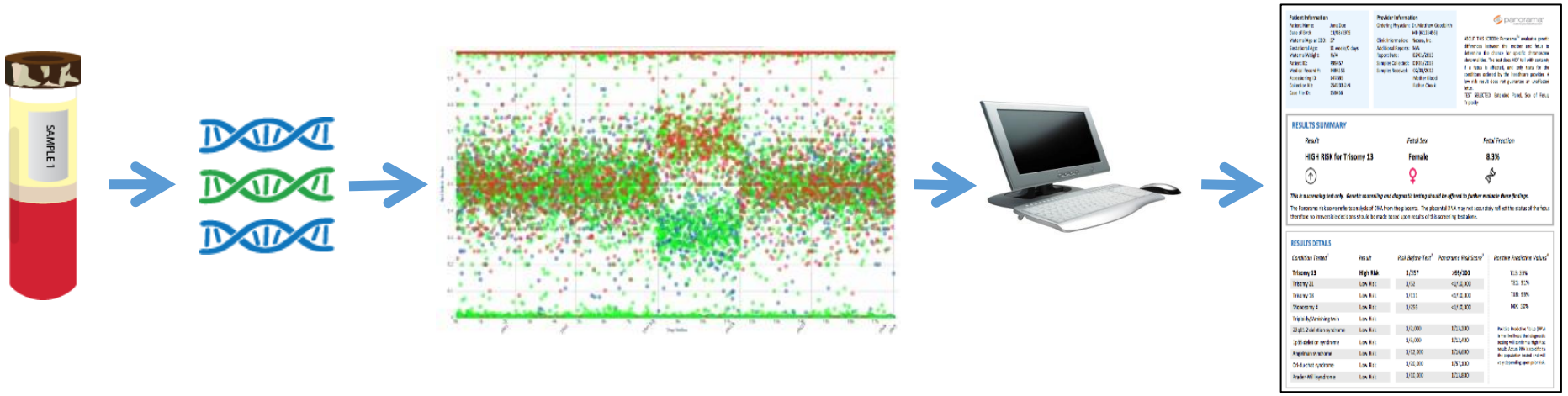
NIPT Methodologies – All NIPTs are not the same

Counting	SNP
harmony PRENATAL TEST	panorama® natera prenatal screen
MaterniT21® PLUS	
Counsyl	
informaSeq℠ non-invasive prenatal test	
verifi™ prenatal test	
BambniTest	
NIFTY® Non-Invasive Fetal TrisomY test	
QNatal™ Advanced NIPS	

SNP = Single Nucleotide Polymorphism

- A DNA sequence variation occurring when a single base pair is changed
- Normal genetic changes that occur in every person
- Panorama® analyzes more than 13,000 SNPs

Proprietary SNP analysis distinguishes between maternal & fetal DNA



Panorama® uniquely differentiates between maternal and fetal DNA

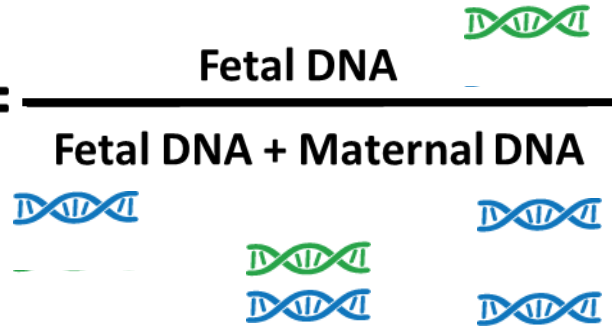
Fetal fraction

Fetal sex accuracy

Triploidy/complete mole

What is fetal fraction?

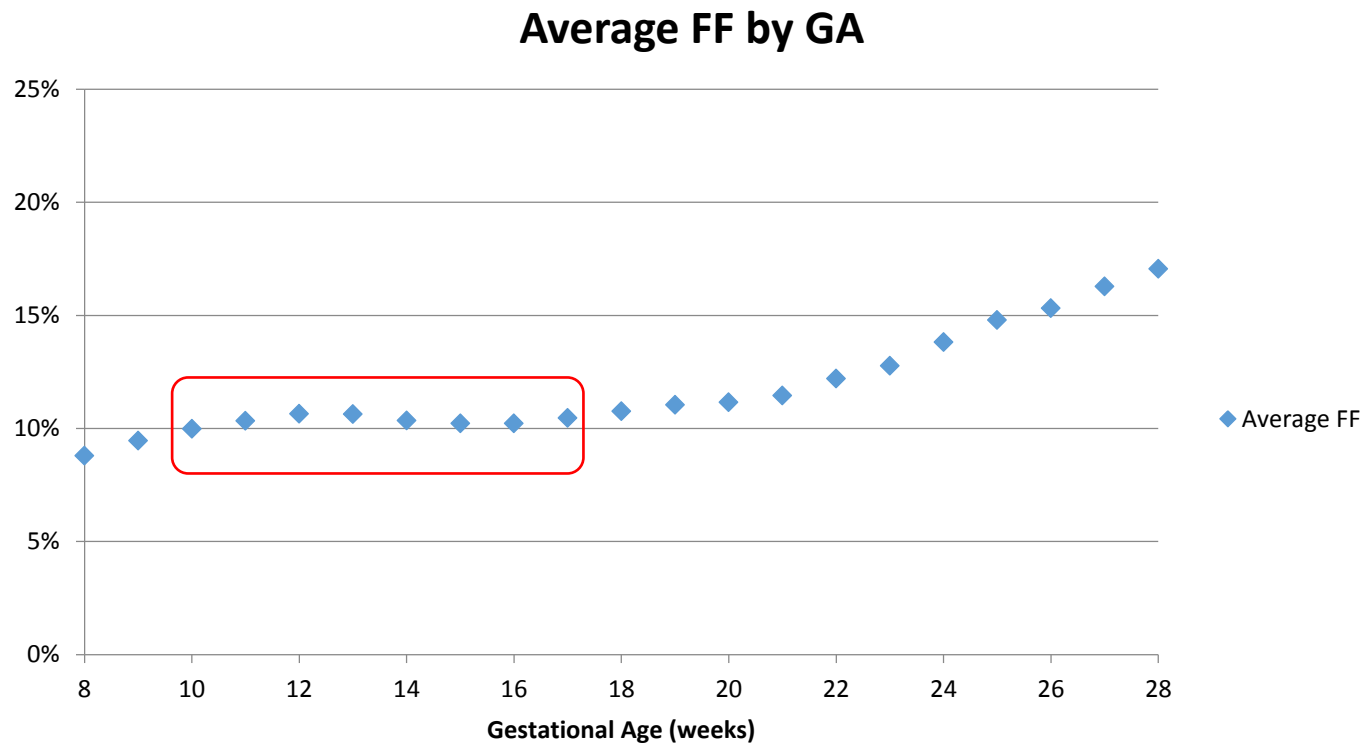
$$\text{Fetal Fraction} = \frac{\text{Fetal DNA}}{\text{Fetal DNA + Maternal DNA}}$$



- Average fetal fraction between 10 and 22 weeks gestation is 10-12%.

"…the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results."[1]

[1]Takoudes et al., *Ultrasound Obstet Gynecol* 2015; 45: 112–116



[1] Dar P et al. Am J Obstet Gynecol 2014 Nov;211(5):527

- Why is fetal fraction so important? – two examples

Example 1

- Samples from two non-pregnant women were sent to five commercial NIPT providers

NIPT results for two non-pregnant women from five commercial laboratories

Lab	Test result available	Details
A	No	Insufficient fetal cfDNA for accurate NIPT evaluation
B	No	Unable to report due to low fetal fraction (fetal fraction reported as 0.6% for both)
C	Yes	Negative, consistent with female fetus (fetal fraction 4.3% reported on request for patient 1, and 3.9% for patient 2)
D	Yes	No aneuploidy detected, two sex chromosomes (XX)
E	Yes	No aneuploidy detected, two sex chromosomes (XX)

Takoudes et al., *Ultrasound Obstet Gynecol* 2015; 45: 112–116

Lessons on NIPT performance in the absence of fetal DNA

- **Natera correctly reported insufficient fetal DNA and did snot provide results.**
- Three MPSS-based laboratories provided results consistent with a *normal female*.
- Fetal fraction matters:
  - One lab reported 4.3% and 3.9% fetal fractions. How accurate is their method for calculating fetal fraction?
  - The other two labs do not measure fetal fraction

"…the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results."[1]

[1]Takoudes et al., *Ultrasound Obstet Gynecol* 2015; 45: 112–116

Example 2: A case study of false negative NIPT

- 35 year old G1P0; 6.9mm NT at 11+ wks gest
- FTS result: high risk for T21 and T18/13
- Follow up by counting NIPT: “No aneuploidy detected” Fetal fraction not analyzed at this lab.
- Amnio elected after abnormal U/S: 47, XY +21
- Retrospective analysis by the NIPT laboratory of the maternal blood sample revealed **low fetal fraction: 1.7%**

Allen R., Kezmarsky P., Lescale K., False Negative NIPT and Potential Implications for Genetic Counseling; (Abstract #47). Presented at the 2013 ACMG Annual Clinical Genetics Meeting, 03/22/2013, Phoenix, AZ

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A key determinant of the reliability of aneuploidy NIPT is the fetal DNA fraction in maternal plasma.

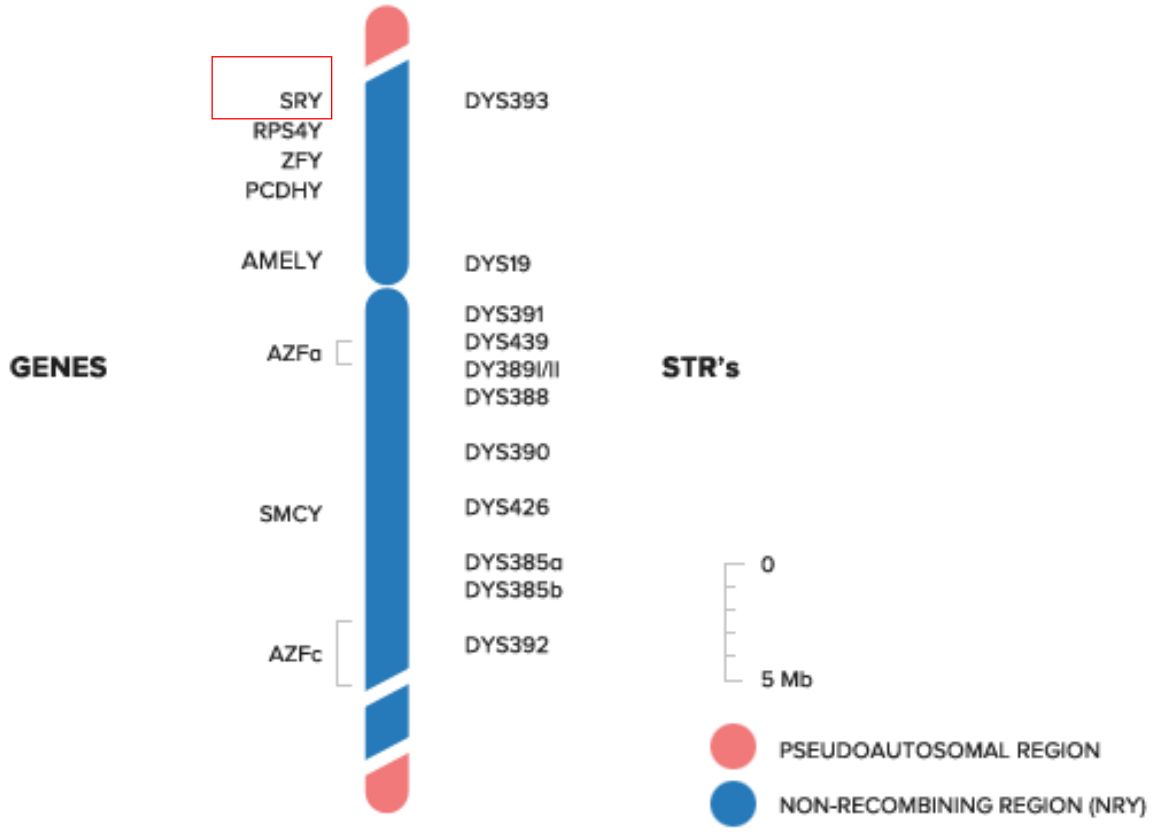
Hudecova I, Sahota D, Heung MMS, Jin Y, Lee WS, Leung TY, et al. (2014) Maternal Plasma Fetal DNA Fractions in Pregnancies with Low and High Risks for Fetal Chromosomal Aneuploidies. PLoS ONE 9(2): e88484.

- How is fetal fraction measured?

Methods of quantifying fetal fraction

- Look for Y-specific markers
- fetal specific methylation markers
- Measure alleles inherited from the father that are absent in the maternal genome

Y-specific markers



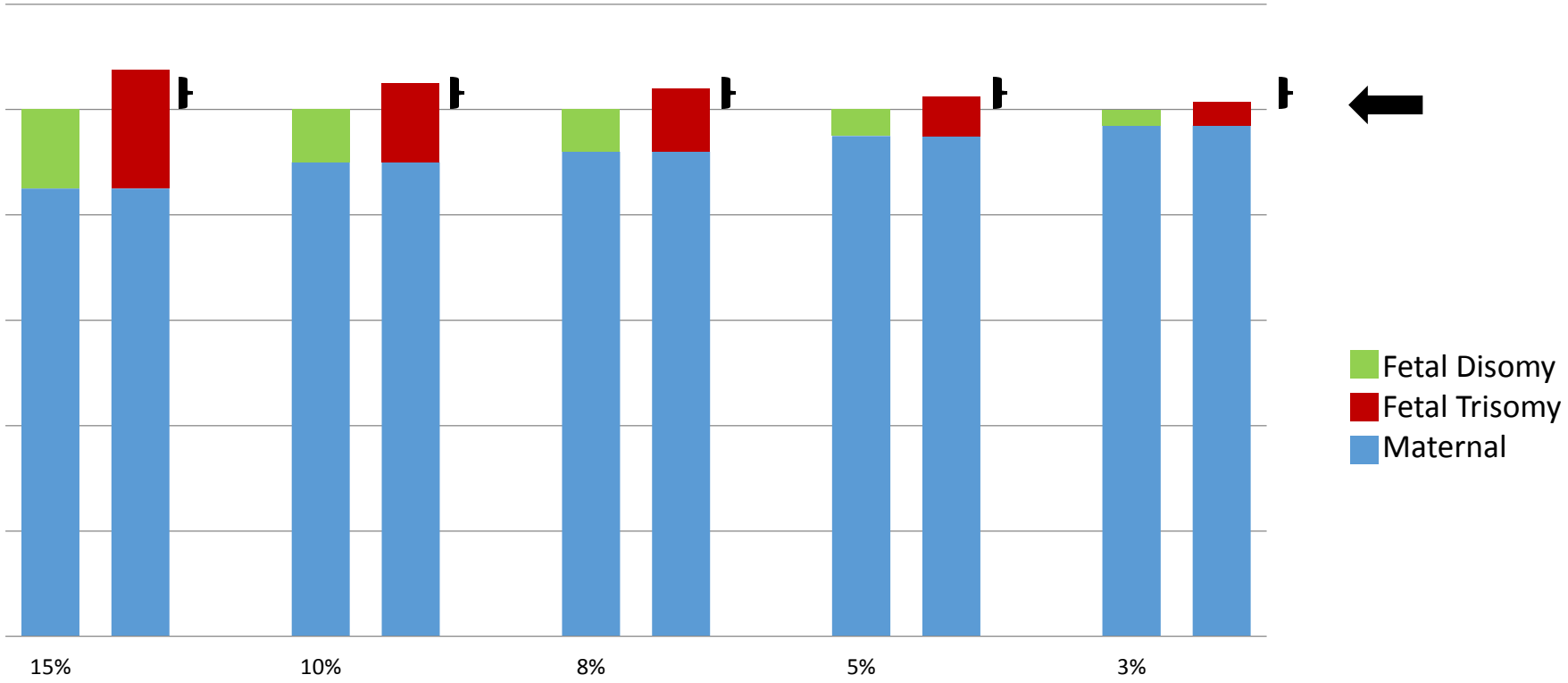
https://genographic.nationalgeographic.com

Methylation

- In certain regions, fetal cfDNA is methylated but maternal cfDNA is unmethylated, so methylation sensitive restriction enzymes are used to eliminate maternal DNA and quantify the fetal contribution

How fetal fraction affects NIPT performance

How Counting is Affected by Fetal Fraction



"Fraction of cfDNA that is fetal is a key component, with trisomy becoming easier to detect at higher fetal fractions" (Norton, et al. 2013)

"Excess maternal DNA could lower the sensitivity of the test" (Futch, et al 2013)

Low Fetal Fraction and Aneuploidy

- Studies show **increased incidence of aneuploidy** with low fetal fractions.[1,2]
- "…data suggests that this "no call" group is at increased risk…Diagnostic testing and/or repeat cfDNA should be strongly considered for such patients."[3]

[1]Pergament E, et al. Obstet Gynecol. 2014 Aug;124(2 Pt 1):210-8
[2]Norton M, et al. N Engl J Med. 2015 DOI: 10.1056/NEJMoa1407349
[3]ACOG Practice Advisory of Cell-Free DNA Screening. April 2, 2015.

Take-home messages – Fetal Fraction

- Fetal fraction is the proportion of fetal DNA present in a cf DNA sample from maternal serum
- At low fetal fractions it becomes more difficult to give accurate aneuploidy results
- Not measuring fetal fraction means you cannot be sure you are assessing fetal DNA!

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

Mary E. Norton, M.D., Bo Jacobsson, M.D., Ph.D., Geeta K. Swamy, M.D., Louise C. Laurent, M.D., Ph.D., Angela C. Ranzini, M.D., Herb Brar, M.D., Mark W. Tomlinson, M.D., Leonardo Pereira, M.D., M.C.R., Jean L. Spitz, M.P.H., Desiree Hollemon, M.S.N., M.P.H., Howard Cuckle, D.Phil., M.B.A., Thomas J. Musci, M.D., and Ronald J. Wapner, M.D.

N Engl J Med. 2015 DOI: 10.1056/NEJMoa1407349

Fetal Fraksyon Calismasi

- 15,000 gebe hasta ustunde
- FF’in onemi

Fetal Fraction	% Women
< 4%	1.2%
Undetectable	0.5%
High Variance or Assay Failure	1.3%
Total	3.0%

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

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True FF (%)	*Frequency (%)*	
0.0–0.9	0.00	0.87%
1.0–1.9	0.00	
2.0–2.9	0.05	
3.0–3.9	0.32	
4.0–4.9	1.08	15.2%
5.0–5.9	2.55	
6.0–6.9	4.62	
7.0–7.9	6.94	
8.0–8.9	9.02	
9.0–9.9	10.44	
≥ 10.0	64.98	
All	100.0	
FF ≥ 4%	99.63	

A unified approach to risk assessment for fetal aneuploidies

D. WRIGHT*, A. WRIGHT* and K. H. NICOLAIDES† *Ultrasound Obstet Gynecol* 2015; 45: 48–54

Panorama NIPT – Clear & Concise Reporting

- ≈ 90% of increased risk results achieve high confidence with algorithm
- Yani PPV

Clinical experience and follow-up with large scale single-nucleotide polymorphism–based noninvasive prenatal aneuploidy testing

Pe'er Dar, MD; Kirsten J. Curnow, PhD; Susan J. Gross, MD; Megan P. Hall, PhD; Melissa Stosic, MS; Zachary Demko, PhD; Bernhard Zimmermann, PhD; Matthew Hill, PhD; Styrmir Sigurjonsson, PhD; Allison Ryan, PhD; Milena Banjevic, PhD; Paula L. Kolacki, MS; Susan W. Koch, MS; Charles M. Strom, MD, PhD; Matthew Rabinowitz, PhD; Peter Benn, DSc

Am J Obstet Gynecol 2014;211:x.ex-x.ex.

Dar (Panorama) study vs Next study (Harmony)

	Dar study	Next study
Hasta sayisi	Yaklasik 32,000	Yaklasik 15,000
T21 PPV	% 90	% 80

panorama®
natera prenatal screen

ABOUT THIS SCREEN: Panorama™ is a screening test, not diagnostic. It evaluates genetic information in the maternal blood, which is a mixture of maternal and placental DNA, to determine the chance for specific chromosome abnormalities. The test does NOT tell with certainty if a fetus is affected, and only tests for the conditions ordered by the healthcare provider. A low risk result does not guarantee an unaffected fetus.

TEST SELECTED: Sex of Fetus, Triploidy

Positive Predictive Values[4]

T21: 91%

T18: 93%

T13: 38%

MX: 50%

Positive Predictive Value (PPV) is the likelihood that diagnostic testing will confirm a High Risk result. PPV provided is NOT personalized for this patient, but calculated from a published study of 17,885 women. PPV for an individual specimen will vary based on prior risk.

This is a screening test only. Genetic counseling and diagnostic testing should be offered to further evaluate these findings.

The Panorama risk score reflects analysis of DNA from the placenta. The placental DNA may not accurately reflect the status of the fetus; therefore, no irreversible decisions should be made based upon results of this screening test alone.

Professional Society Guidelines on NIPT

Organization	Policy	Date
ACOG	"any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risk status, the patient choosing this testing should understand the limitations and benefits of this screening paradigm in the context of alternative screening and diagnostic options."	2015
ASHG/ESHG	"Different scenarios are possible, including NIPT as an alternative first tier option"	2015
ACMG	"ACMG recommendation that NIPS can be used as a first line screening tool."	Webinar 2015
ISPD	"The following protocol options are currently considered appropriate: 1. cfDNA screening as a primary test offered to all pregnant women."	2015

Incorporating NIPT into Clinical Practice

Let’s Practice!

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Case #1

- 36 year old G2P1
- cfDNA 13 weeks high risk Trisomy 21
- Female

- Ultrasound 20 weeks unremarkable

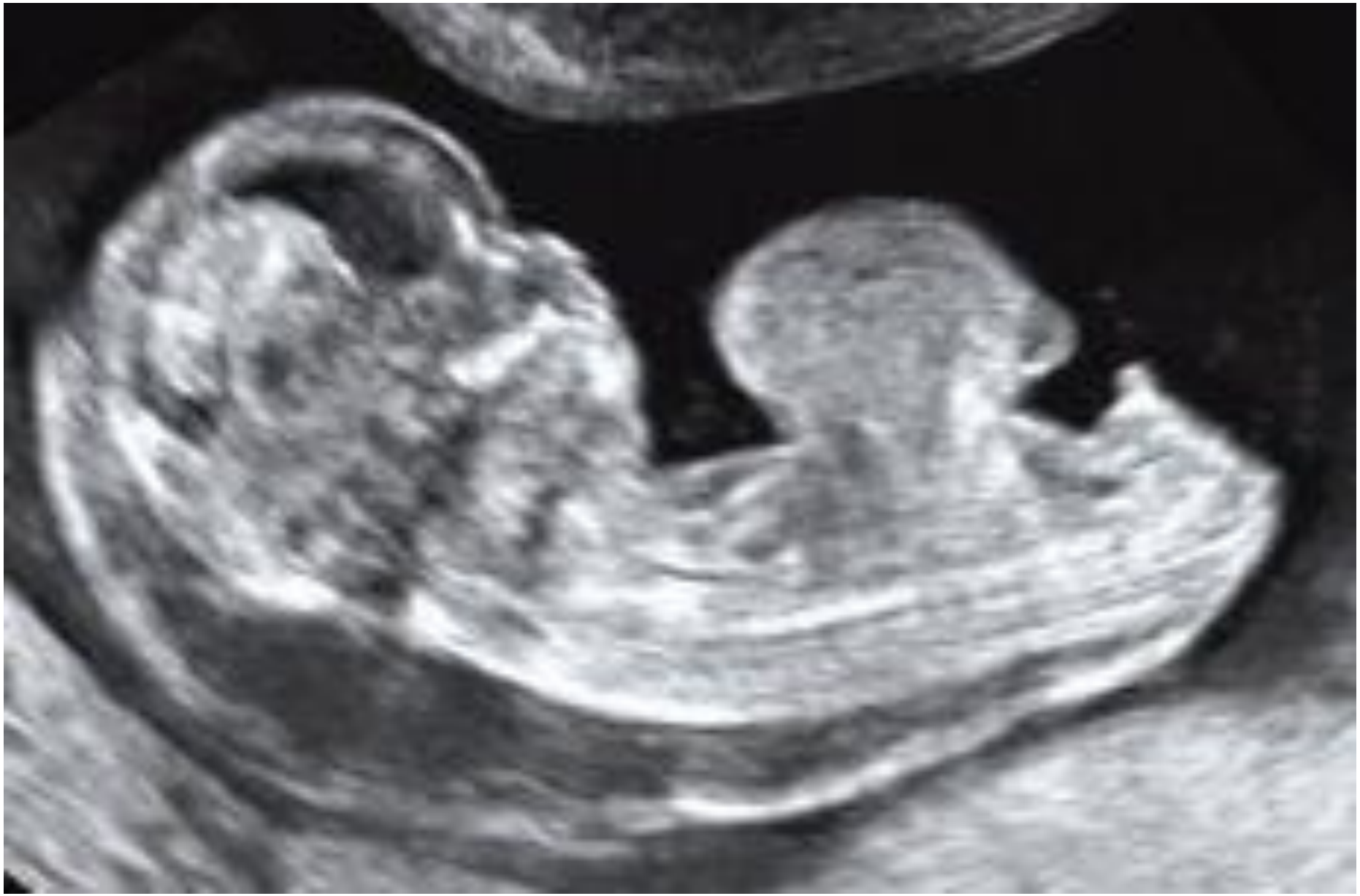
- Amniocentesis ; Trisomy 21

Case #1

- Yuksek sensitivite, PPV

- Testin teknik limitasyonu olmamali

Case # 2

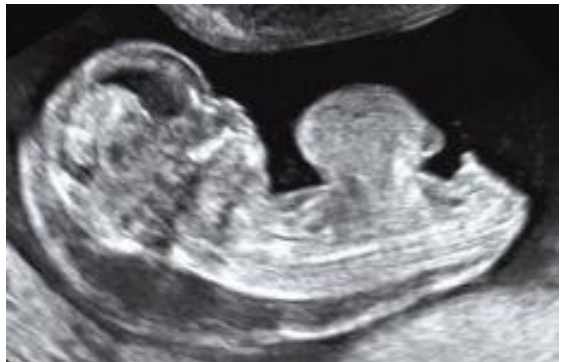


Case # 2

- Counseled increased risk of chromosome abnormalities & offered cfDNA
- FF 10.5%
- Trisomy 18 risk

- Preterm labor & delivery at 29 weeks
- Obvious features of Trisomy 18
- Expired 35 hours

- Postmortem kromozom; T18



Case #2

Sonucta tarama testi

Diger taramalara gore dogruluk orani en yuksek

Fakat gene tani; diyagnostik test onerilmeli

A Major Malformation Requires A Diagnostic Test!!

Case #3

- 36 year old G3P2
- Panorama at 10 weeks: fetal fraction less than 1%
- What Next?

What are the causes of low Fetal Fraction?

- Is She Pregnant?
- Technical Limitations (draw technique, transport, ??)
- Maternal Obesity
- Chromosome Abnormality (13, 45X, 18, triploidy, 21?)

- Redraw Panorama @ 12 weeks: fetal fraction 1.0%
- Counting NIPT @ 14 weeks: normal female fetus

COMMITTEE OPINION

Number 640 • September 2015 *(This Committee Opinion Replaces Committee Opinion Number 545)*

Committee on Genetics
Society for Maternal–Fetal Medicine

Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Although repeat screening can be performed, it may delay the diagnosis of aneuploidy, potentially limiting reproductive options, and only 50–60% of repeat screens will provide a result (14, 20).

PANORAMA

Fetal fraksyon yonteminin yarari

Tesekkur ederim