Genetic Testing and Screening

Part 1 – Fetal Aneuploidy and Birth Defects

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OUTLINE:

1. Screening vs. diagnostic testing
2. Aneuploidy screening
    a. Maternal age
    b. 1st and 2nd trimester (quad) screens
    c. AFP
    d. Ultrasound
    e. Cell free fetal DNA (aka noninvasive prenatal testing (NIPT))
3. Diagnostic testing
    a. Chorionic villus sampling (CVS)
    b. Amniocentesis

OBJECTIVES: After studying these lectures you should be able to:

1. Compare and contrast characteristics of “screening” versus “diagnostic” testing.
2. Outline key components of NIPT, first and second trimester screening including timing, elements used to assess risk and interpretation of results.
3. Describe diagnostic testing including timing, technique and specimen obtained.
4. Discuss the use of ultrasound in the second trimester as a screening tool.
5. Recognize the most appropriate testing option based on indication and patient goals.

READING REFERENCE:

N/A

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Laura Bulmer, MS, CGC

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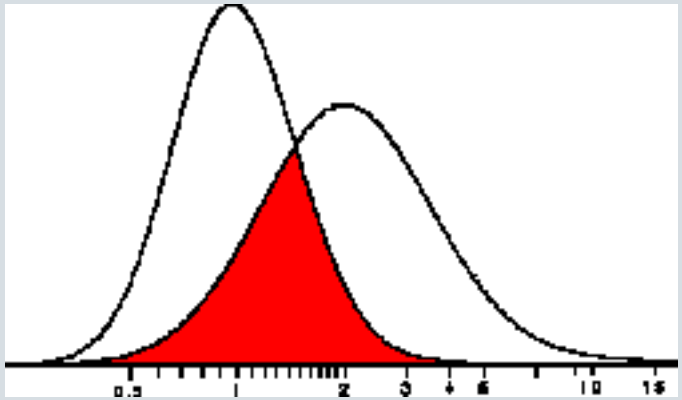
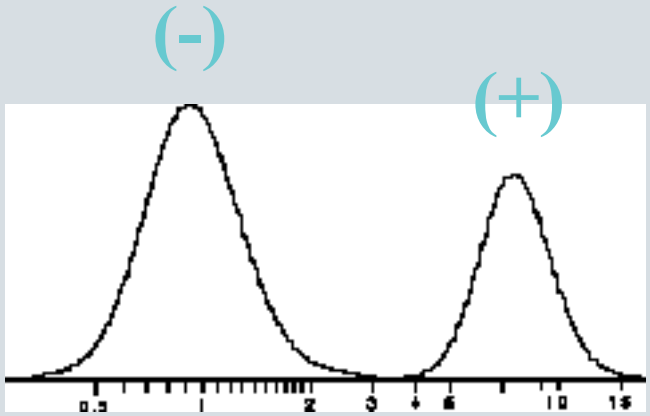
Screening Vs. Diagnostic Defined

Screening tests	Diagnostic tests
Tests carried out to identify, from a population of apparently healthy individuals, those at increased risk for a specific disorder as to justify a subsequent diagnostic procedure	Tests used to confirm or exclude whether an individual fetus is affected by a specific disorder

Screening vs. Diagnostic

Screening	Diagnostic
	

Screening vs. Diagnostic

Screening	Diagnostic
•Cost effective •Easy to perform •Non-invasive •Able to define at-risk population •No definitive answers or diagnosis •Reliable	•Expensive •Invasive •Generally for at-risk population •Provides definitive answers/diagnosis

Screening Tests

- Maternal Age
- 1st trimester combined screening
- Second trimester screening (AKA Quad Screening)
- AFP only (single marker)
- Cell free DNA testing (AKA non-invasive prenatal testing, NIPT)
- Ultrasound

Screening – Maternal Age

“Advanced Maternal Age” “Elderly Gravida”

- Increased risk of fetal aneuploidy
  - Down syndrome, trisomy 13, trisomy 18, 47,XXX, 47,XXY, any trisomy
- 35 or older at delivery (singleton pregnancies)
  - 33 or older for multiples
- Why 35?
  - Age at which the rate of aneuploidy = the miscarriage rate associated with a diagnostic procedure (amniocentesis/CVS)
- Detection: 30%
  - Using age alone is a bad/ineffective screening tool

Maternal Age and Risk for Chromosome Abnormalities

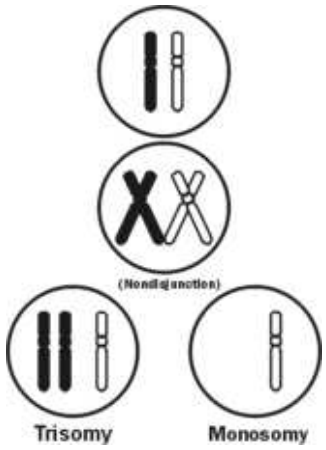
Low Risk

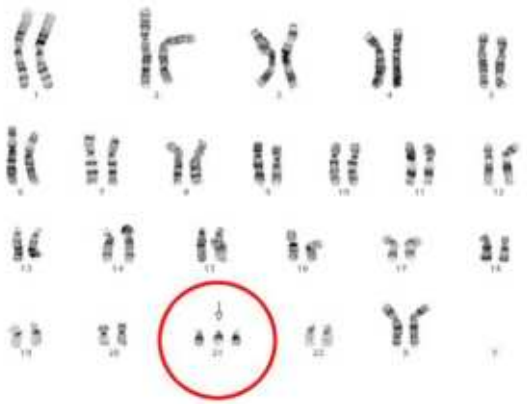
High Risk

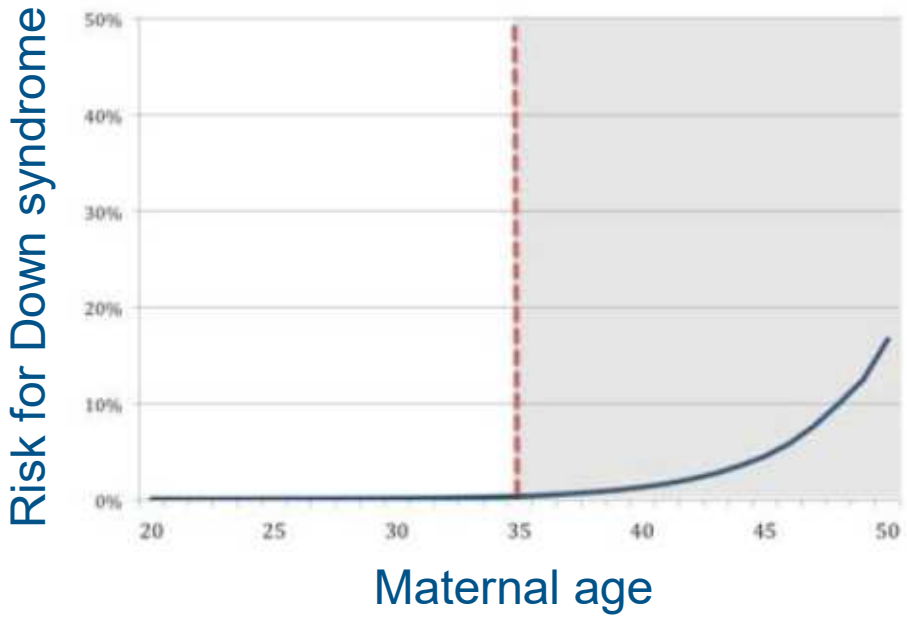
	First Trimester		Second Trimester		Live-birth	
Maternal Age	Down Syndrome	All	Down Syndrome	All	Down Syndrome	All
25	1 in 616		1 in 906		1 in 1250	1 in 476
26	1 in 586		1 in 863		1 in 1190	1 in 476
27	1 in 551		1 in 811		1 in 1111	1 in 455
28	1 in 510		1 in 751		1 in 1031	1 in 435
29	1 in 464		1 in 683		1 in 935	1 in 417
30	1 in 415		1 in 610		1 in 840	1 in 385
31	1 in 363		1 in 535		1 in 741	1 in 385
32	1 in 311		1 in 459		1 in 637	1 in 323
33	1 in 262		1 in 386		1 in 535	1 in 286
34	1 in 216		1 in 318		1 in 441	1 in 244
35	1 in 238	1 in 114	1 in 256	1 in 141	1 in 356	1 in 179
36	1 in 175	1 in 87	1 in 200	1 in 111	1 in 281	1 in 149
37	1 in 133	1 in 66	1 in 156	1 in 88	1 in 217	1 in 123
38	1 in 100	1 in 51	1 in 123	1 in 70	1 in 166	1 in 105
39	1 in 75	1 in 38	1 in 96	1 in 56	1 in 125	1 in 81
40	1 in 56	1 in 29	1 in 75	1 in 44	1 in 94	1 in 63
41	1 in 42	1 in 22	1 in 59	1 in 35	1 in 70	1 in 49
42	1 in 32	1 in 17	1 in 46	1 in 28	1 in 52	1 in 39
43	1 in 24	1 in 13	1 in 36	1 in 22	1 in 40	1 in 31
44	1 in 18	1 in 10	1 in 28	1 in 18	1 in 30	1 in 24
45	1 in 13	1 in 8	1 in 22	1 in 14	1 in 24	1 in 19
46	1 in 10	1 in 6	1 in 17	1 in 11	1 in 19	1 in 15
47	1 in 7	1 in 4	1 in13	1 in 9	1 in 16	1 in 11
48	1 in 6	1 in 3	1 in 11	1 in 7	1 in 14	1 in 9
49			1 in 8	1 in 6	1 in 13	1 in 7

Adapted from published tables referenced on the back of this page. Numbers do not include mosaicism, translocations, and marker chromosomes

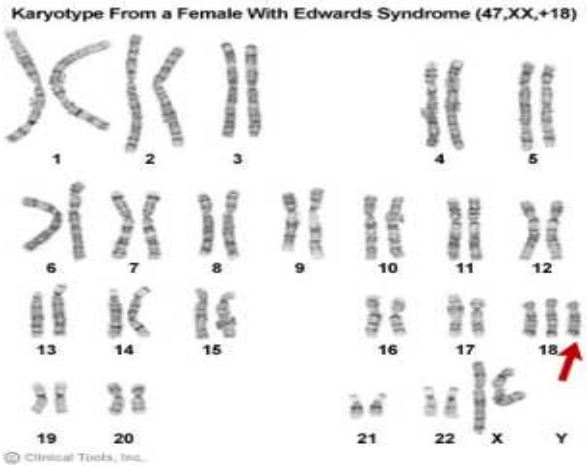
Trisomy 21 (Down syndrome)

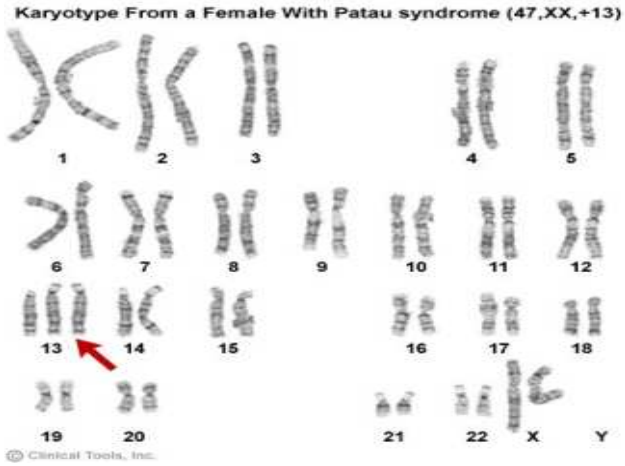




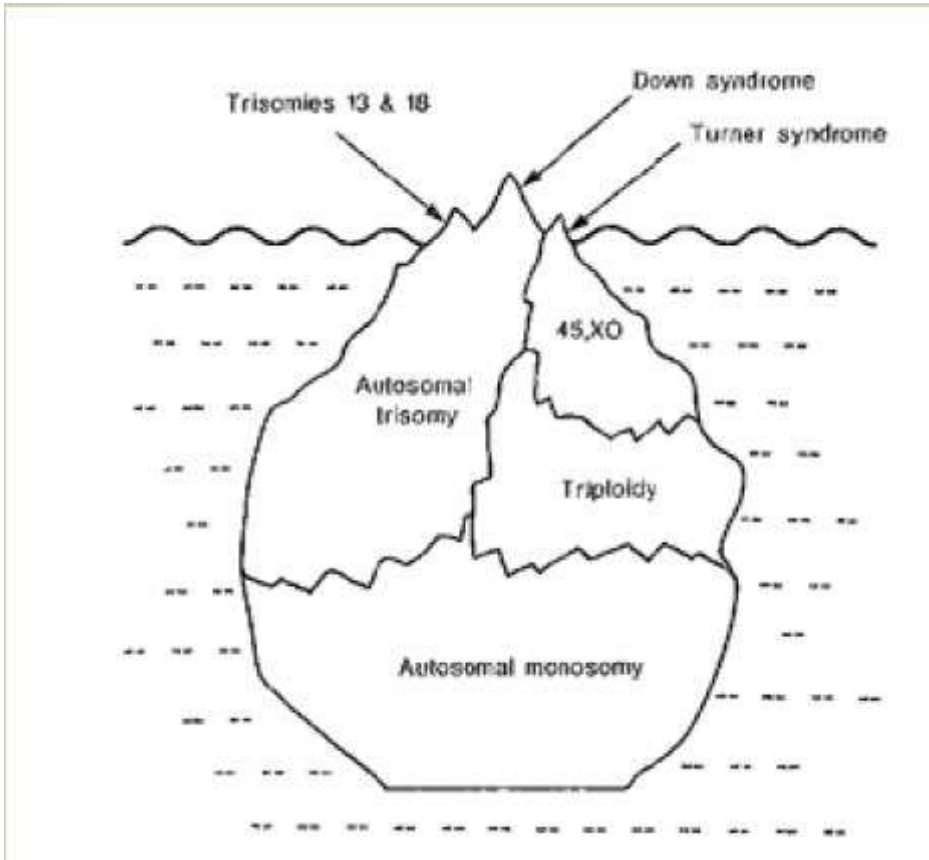


Trisomy 18, trisomy 13





Chromosomal Iceberg



Screening- First Trimester Screen

- Performed between 11 0/7 - 13 6/7 weeks gestation
- Two components measured
  - Ultrasound (Nuchal translucency measurement)
  - Biochemical analysis (hCG and PAPP-A)
- Disorders screened
  - Down syndrome
  - Trisomy 13
  - Trisomy 18

First trimester screen- NT

First trimester Screen Interpretation

	Down Syndrome	Tri 13/Tri 18
NT	↑	↑
hCG	↑	↓
PAPP-A	↓	↓

Nuchal translucency (NT)

- Increased NT measurement
  - increases risk of chromosome abnormality, heart defect, and some genetic conditions
- NT 3.5mm +
  - automatic “screen positive” result

Biochemical analysis

- hCG
  - Increased in babies with Down syndrome
  - decreased in babies with Tri13/Tri18
- PAPP-A
  - decreased in babies with Down syndrome, Tri 13, and Tri 18

First trimester screen

- Detection
  - First trimester combined screening
    - Down syndrome: 85%
    - Trisomy 13/Trisomy 18: 90-95%
- 5% positive rate; most are “false positives”
- Provides a “1 in X” numerical risk assessment, not a “yes” or “no”
  - Risk greater than the average 35-year-old’s risk is “positive”
- Benefits of first trimester screening
  - Earlier result- allows more time for follow up options
  - Better detection rates than second trimester serum screening

Sample FTS Report

Notes:

- MoM= multiple of the median
- Expected analyte values change with gestational age
- You do not need to know “normal” values, just high or low (e.g. 4.2 MoM is high, 0.3 MoM is low)

Sono Supervisor: CHANG Smoker: NO

Marker/Analyte	Value	MoM/Delta	Percentile
Free Beta hCG	76.24 ng/ml	1.49 MoM	70
PAPP-A	0.86 mIU/ml	0.80 MoM	40
NT	1.9 mm	+0.32 Delta	75
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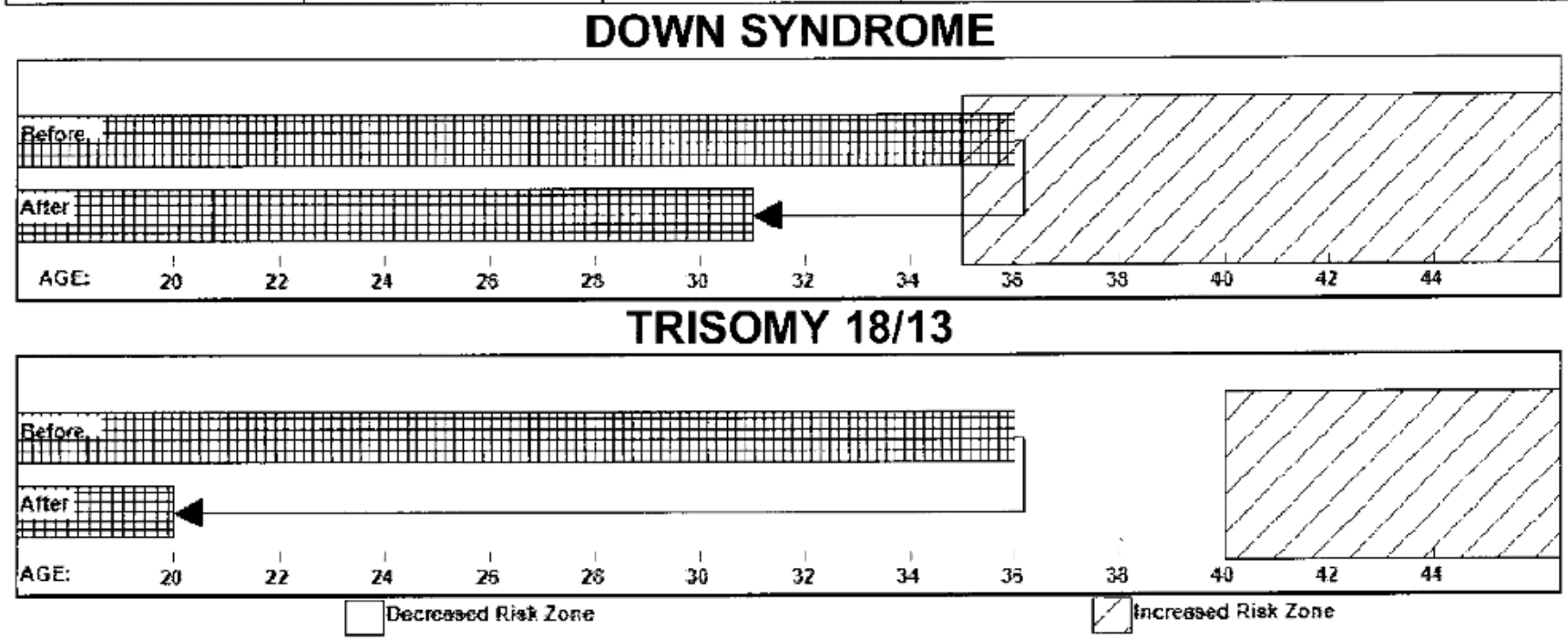
RISK TABLE	1st Trimester Cut-Off	Risk Before Screening	Risk After Screening	Results
DOWN SYNDROME	1 in 307	1 in 244	1 in 591	**WITHIN RANGE**
TRISOMY 18/13	1 in 150	1 in 443	1 in 8,841	**WITHIN RANGE**



Second trimester screen

AKA: multiple marker, maternal serum screening, quad screening

15 weeks 0 days to 21 weeks 6 days

Analysis of 4 analytes detected in maternal serum during pregnancy

- AFP – produced by baby's liver
- hCG – produced by placenta
- uE3 – produced by placenta and baby's liver
- DIA – produced by placenta

Conditions screened via this method

- Neural tube defects
- Down syndrome
- Trisomy 18 (not trisomy 13)

Second trimester screen

Interpretation

	Down Syndrome	Trisomy 18	ONTD
AFP	↓	↓	↑
hCG	↑	↓	x
uE3	↓	↓	x
DIA	↑	x	x

Second trimester screen example

- AFP 0.37 MoM
- hCG 3.74 MoM
- UE3 0.59 MoM
- DIA 2.08 MoM

Example values only – much less than 1 = low, much > 1 = high, greater deviance from 1 will have greater impact on risk assessment

Patterns associated with Down syndrome

- **AFP ↓**
- **hCG ↑**
- **UE3 ↓**
- **DIA ↑**

Know patterns, not numbers!

"Multiple of the Median" – ratio of measured analyte to expected quantity for gestational age and other factors

Second trimester screen

Detection

- Down Syndrome: 80%
- Trisomy 18: 60%
- ONTD: 80%

5% false positive rate

Interpretation

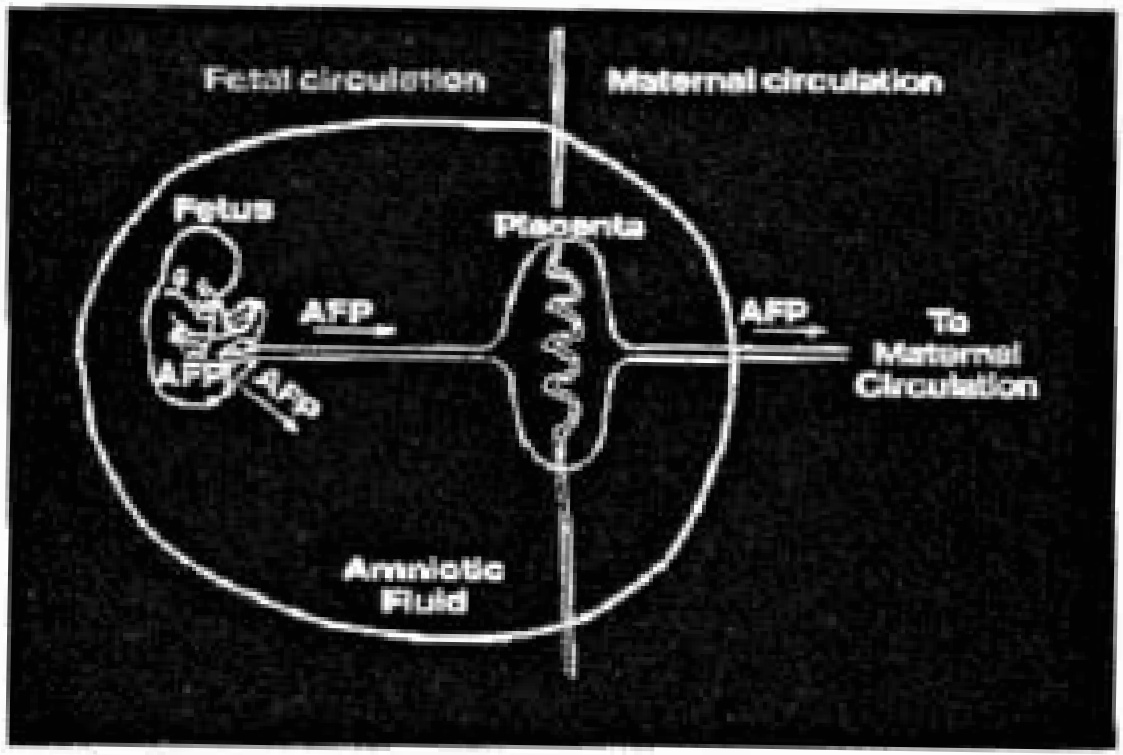
- Risk assessment; 1 in X chance, not a “yes” or “no”
- Cut offs for screen positive result
  - Down syndrome: 1 in 270 (same age related risk of a 35 year old)
  - Trisomy 18: 1 in 100
  - Neural tube defect: 2.5 MoM +

Screening- AFP alone

AFP (alpha fetoprotein)

- Protein first produced by the yolk sac then the fetal GI tract and liver
- Can be detected in maternal blood for analysis of risk for
  - Neural tube defects (~1 in 700 background risk)
  - Abdominal wall defects
  - Down syndrome, trisomy 18
  - Some genetic conditions (Finnish nephrosis)
  - Unexplained elevated AFP → increased incidence of placental insufficiency (IUGR, oligohydramnios, IUFD), placenta abruption, and gestational hypertensive disorders

Screening- AFP



Screening- Factors that influence screening

Gestational age:

- Expected amount of analytes change throughout pregnancy

Maternal Age:

- Starting aneuploidy risk is factored in for FTS and quad screen

Maternal Weight:

› Increased maternal weight = greater blood volume (dilutes analytes)

Race:

› AFP is 10-15% HIGHER in African Americans than Caucasians

IDDM:

› Diabetics have LOWER levels of AFP on average

› Diabetics have HIGHER risk of ONTD

Multiple gestations

Screening- What causes high MS-AFP?

Incorrect dating

- AFP levels increase naturally through course of pregnancy

Placental insufficiency

- Normal fetus but placenta not functioning properly

Birth defect

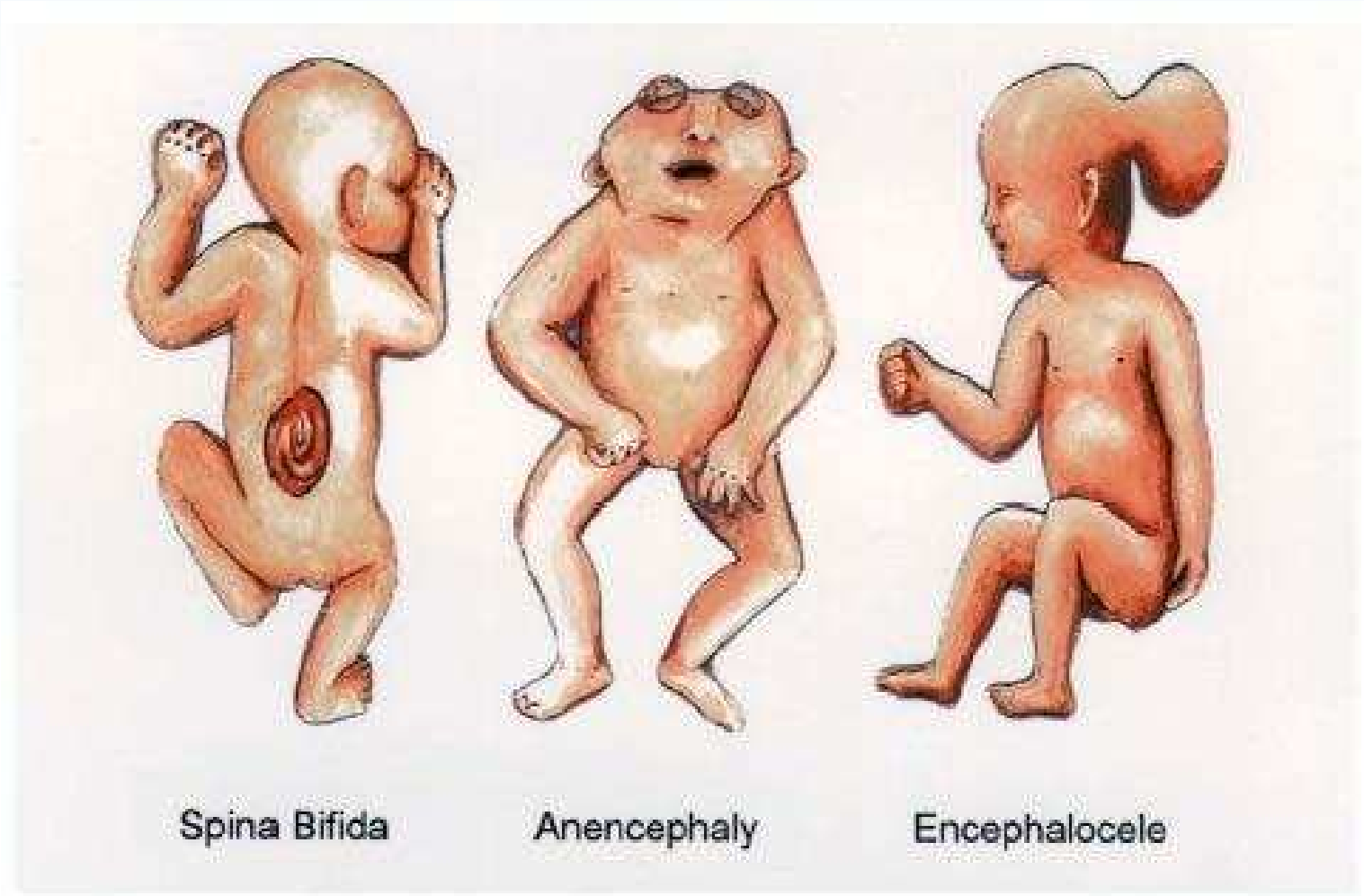
- Neural tube defects, abdominal wall defects
- opening in the baby's body = additional place for AFP to leak out from the baby's body into amniotic fluid and ultimately into maternal circulation.

Low amniotic fluid

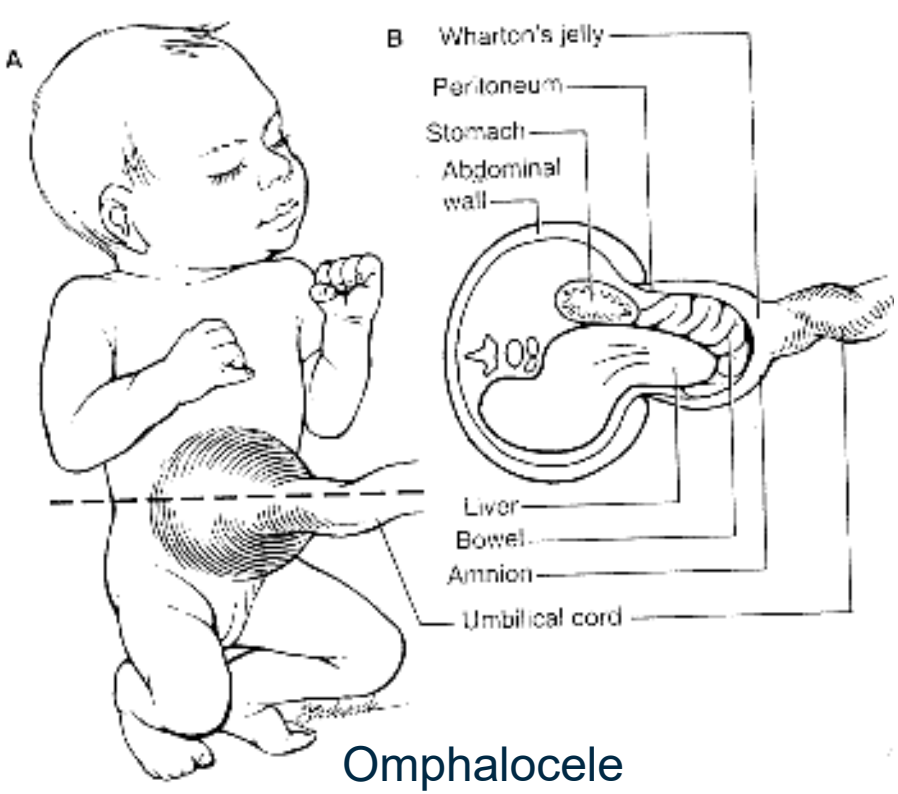
- AFP more concentrated

Normal variation

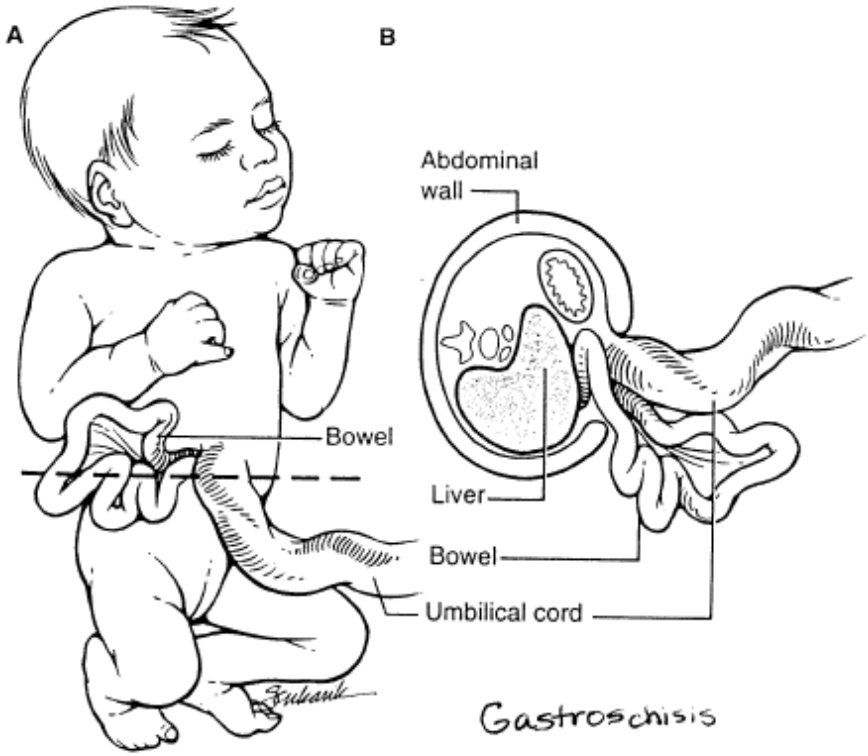
Screening – Neural tube defects



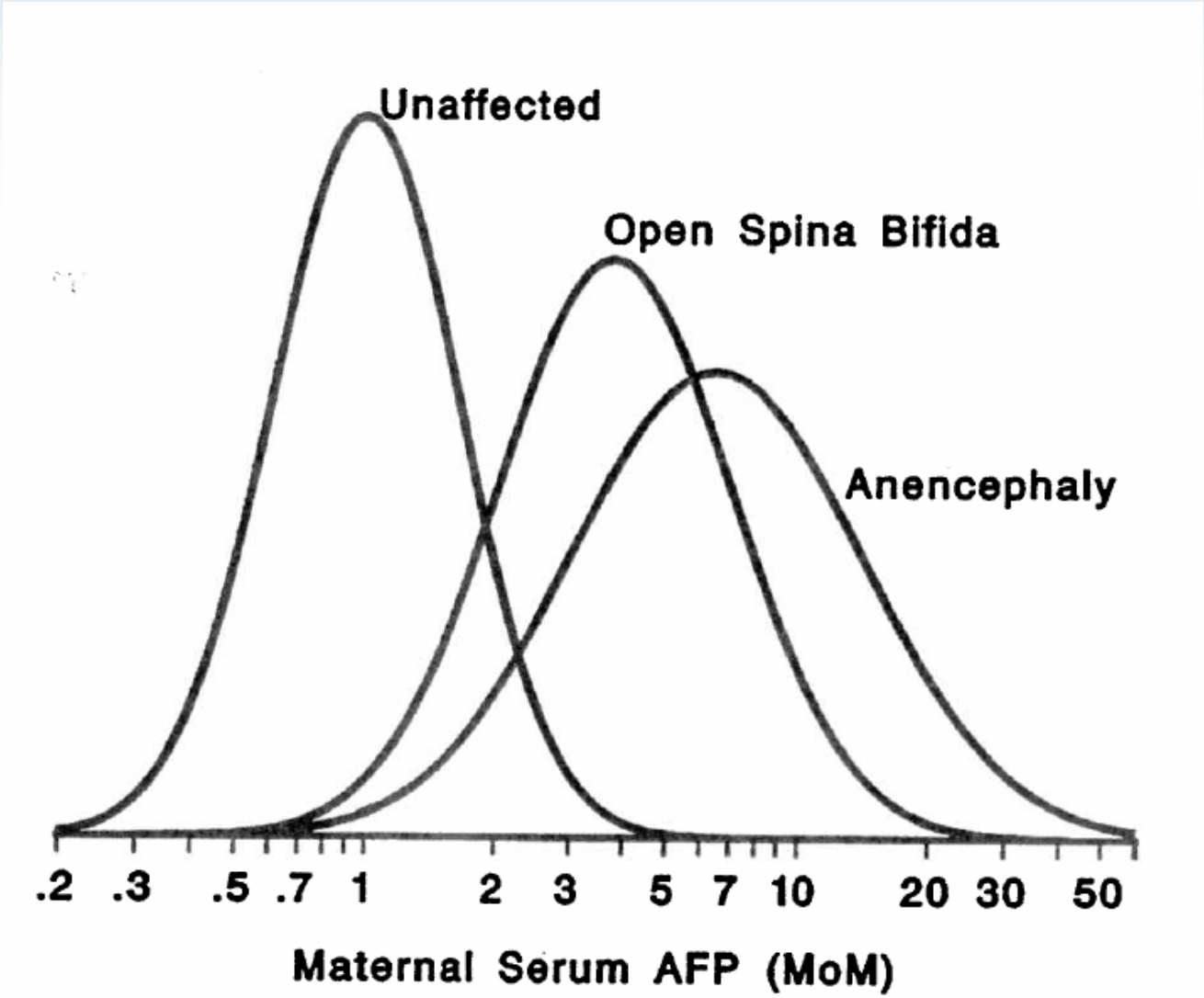
Screening – Abdominal wall defects



Omphalocele



Screening- AFP & birth defects



Second trimester ultrasound

Screening tool used to detect

- birth defects
- soft markers

Performed at ~20 weeks gestation

Birth defects- structural malformations of the baby

- some birth defects associated with an increased risk of chromosome abnormalities

Soft markers- not birth defects or malformations but features associated with an increased risk of chromosome abnormality

- 60% with Down syndrome have ultrasound markers
- >95% with trisomy 18/13 have ultrasound abnormalities

Ultrasound birth defects

Congenital heart defects

- Increased risk for chromosome abnormalities and other genetic syndromes (e.g. 22q-, Noonan)

Duodenal atresia (“double bubble”)

- 30-40% risk of Down syndrome

Omphalocele

- 30-50% risk of chromosome abnormality
- (Gastroschisis is typically sporadic)

Other birth defects can also be associated with chromosome problems

- Neural tube defects, facial clefts, limb abnormalities, etc.

Ultrasound Markers/Soft Markers

Variations in normal anatomy

- ~5% general population have marker(s)

In isolation, unlikely to be clinically significant

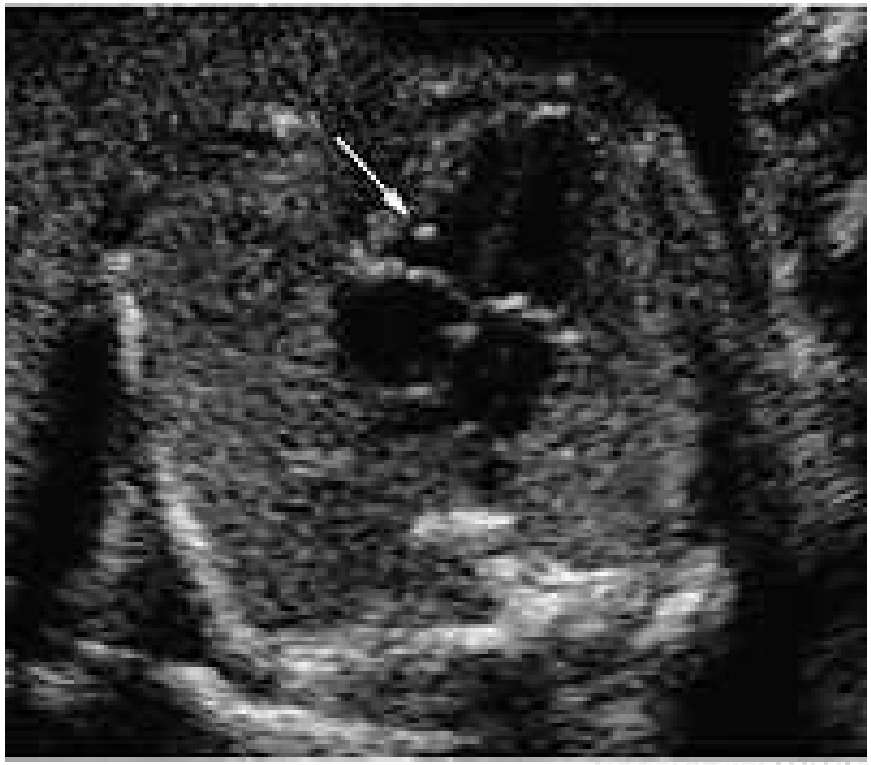
Likelihood ratios available to calculate risk

Ultrasound Soft Markers

Soft Marker	Associated risk
Thickened nuchal fold	11 fold ↑ risk for DS
Short humerus to head circumference ratio	5.1 fold ↑ risk for DS
Short femur to head circumference ratio	1.5 fold ↑ risk for DS
Intracardiac Echogenic Focus	1.8 fold ↑ risk for DS
Echogenic bowel	6.7 fold ↑ risk for DS
Renal pyelectasis	1.5 fold ↑ risk for DS
Ventriculomegaly	9 fold ↑ risk for DS
Choroid plexus cysts	7 fold ↑ risk for Tri18

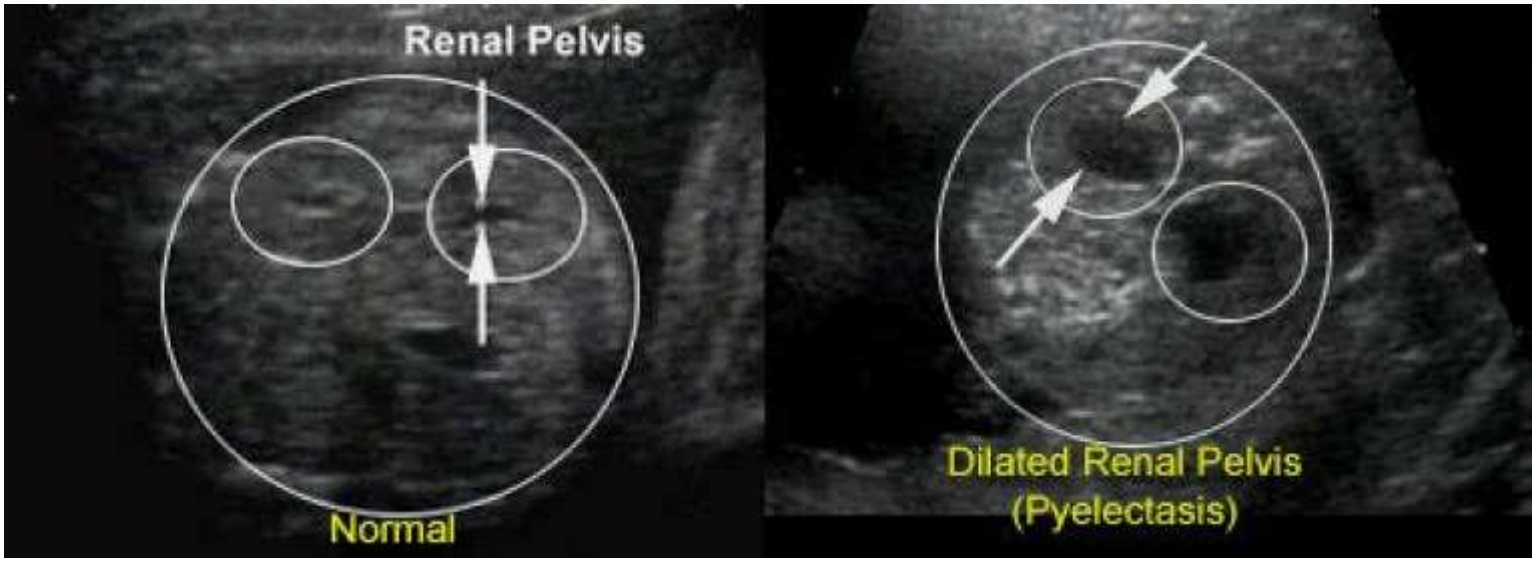
Soft Marker Identification

Intracardiac echogenic focus



Soft Marker Identification

Renal pyelectasis



Screening

REMEMBER: These tests DO NOT diagnose a problem. They only signal that further testing/investigation should be offered.

Adjusting a patients risk

1) Start with patient's a priori risk

- Ex: Patient is 38 at delivery, her age related risk of Down syndrome in the first trimester is 1 in 50.

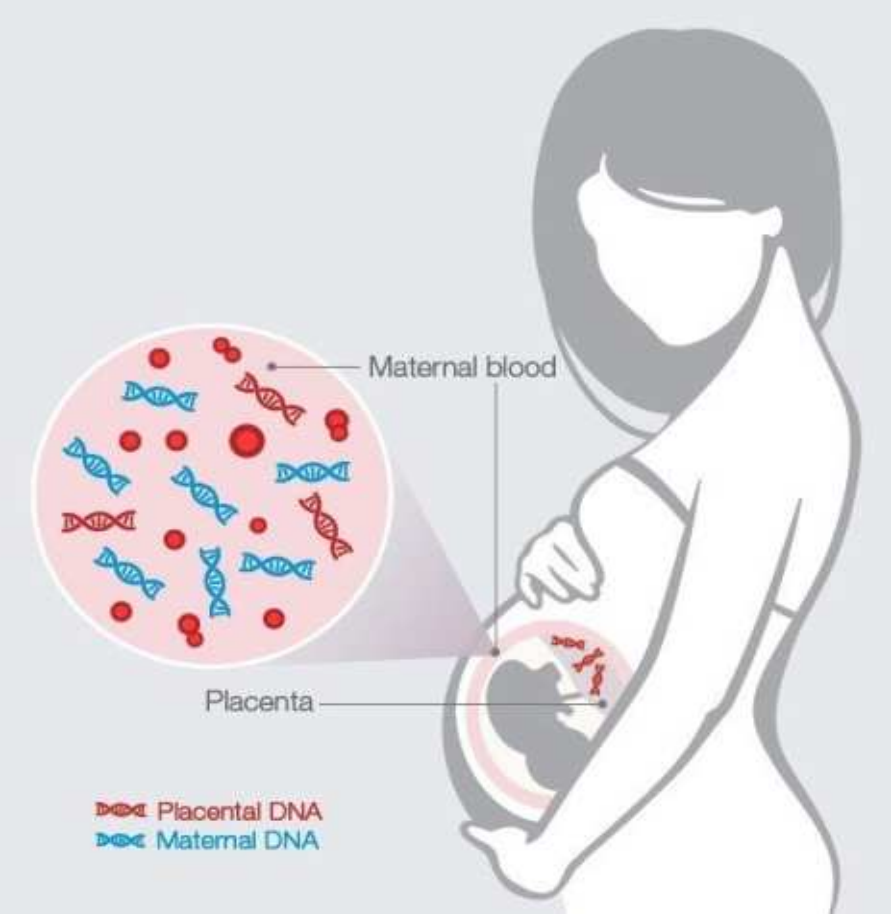
2) Patient has a first trimester screen. This analysis provides a risk assessment of 1 in 532 for Down syndrome

3) Patient returns to clinic for her 20 week ultrasound. An echogenic focus is observed. No other fetal malformations or markers were identified.

What is her overall risk of Down syndrome?

- 1/50 → 1/532 → (1/532 x 1.8 = 1/295) → FINAL RISK 1 in 295
- 1/295 = 0.3% risk of Down syn. = 99.7% chance of NO Down syn.

Cell Free DNA / Non-Invasive Prenatal Testing/Screening



Cell free DNA testing

- AKA noninvasive prenatal testing/screening (NIPT/NIPS)
- A very accurate screening test
- Best noninvasive test for those who meet criteria
- Any time after 9 weeks gestation
- Sample is maternal blood only
- Low false positive and false negative rate
- Tests for more aneuploid conditions than traditional screening (e.g. sex chromosome aneuploidies)
- Does not test for neural tube defects (consider 10-13w ultrasound – anencephaly and later msAFP)

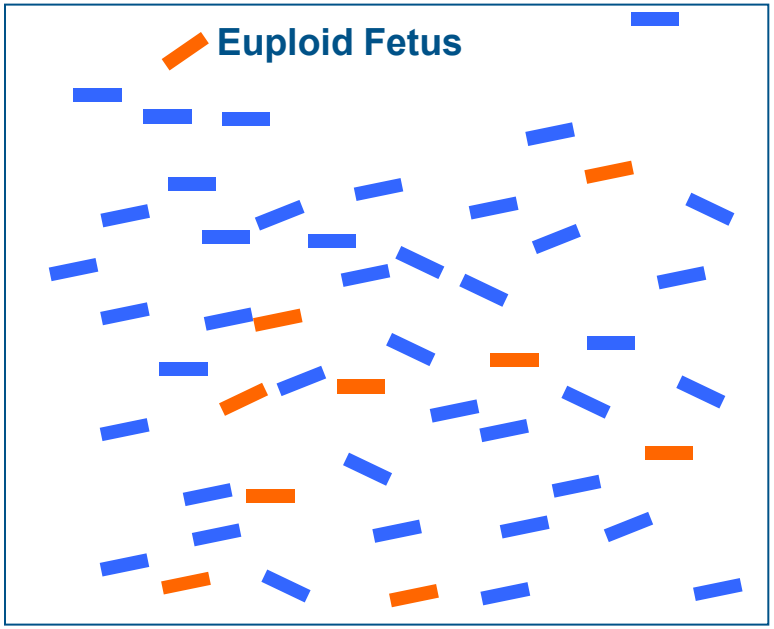
cfDNA

- Placental cells break down and release fetal DNA fragments into maternal circulation
- Fragments of circulating cfDNA are sequenced to determine the chromosome of origin
- cfDNA analyzed for chromosome material
  - detects aneuploidy of chromosome 21,18,13 and sex chromosomes (XXX, XXY, X, XYY)
  - Evolving test capabilities

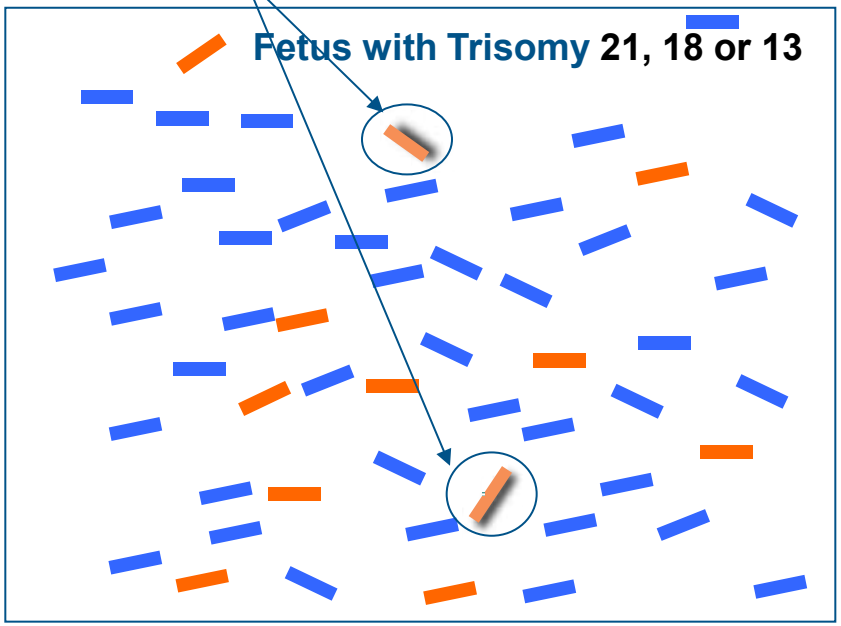
Principles of Fetal Trisomy Testing From a Maternal Blood Sample Using DNA Sequencing

- ~10% of the DNA fragments in a pregnant woman's blood are from the fetus (—)
- ~90% are from the mother (—)

Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA and Euploid Fetal DNA



Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA, Fetal DNA and Extra Fragments of Chromosome 21, 18 or 13 Contributed by a Fetal Trisomy 21, 18 or 13



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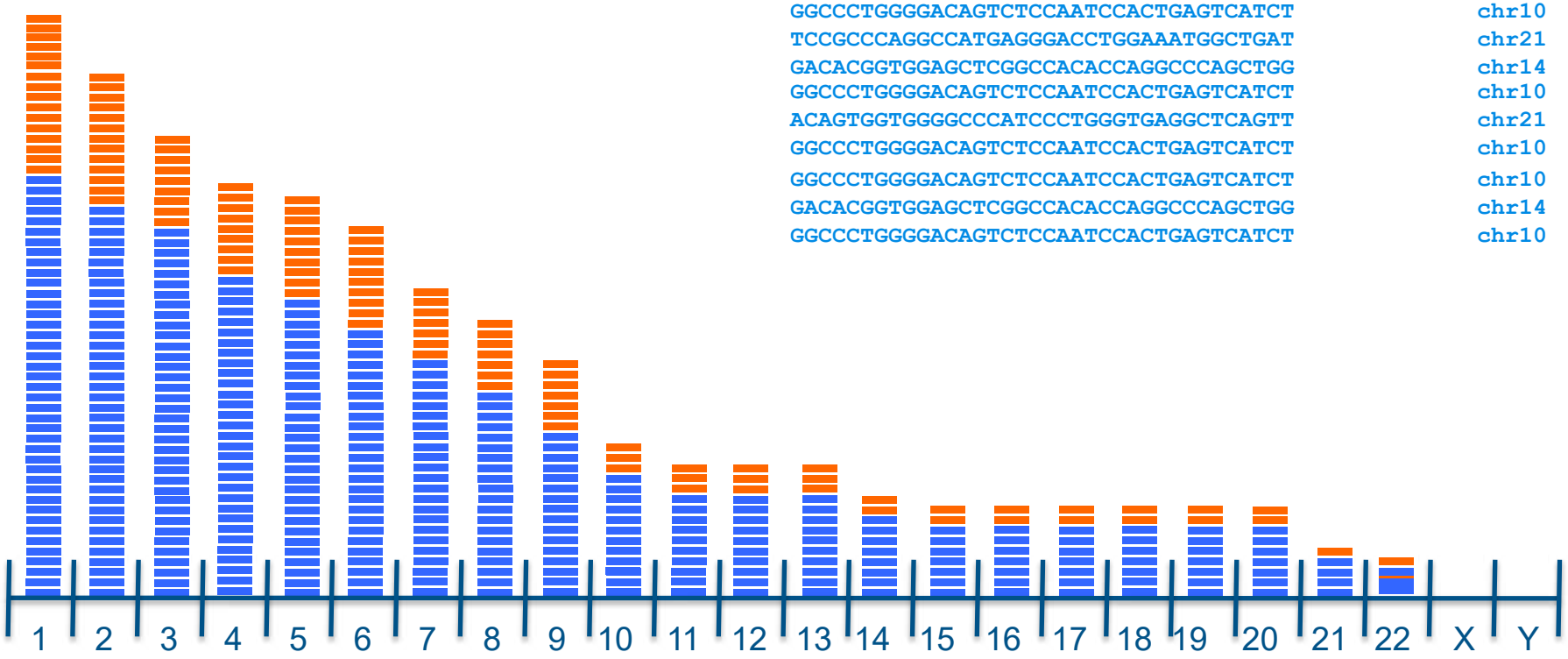
Principles of Fetal Trisomy Testing From a Maternal Blood Sample Using DNA Sequencing

Sequencing tells you which chromosome the ccf fragment comes from.

TCCGCCCAGGCCATGAGGGACCTGGAAATGGCTGAT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
ACAGTGGTGGGGCCCATCCCTGGGTGAGGCTCAGTT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
TCCGCCCAGGCCATGAGGGACCTGGAAATGGCTGAT	chr21
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
ACAGTGGTGGGGCCCATCCCTGGGTGAGGCTCAGTT	chr21
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10
GACACGGTGGAGCTCGGCCACACCAGGCCCAGCTGG	chr14
GGCCCTGGGGACAGTCTCCAATCCACTGAGTCATCT	chr10



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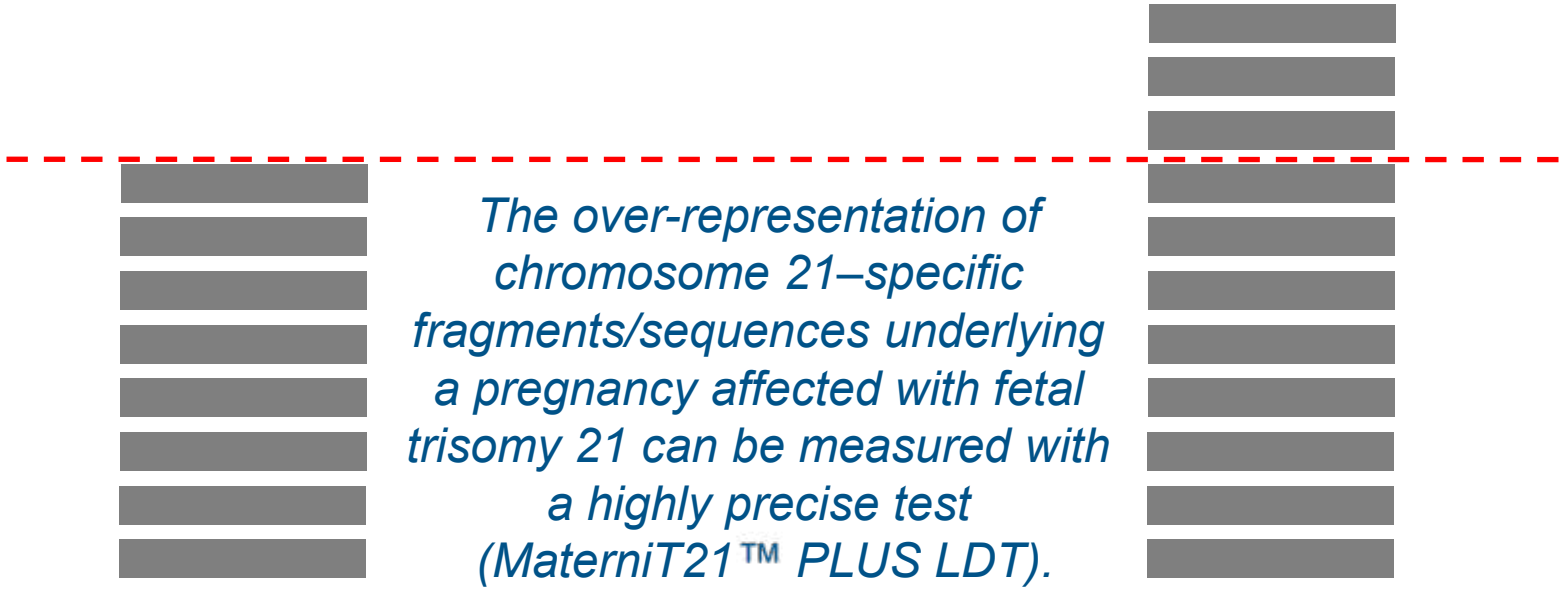
Principles of Fetal Trisomy Testing From a Maternal Blood Sample Using DNA Sequencing



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Performance of cfDNA/NIPT

Varies by laboratory

Trisomy 21

- Sensitivity: >99%
- Specificity: >99%

Trisomy 18

- Sensitivity: >99%
- Specificity: >99%

Trisomy 13

- Sensitivity: >91%
- Specificity; >99%

- How results are reported (varies by laboratory)
  - "positive" or "negative"
  - Risk calculation (1/10,000)

SCREEN RESULT: NEGATIVE

Predicted sex: MALE | Fetal fraction: 8%

ANEUPLOIDIES	RESULTS	PPV	NPV
Down syndrome (Trisomy 21)	Negative: Result consistent with two copies of chromosome 21	–	99.9%
Edwards syndrome (Trisomy 18)	Negative: Result consistent with two copies of chromosome 18	–	99.9%
Patau syndrome (Trisomy 13)	Negative: Result consistent with two copies of chromosome 13	–	99.9%
Sex Chromosome	Negative: Result consistent with two copies of sex chromosomes (XY). Predicted sex is male	–	99.9%

About this test

This screening test evaluates whether your pregnancy is at increased risk for certain types of chromosomal disorders. Because this is a screen, false posit
of Invitae's non-inva
the negative predict

SCREEN RESULT: POSITIVE

Predicted sex: FEMALE | Fetal fraction: 15%

ANEUPLOIDIES	RESULTS	PPV	NPV
Down syndrome (Trisomy 21)	**POSITIVE: Result suggestive of trisomy of chromosome 21**	**96.1%**	–
Edwards syndrome (Trisomy 18)	Negative: Result consistent with two copies of chromosome 18	–	99.9%
Patau syndrome (Trisomy 13)	Negative: Result consistent with two copies of chromosome 13	–	99.9%
Sex Chromosome	Negative: Result consistent with two copies of sex chromosomes (XX). Predicted sex is female	–	99.9%

About this test

This screening test evaluates whether your pregnancy is at increased risk for certain types of chromosomal disorders. Because this is a screen, false positives and false negatives can occur. The estimated fetal fraction of DNA present in this sample is one component of Invitae's non-invasive screening algorithm. The positive predictive value (PPV) is the likelihood that a positive result is true, and the negative predictive value (NPV) is the likelihood that a negative result is true.

cfDNA/NIPT

Indications for cfDNA:

- Advanced maternal age
- Previous child with chromosome abnormality
- Ultrasound markers or abnormalities suggestive of aneuploidy
- Positive serum screen
- Gender determination*
  - when information would affect clinical management, e.g. CAH
  - Determined on a case by case basis


ACOG

ACOG PRACTICE BULLETIN

Clinical Management Guidelines for Obstetrician-Gynecologists

Number 226

Screening for Fetal Chromosomal Abnormalities

2020 ACOG Guidelines recommend cfDNA as an option to be “discussed and offered to all patients early in pregnancy, regardless of maternal age or baseline risk.”

Newer cfDNA Applications

- Detection of select microdeletion syndromes and screen genome-wide for aneuploidy or gains/losses at 7 Mb or greater
- Detection of select single gene disorders (primarily de novo autosomal dominant)

ACOG 2019

The continued innovation in cell-free technology combined with the desire for a maternal blood test to predict the risk for fetal genetic disorders during a pregnancy has broadened the application of cell-free DNA screening beyond aneuploidy to single-gene disorders. Examples of single-gene disorders include various skeletal dysplasias, sickle cell disease and cystic fibrosis. Although this technology is available clinically and marketed as a single-gene disorder prenatal screening option for obstetric care providers to consider in their practice, often in presence of advanced paternal age, there has not been sufficient data to provide information regarding accuracy and positive and negative predictive value in the general population. For this reason, single-gene cell-free DNA screening is not currently recommended in pregnancy.

Diagnostic Testing

PRENATAL DIAGNOSIS is performed to help at-risk families gain definite fetal genetic information and make informed choices during pregnancy

Confirm or rule out genetic condition by obtaining fetal cell sample

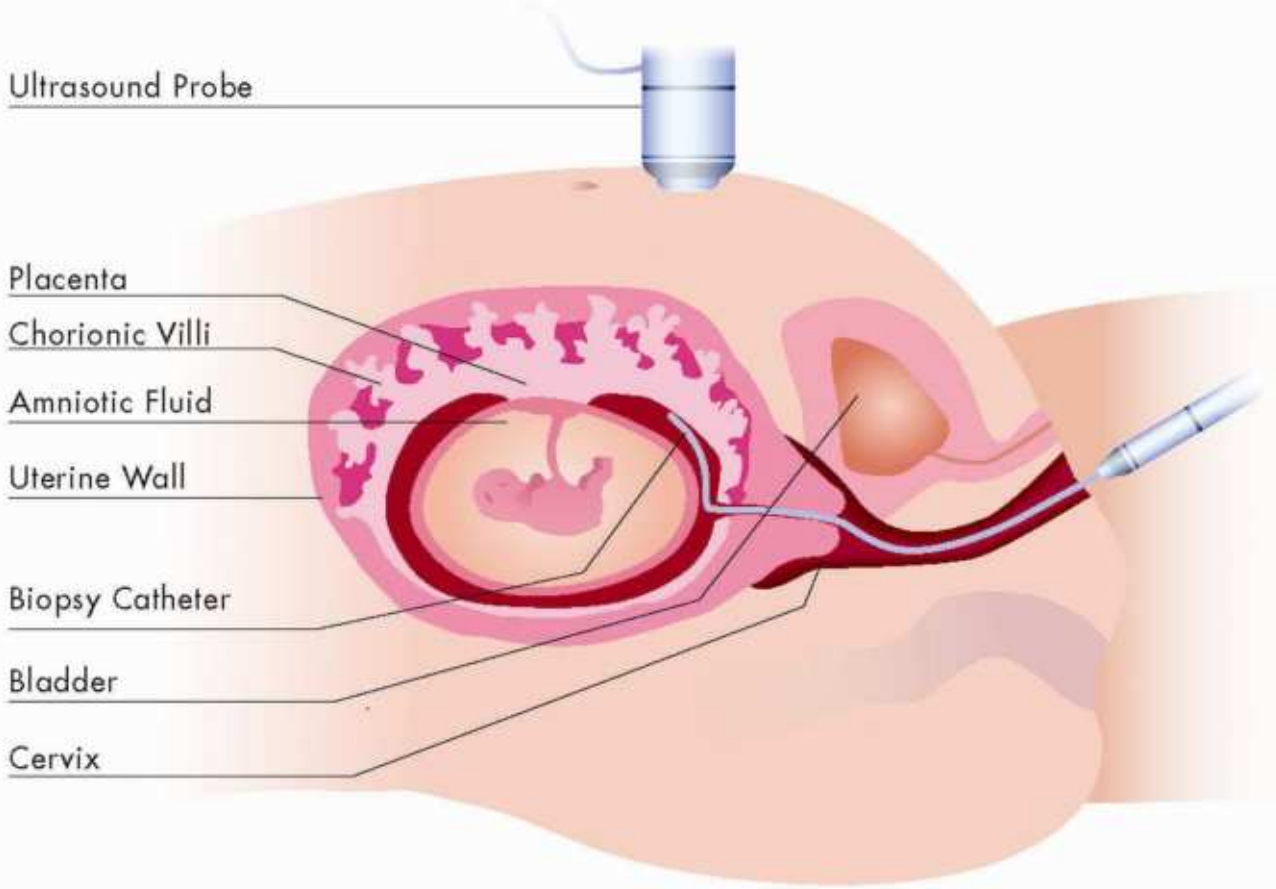
Procedures

- Chorionic Villus Sampling (CVS)
- Amniocentesis

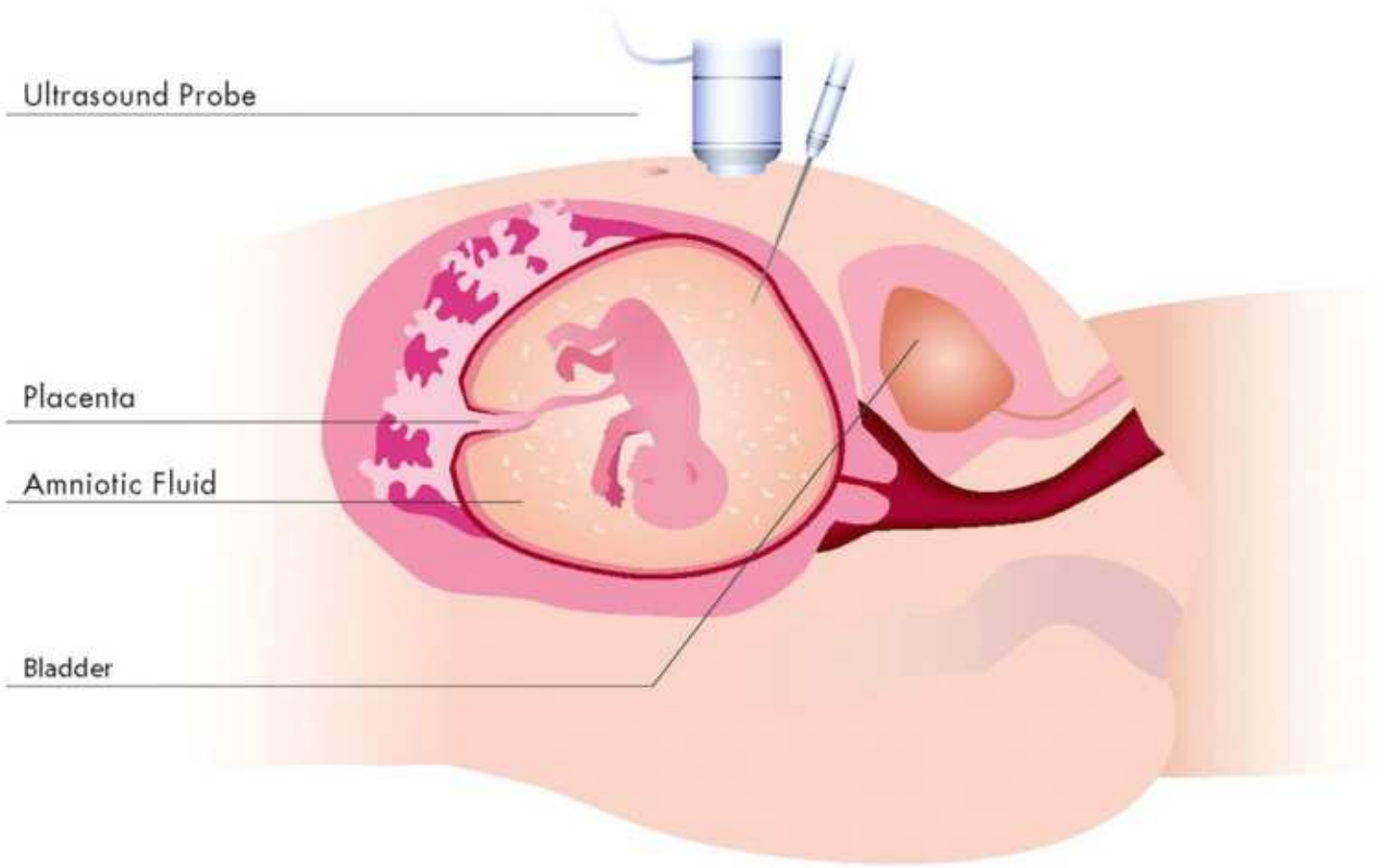
Diagnostic Procedures

	CVS	Amnio (Traditional)
Time Frame	10 – 14 weeks gestation	15+ weeks for traditional amnio (earlier is possible)
Sample source	Chorionic villi	Fetal cells (skin, intestinal tract) that are in amniotic fluid
Risk of complication or loss	0.2 – 0.5%	0.2 – 0.5%
Results	Chromosomes/karyotype, microarray, genetic testing for known familial mutations	Chromosomes/karyotype, microarray, AFP/acetylcholinesterase for ONTD; genetic testing for known familial mutations

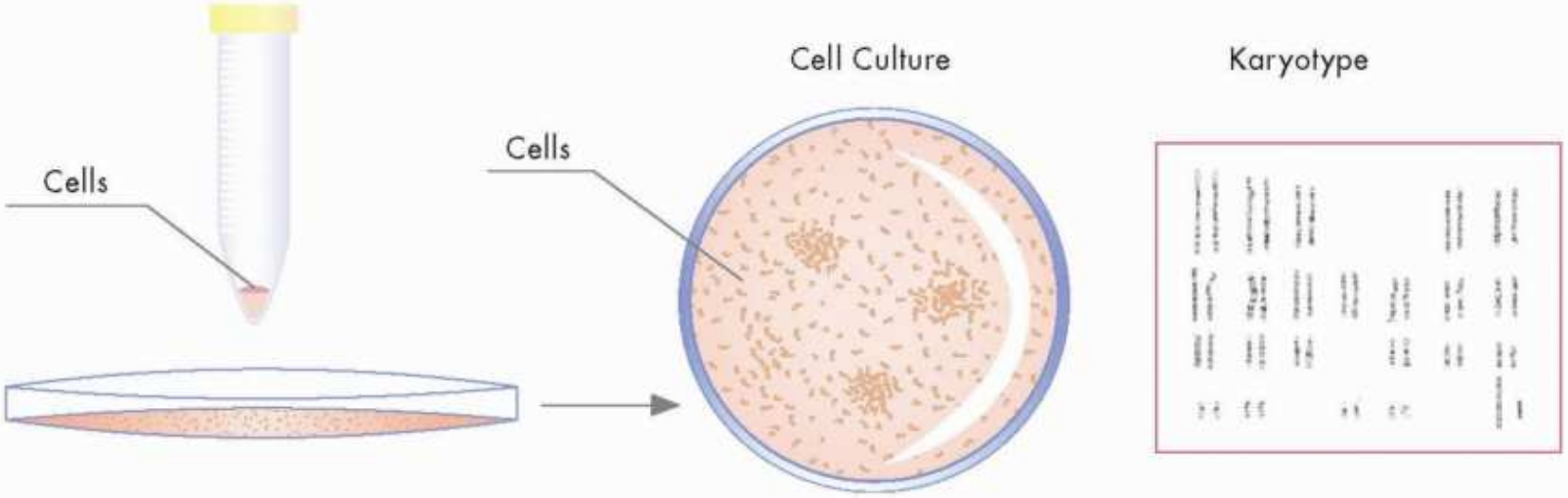
Transcervical Chorionic Villus Sampling



Amniocentesis



Test Results



CVS and Amnio

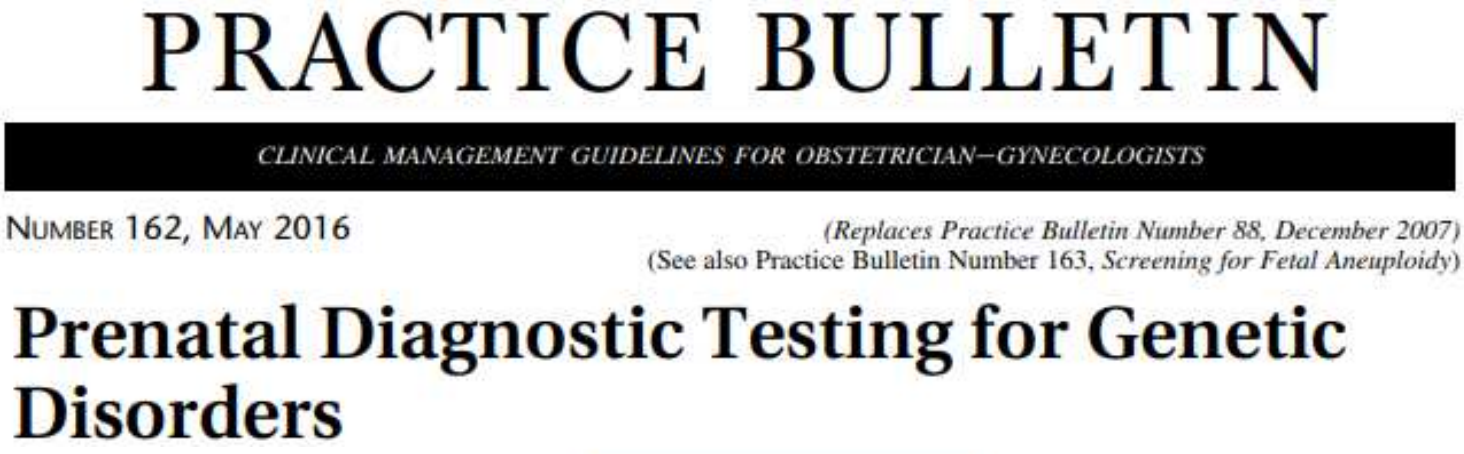
- Definite Y or N for aneuploidy (FISH for rapid results)
- Follow-up to positive cfDNA/other screening
- Offer for abnormalities
- Microarray now offered to all having invasive testing, encouraged for known abnormalities if testing
  - Detects submicroscopic deletions and duplications of genetic material
- Single gene testing when indicated
  - E.g. cystic fibrosis, sickle cell disease, muscular dystrophy
- Benefits: definitive answers
- Limitations: procedure and related risk

ACOG position on testing

"All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors."

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

Society for Maternal-Fetal Medicine



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Prenatal Diagnostic Testing for Genetic Disorders

Questions?

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