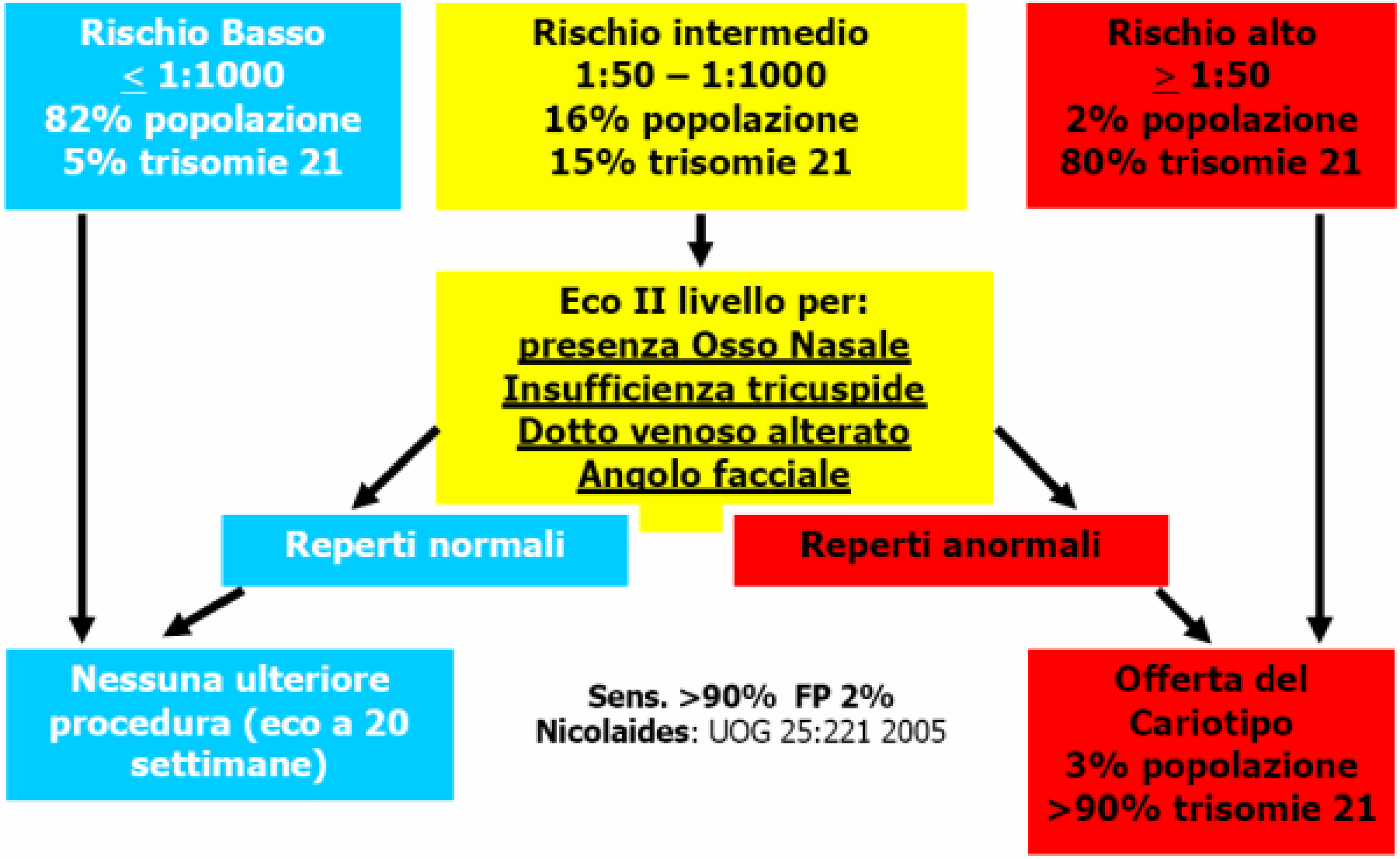
NT: translucenza nucale; AFP: alfafetoproteina; DR: Detection Rate;DTN: difetti tubo neurale

Detection rate dell'età materna e dei test di screening della trisomia 21 (5% falsi positivi)

Da Messerlian e coll;; https://www.uptodate.com/contents/down-syndrome-overview-of-prenatal-screening; 06/11/2016



Esempio di test contingente basato sul test combinato



Dai nuovi LEA 2017 per la gravidanza

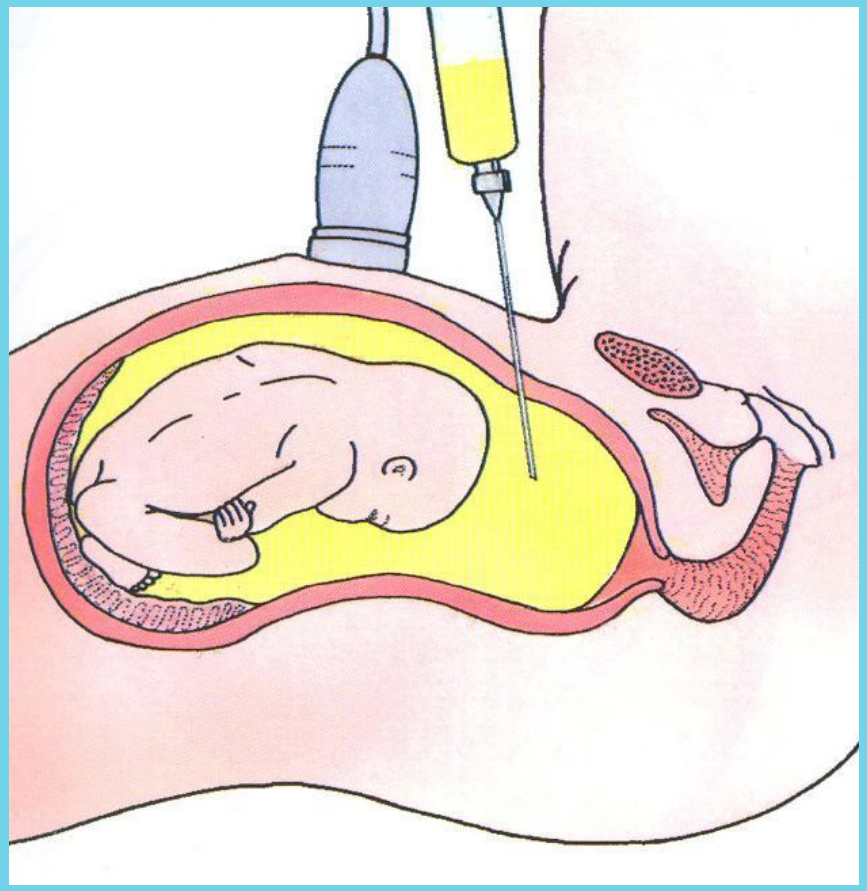
ALLEGATO 10C

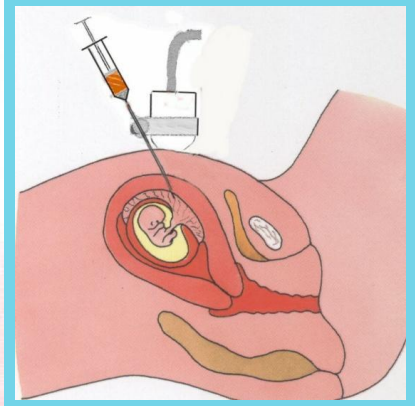
CONDIZIONI DI ACCESSO ALLA DIAGNOSI PRENATALE INVASIVA, IN ESCLUSIONE DALLA QUOTA DI PARTECIPAZIONE AL COSTO

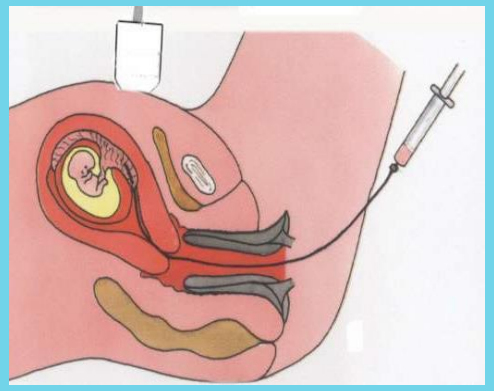
1) probabilità di trisomia 21, o di altre anomalie cromosomiche ≥1/300 al momento del test per la valutazione del rischio nel primo trimestre (o ≥1/250 in caso di test nel secondo trimestre)

- anomalie fetali/della gravidanza evidenziate mediante ecografia

Amniocentesi vs villocentesi



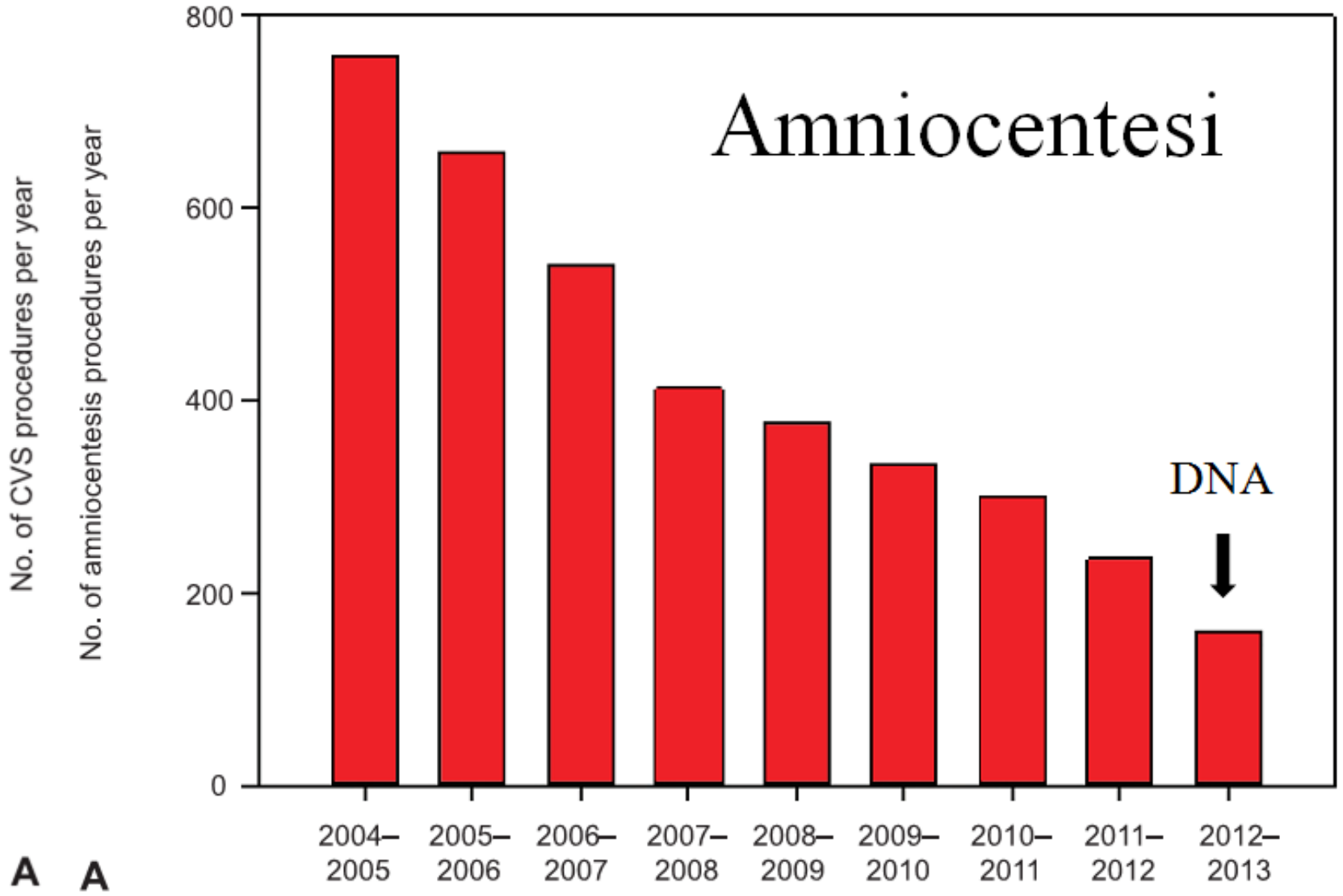




Association of Combined First-Trimester Screen and Noninvasive Prenatal Testing on Diagnostic Procedures

Sebastian Larion, MS, Steven L. Warsof, MD, Letty Romary, MD, Margaret Mlynarczyk, MD, PhD, David Peleg, MD, and Alfred Z. Abuhamad, MD

(Obstet Gynecol 2014;123:1303–10)



Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis

R. AKOLEKAR*†, J. BETA*, G. PICCIARELLI*, C. OGILVIE‡ and F. D'ANTONIO§

Only studies reporting data on more than 1000 procedures were included in this review

The weighted pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95% CI, –0.04 to 0.26%) and 0.22% (95% CI, –0.71 to 1.16%), respectively.

ABSTRACT

included in this review to minimize the effect of bias from smaller studies. Heterogeneity between studies was estimated using Cochran's Q, the I^2 statistic and Egger bias. Meta-analysis of proportions was used to derive weighted pooled estimates for the risk of miscarriage before 24 weeks' gestation. Incidence–rate difference meta-analysis was used to estimate pooled procedure-related risks.

Results The weighted pooled risks of miscarriage follow- (95% CI, 0.46–0.91%) for amniocentesis and 1.79% (95% CI, 0.61–3.58%) for CVS. The weighted pooled procedure-related risks of miscarriage for amniocentesis and CVS were 0.11% (95% CI, –0.04 to 0.26%) and 0.22% (95% CI, –0.71 to 1.16%), respectively.

Conclusion *The procedure-related risks of miscarriage following amniocentesis and CVS are much lower than are currently quoted.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

PRACTICE BULLETIN

CLINICAL MANAGEMENT GUIDELINES FOR OBSTETRICIAN–GYNECOLOGISTS

NUMBER 162, MAY 2016

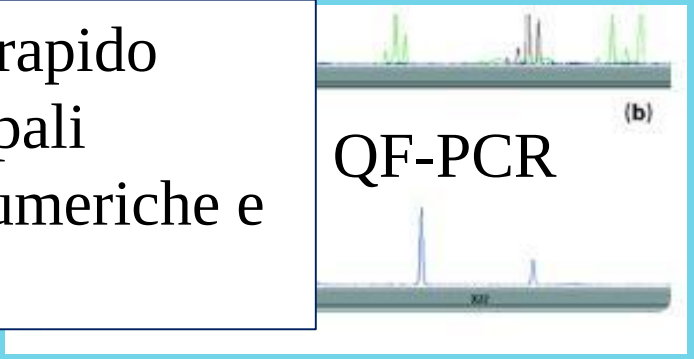
(Replaces Practice Bulletin Number 88, December 2007)
(See also Practice Bulletin Number 163, *Screening for Fetal Aneuploidy*)

Prenatal Diagnostic Testing for Genetic Disorders

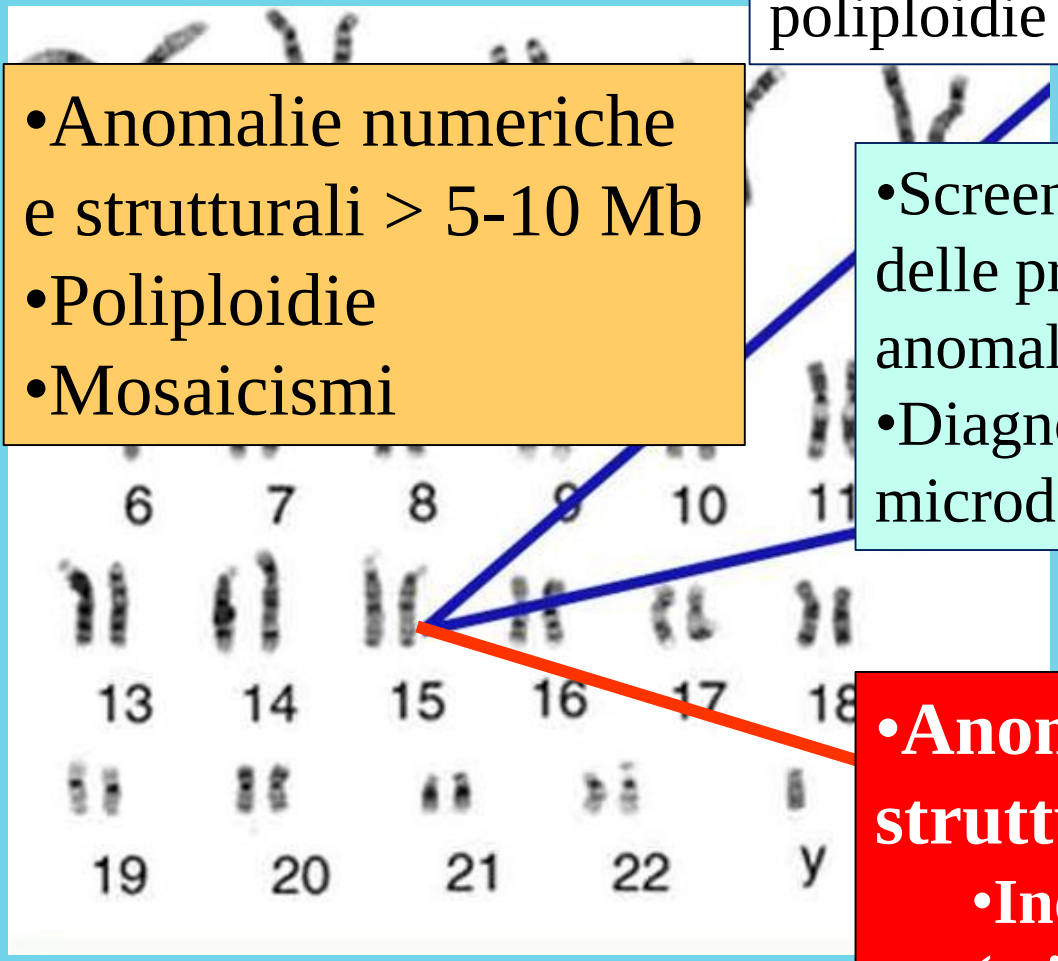
The rate of procedure-related pregnancy loss that is attributable to a prenatal diagnostic procedure currently is estimated to be approximately 0.1–0.3% in procedures performed by experienced health care providers. The loss rates for amniocentesis and CVS are both very low.

Cariotipo e tecn
complementa

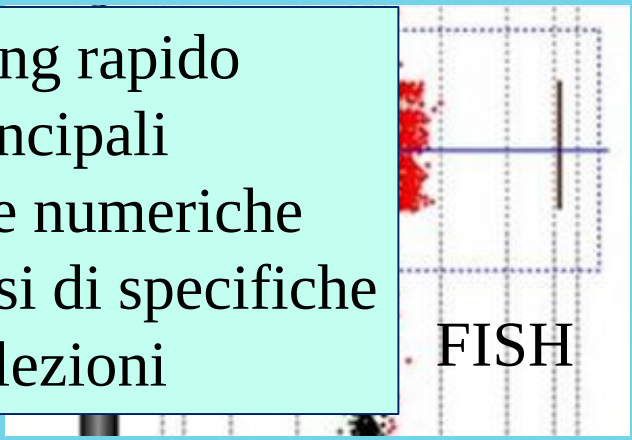
•Screening rapido delle principali anomalie numeriche e poliploidie

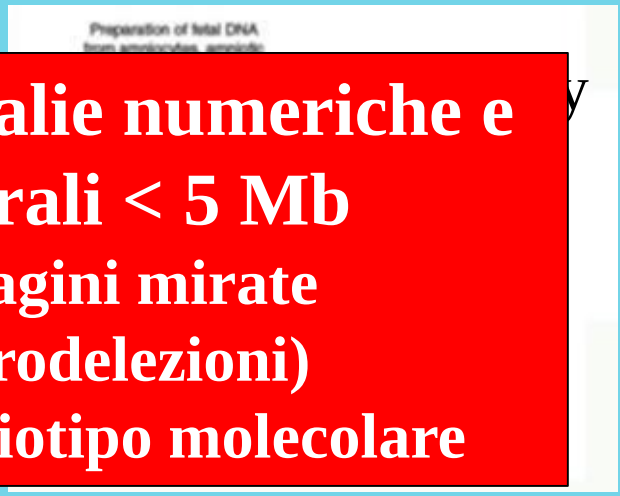


•Anomalie numeriche e strutturali > 5-10 Mb
•Poliploidie
•Mosaicismi



•Screening rapido delle principali anomalie numeriche
•Diagnosi di specifiche microdelezioni





•Anomalie numeriche e strutturali < 5 Mb
- **•Indagini mirate (microdelezioni)**
- **•Cariotipo molecolare**

Sindromi da microdelezione diagnosticabili con le tecniche di ibridazione (Prenatal Bobs)

Delezione	Sindrome	Segni Clinici	Incidenza
4p16.3	Wolf-Hirschhorn	Ritardo mentale, malformazioni, iposviluppo	1/50,000
5p15.3-p15.2	Cri du Chat	Ritardo mentale grave, malformazioni	1/15,000–50,000
7q11.2	Williams-Beuren	Ritardo mentale, malformazioni	1/7,500–20,000
8q23-q24	Langer-Giedion	Ritardo mentale, dismorfismi	nv
15q11-q12	Prader-Willi	Difficoltà apprendimento, obesità, ipogonadismo	1/10,000–30,000
15q11-q12	Angelmann	Ritardo mentale grave, autismo, epilessia	1/12,000–25,000
17p13.3	Miller-Dieker	Ritardo mentale grave, malformazioni	1/100,000–300,000
17p11.2	Smith-Magenis	Ritardo mentale grave, malformazioni	1/25,000–50,000
22q11.2	DiGeorge-1	Ritardo mentale, malformazioni cardiache	1/4,000–5,000
10p14	DiGeorge-2	Ritardo mentale, malformazioni cardiache	1/2,000–4,000

Sindromi da microdelezione diagnosticabili con CGH array

43 varianti con piattaforma Easychip

Syndromic regions	incidence	critical genes
1p36 deletion syndrome	1/5,000	/
1q41q42 microdeletion syndrome	unknown	DISP1
2p15-16.1 microdeletion syndrome	unknown	BCL11A
2q23.1 microdeletion syndrome	unknown	MBD5, EPC2
2q33.1 deletion (Glass syndrome)	unknown	STAB2
2q37 deletion syndrome	<1/10,000	HDAC4
3pter-p25 deletion syndrome	unknown	CNTN4, ITPR1, SRGAP3, VHL
3q29 deletion/duplication syndrome	unknown	FBXO45, PAK2, DLG1
4p16.3 deletion syndrome (Wolf-Hirschhorn)	1/20,000 - 1/50,000	LETM1, WHSC1
4q21 deletion syndrome	unknown	PRKG2, RASGEF1B
5p deletion syndrome (Cri du chat)	1/20,000 – 1/50,000	CTNND2, TERT
5q14.3 deletion syndrome	unknown	MEF2C
5q35 deletion syndrome (Sotos)	1-9/100,000	NSD1
6q13-q14 deletion syndrome	unkown	COL12A1
7q11.23 deletion syndrome (Williams-Beuren)	1/10,000	ELN
8p23.1 deletion syndrome	unknown	GATA4
8q21.11 Microdeletion Syndrome	unknown	ZFHX4, PEX2
8q24.1 deletion syndrome (Langer-Giedion)	unknown	TRPS1, EXT1
9q34.3 deletion syndrome (Kleefstra)	unknown	EHMT1
10p14p13 deletion syndrome (DiGeorge type 2)	unknown	GATA3
11p13 deletion syndrome (WAGR)	unknown	PAX6, WT1
11p11.2 deletion syndrome (Potocki-Shaffer)	unknown	ALX4
11q deletion syndrome (Jacobsen)	1/100,000	/
14q12 microdeletion syndrome	unknown	FOXG1
15q11q13 deletion syndrome (Prader-Willi)	1/25,000	SNRPN
15q11q13 deletion syndrome (Angelman)	1/10,000 - 1/20,000	UBE3A
15q24 deletion/duplication syndrome	unknown	/
16p deletion syndrome (ATR-16)	unknown	HBA1, HBA2
16q24.1 micorodeletion syndrome	unknown	FOXF1, FOXC2
17p13.3 deletion syndrome (Miller dieker)	unknown	PAFAH1B1, YWHAE
17p11.2 deletion syndrome (Smith-Magenis)	1/25,000	RAI1
17p11.2 duplication syndrome (Potocki Lupski)	unkown	RAI1
17q11.2deletion/duplication syndrome	unkown	NF1, SUZ12
17q21.31 deletion syndrome (Koolen-De Vries)	1/16,000	KANSL1
17q23.1-q23.2 deletion syndrome	unknown	TBX2, TBX4
19q13.11 deletion syndrome	unknown	LSM14A, UBA2
Down Sndrome critical region (21q22.12q22.2)	1/650 - 1,000	/
22 partial tetrasomy (Cat eye)	1/50,000 - 1/150,000	/
22q11.2 deletion syndrome (DiGeorge)	1/2,000 – 1/4,000	HIRA, TBX1
22q11.2 distal deletion syndrome	unknown	MAPK1
Xp11.3 deletion syndrome	unknown	RP2
Xp11.22 microduplication syndrome	unknown	HUWE1
Xq12 deletion/duplication (OPHN1)	unknown	OPHN1
Xq22.3 deletion syndrome (AMME COMPLEX)	unknown	COL4A5, ACS4
Xq28 duplication syndrome	unknown	MECP2

Alesi et al., J Genet Syndr Gene Ther 2016, 7:1

Vantaggi della tecnica CGH array

- Spettro diagnostico più ampio rispetto al cariotipo convenzionale
  - *+1,7% di anomalie rilevate in casi senza malformazioni ecografiche*
  - *+6,5% di anomalie rilevate in casi con malformazioni ecografiche*
- Possibilità di analisi diretta senza coltura
  - *Utilizzabile anche in caso di MEF e mortalità neonatale*
- Tempi di risposta più rapidi

Svantaggi e della tecnica CGH array

- Incapacità di rilevare anomalie cromosomiche bilanciate e disomie uniparentali
- Rilievo occasionale di varianti di significato incerto (VOUS)
- Costi più elevati

Indicazioni preferenziali della tecnica CGH array in ostetricia

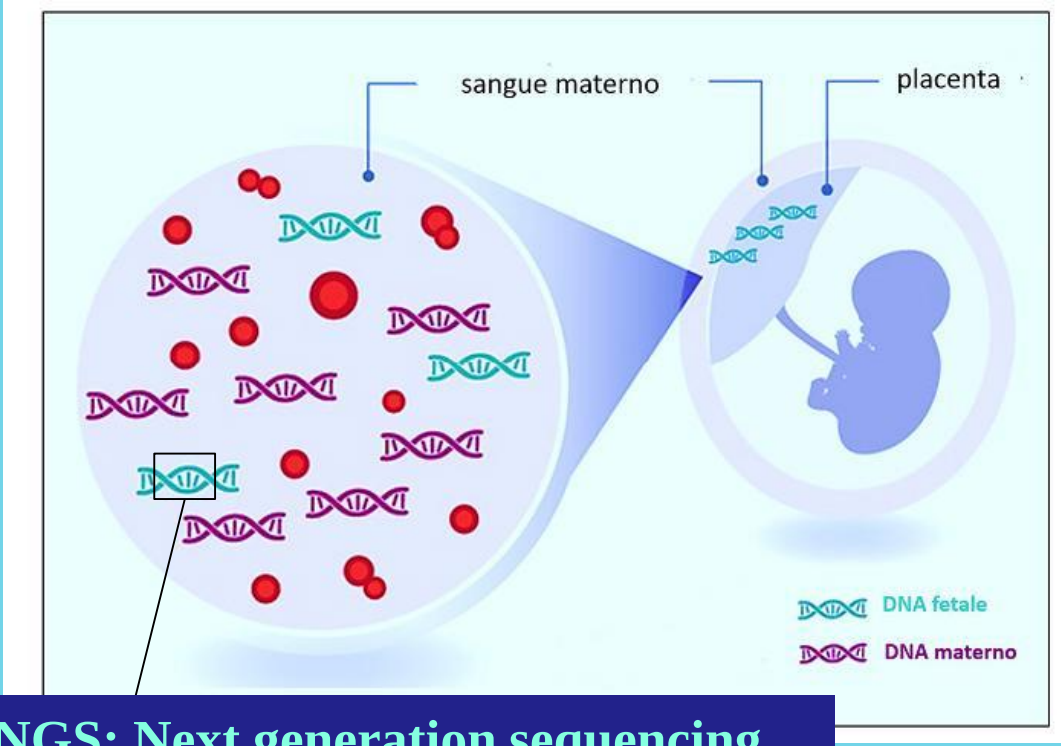
- Valutazione di feti con sindromi malformative o difetti di crescita rilevati all'ecografia
- Conferma di microdelezioni rilevate con il DNA fetale
- Casi di MEF (in cui si ritenga indicata l'indagine cromosomica)
- ***Alternativa al cariotipo tradizionale?***

Test del DNA libero circolante

NIPT (Non Invasive Prenatal Testing)

Screening prenatale non invasivo basato sul DNA

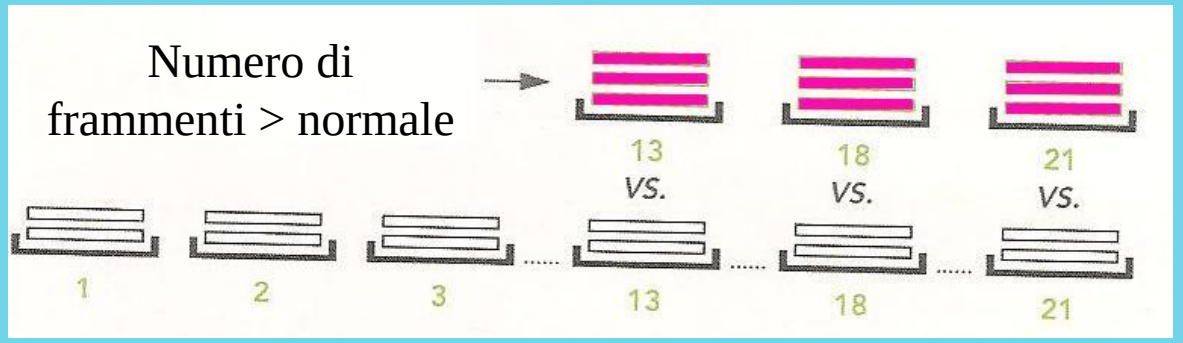
DNA libero circolante nel sangue materno



>10 settimane: DNA fetale > 4%

NGS: Next generation sequencing

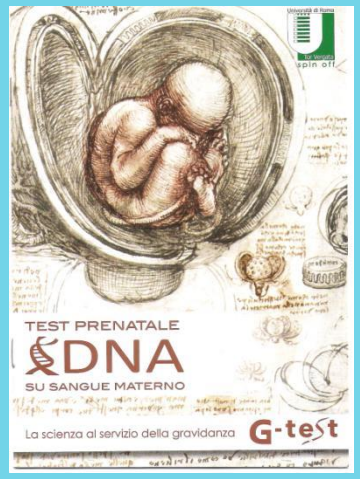





ames
Centro Polidiagnostico Strumentale srl
800 586 368
Vera
PRENATAL TEST
INDIVIDUA LE PRINCIPALI ANOMALIE CROMOSOMICHE FETALI CON UN SEMPLICE PRELIEVO DEL SANGUE MATERNO
Fattore RH fetale
INDIVIDUA IL FATTORE RH DEL FETO CON UN SEMPLICE PRELIEVO DEL SANGUE MATERNO


Harmony
PRENATAL TEST
Test Prenatale Non Invasivo:
La nuova era nell'individuazione delle trisomie fetali


ANALISI PRENATALE NIPT
UN TEST SICURO E NON INVASIVO
PER TE ED IL TUO BAMBINO
W&B


TEST PRENATALE
DNA
SU SANGUE MATERNO
La scienza al servizio della gravidanza
G-test


Aurora
Il test prenatale non invasivo
Aurora
Aurora
Aurora


verifi
prenatal test
Non-invasive prenatal testing
for trisomies 21, 18, 13
and sex chromosomes


panorama
natera prenatal screen


percept
cell-free DNA prenatal test


MaterniT21
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Sequenom
Product Branding
LEARN MORE ABOUT THIS PROJECT


Tranquility
The most Accurate Non-Invasive Prenatal Trisomy Test
Avoid the risk of Amniocentesis
Maternal blood DNA collection kit
Genoma


PrenatalSafe
PrenatalSafe
800 581 651
GENOMA

Possibilità di screening con DNA fetale

- Trisomie 13,18,21
- Diagnosi di sesso
- Anomalie numeriche dei cromosomi sessuali (X0, XXY, XXX, XYY)
- Rh fetale
- *Microdelezioni (1p,4p,5p,15q,22q)*
- *Trisomia 9 e 16*
- *Anomalie numeriche e strutturali di tutti i cromosomi*

Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis

M. M. GIL*, M. S. QUEZADA*, R. REVELLO*, R. AKOLEKAR*† and K. H. NICOLAIDES*†

*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK; †Fetal Medicine Unit, Medway Maritime Hospital, Gillingham, Kent, UK

Ultrasound Obstet Gynecol 2015; 45: 249–266

Results *In total, 37 relevant studies were identified and these were used for the meta-analysis on the p mance of cfDNA testing in screening for aneupl 0.13% (95% CI, 0.07–0.20) for trisomy 18, 91.0% CI, 85.0–95.6%) and 0.13% (95% CI, 0.05–0. for trisomy 13, 90.3% (95% CI, 85.7–94.2% 0.23% (95% CI, 0.14–0.34%) for monosomy X and 93.0% (95% CI, 85.8–97.8%) and 0.14% (95 0.06–0.24%) for sex chromosome aneuploidies than monosomy X. For twin pregnancies, the D trisomy 21 was 93.7% (95% CI, 83.6–99.2%) a FPR was 0.23% (95% CI, 0.00–0.92%).*

TEST COMBINATO (T21)*:
< 35 aa - DR 75% FPR 5,0%
> 35 aa - DR 95% FPR 22,0%

- **T21 - DR 99,2% FPR 0,09%**
- **T18 - DR 96,3% FPR 0,13%**
- **T13 - DR 91,0% FPR 0,13%**

- **45,X - DR 90,3% FPR 0,23%**
- **47XXX, 47XXY, 47XYY : DR 93,0% FPR 0,14%**

GEMELLARI:
- **T21 - DR 93,7% FPR 0,23%**

*Malone, FD, Canick, JA, Ball, RH, et al. N Engl J Med 2005; 353:2001

Principali problemi nello screening con DNA fetale

- Fallimento dell'esame: 1-5% (frazione fetale insufficiente <4%)
  - ✓Età gestazionale < 10 settimane
  - ✓Prelievo errato
  - ✓Obesità materna (> 80 kg)
  - ✓Anomalie cromosomiche fetali (13-18, X0, Triploidie)
- Falso positivo:
  - ✓Mosaicismo placentare
  - ✓"Vanishing twin"
  - ✓Anomalie cromosomiche materne costituzionali o acquisite (mosaicismi, anomalie strutturali,neoplasie, trapianti, trasfusioni recenti...)
- Falso negativo:
  - ✓Mosaicismo placentare
  - ✓Frazione fetale borderline

Diagnosi Prenatale: Quale Futuro?

