Prenatal diagnosis

WHEN? WHY? AND HOW

DR.NADIA NAEEF

Definition

- 'Prenatal diagnosis is defined as the detection of abnormalities in the fetus, before birth'

To make prenatal diagnosis more effective

- Less invasive techniques
- Earlier testing
- Individual diagnosis

Why Prenatal screening/diagnosis

- All the pregnant women are at risk of carrying a fetus with genetic abnormalities.
- Incidence of major abnormality apparent at birth is 2 to 3%.
- Principle goal of prenatal diagnosis is to supply information to at risk families so they can make informed choice during pregnancy.
- Potential benefits of prenatal diagnosis are

1) early reassurance to at risk families if results are normal.
2) Risk information to couples who would not have child without defects.
3) Allowing couples to prepare for the birth of an affected child.
4) Risk information to couples for whom termination is an option

Decision making, parental rights and responsibilities

- The couple decide whether or not to undertake pregnancy (before 12th week)
- The couple decide whether or not to accept the offer of prenatal diagnosis •
- Genetic counselor: gives the adequate information .
- Parents: make the decision .
- Factors influencing the decision: optimistic/pessimistic attitude, ethical or religious principles etc

Some Disorders for which PRENATAL DIAGNOSIS is available:

1. Congenital malformations

2. Chromosomal disorders

3. Non genetic Fetal disorders

***Fetal infections, Hydrocephalus, Fetal effects of maternal drugs e.g valproic acid**

4. Single gene disorders -Multiple malformation syndromes

***Holt oram, Craniosynostosis, Orofacial digital syndromes**

-Hematological disorders

***Thalassemias, Hemophilia**

-Metabolic Disorders

***Tay sachs, Wilson, CAH.**

-Neuromuscular disorders

***Huntington chorea, Myotonic dystrophy, Fragile X**

Holt Oram syndrome

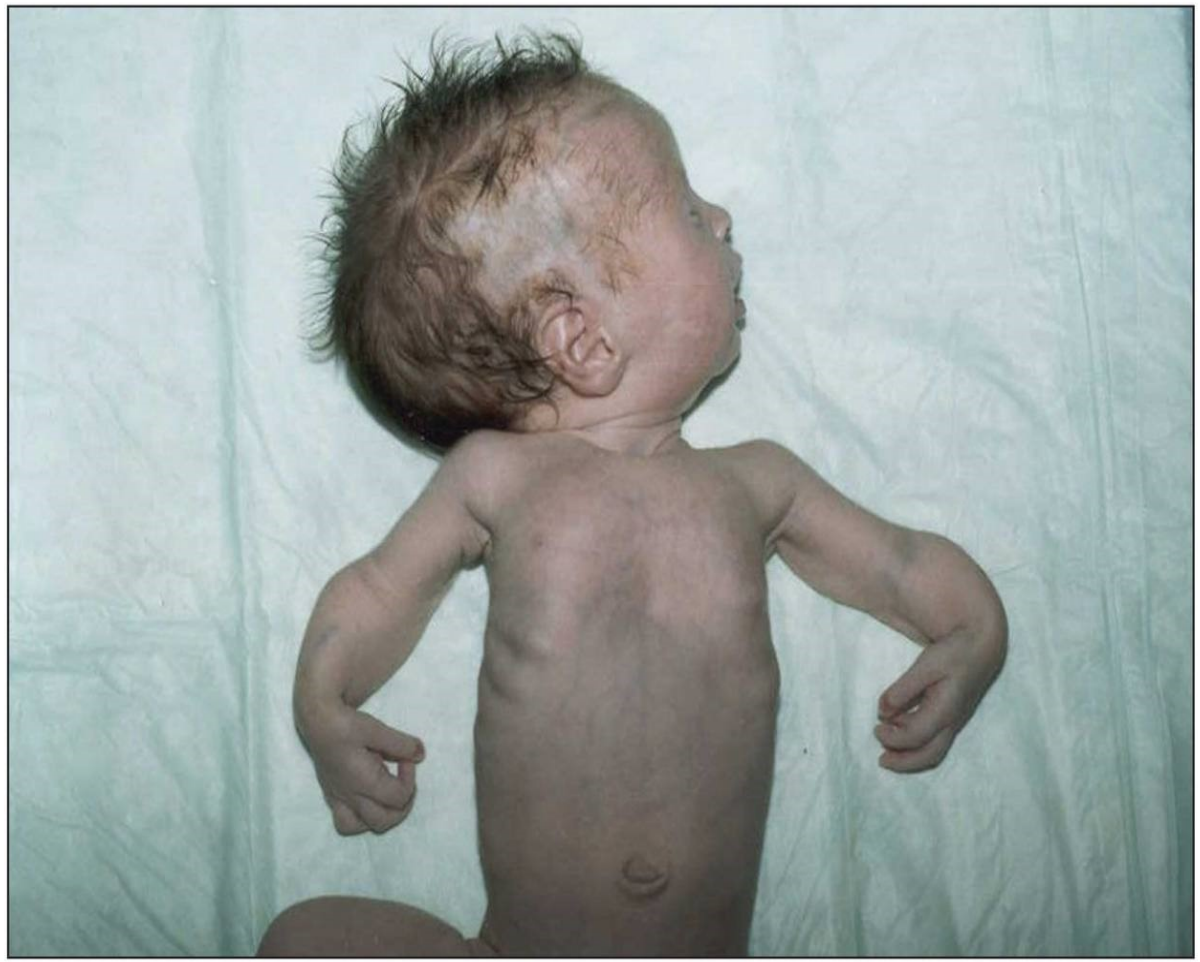
Holt-Oram syndrome (HOS) is an autosomal dominant syndrome that results in :
congenital heart defects:

- atrial septal defect (ASD) (commonest cardiac defect)
- ventricular septal defect (VSD)

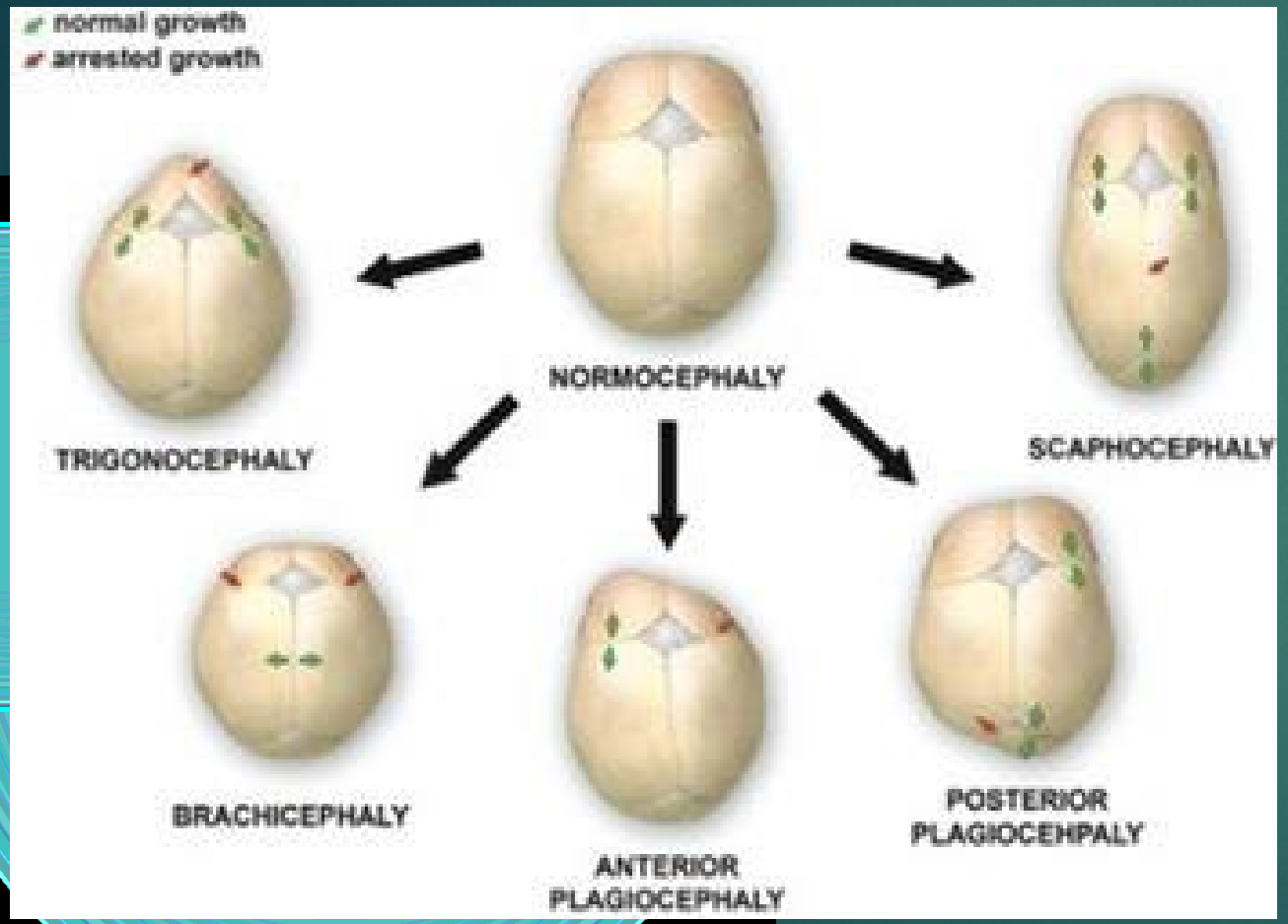
upper limb abnormalities:

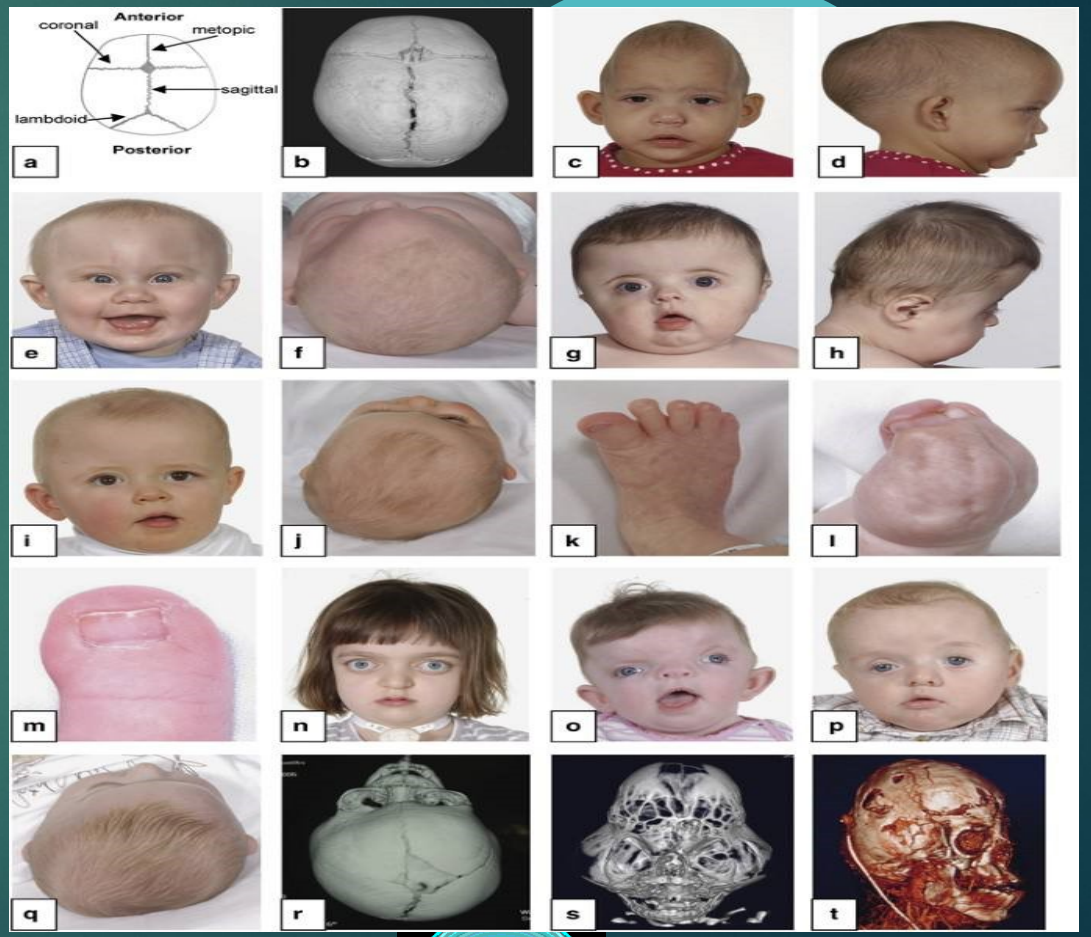
- radial ray anomalies, e.g. radial aplasia, radial hypoplasia
- thumb anomalies, e.g thumb aplasia

Dr Jeremy Jones and Dr Yuranga Weerakkody et al.

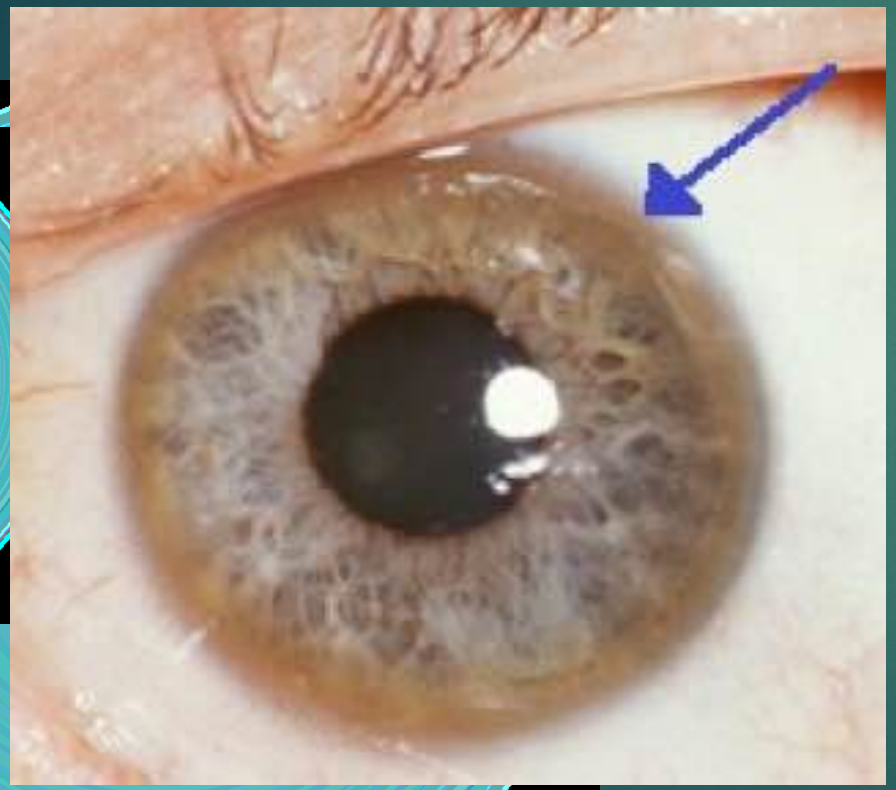


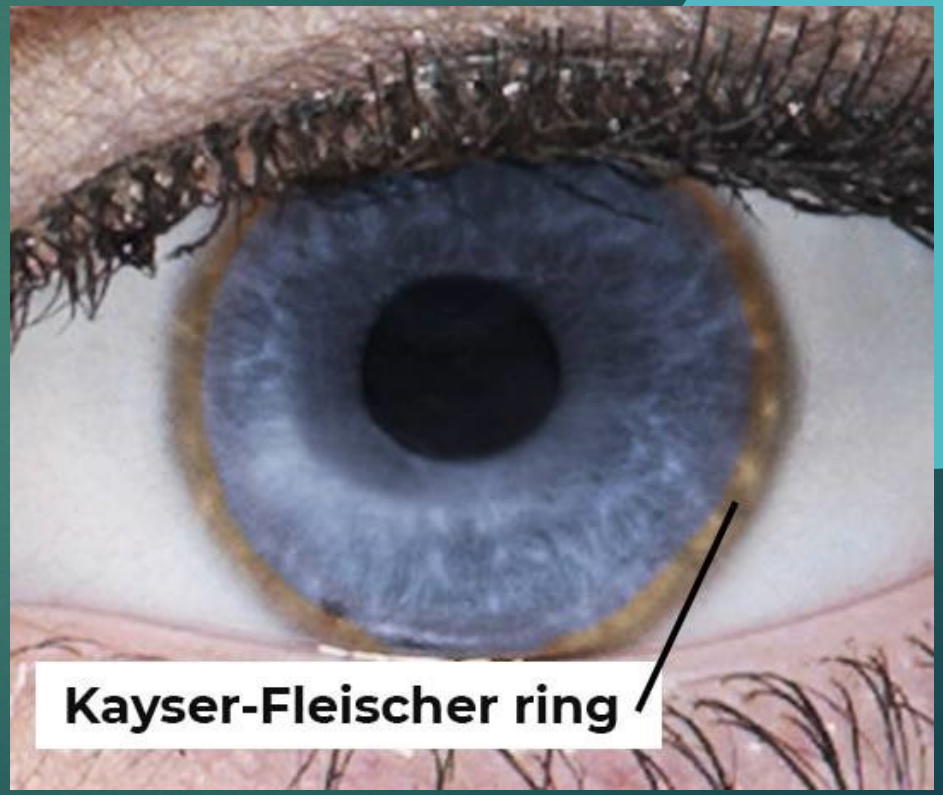
Craniosynostosis





Wilson disease





FRAGILE X SYNDROME

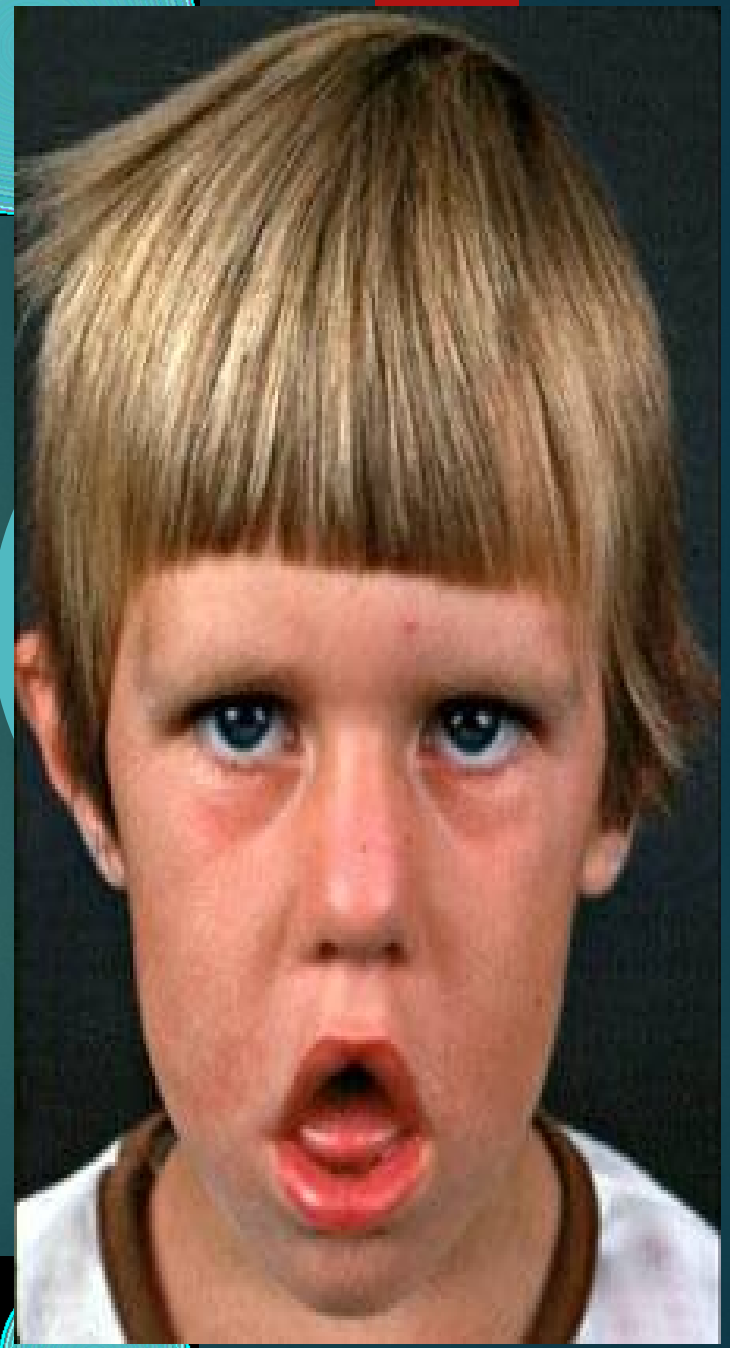
Broad forehead
Elongated face
Large prominent ears
Strabismus (crossed eyes)
Highly arched palette

Hyperextensible Joints
Hand calluses
Pectus Excavatum
(indentation of chest)
Mitral valve prolapse

Hypotonia (low muscle tone)
Soft, fleshy skin
Enlarged testicles
Flat feet
Seizures in 10%

Congenital Myotonic Dystrophy

- Marked facial weakness
- Hypotonia, may disappear
- Clinical myotonia absent
- Neonatal respiratory distress
- Feeding difficulties
- Developmental delay
- Mental retardation (nonprogre
- Maternally inherited
- Large repeat expansions



-Renal Disoders

*AD/AR polycystic kidney disease

-Connective tissue diseases/Skeletal dysplasia

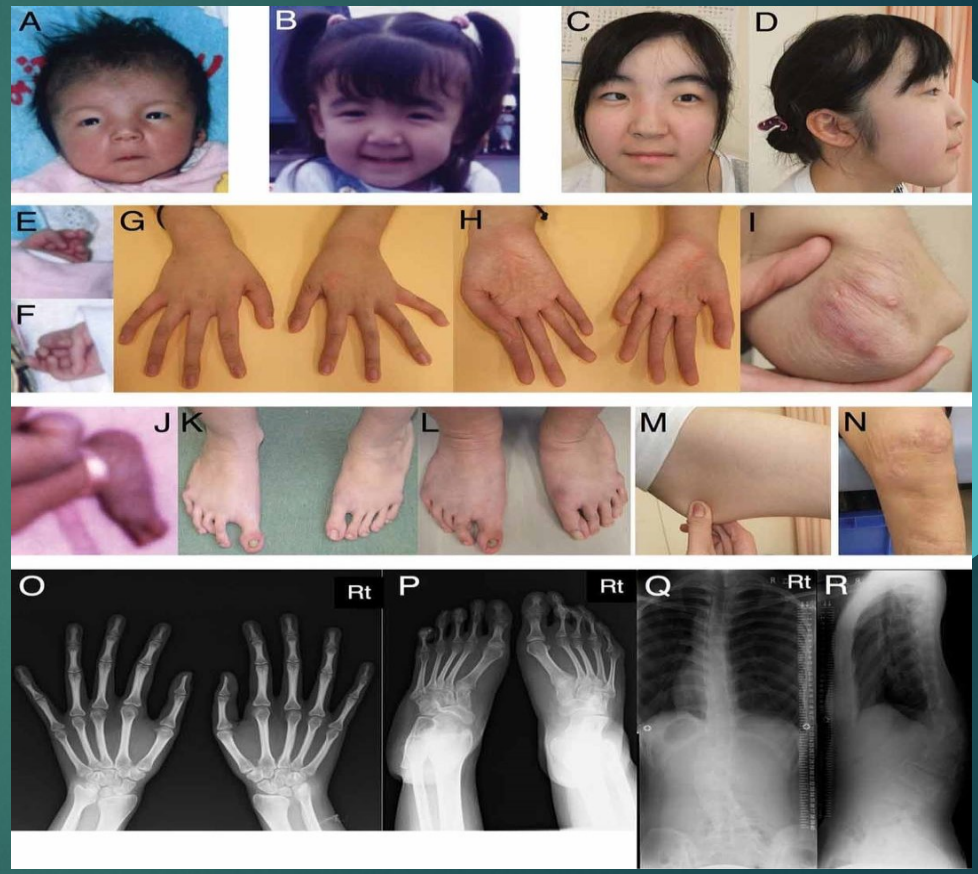
*Osteogenesis imperfecta, Ehlers Danlos, Achondroplasia, Marfan.

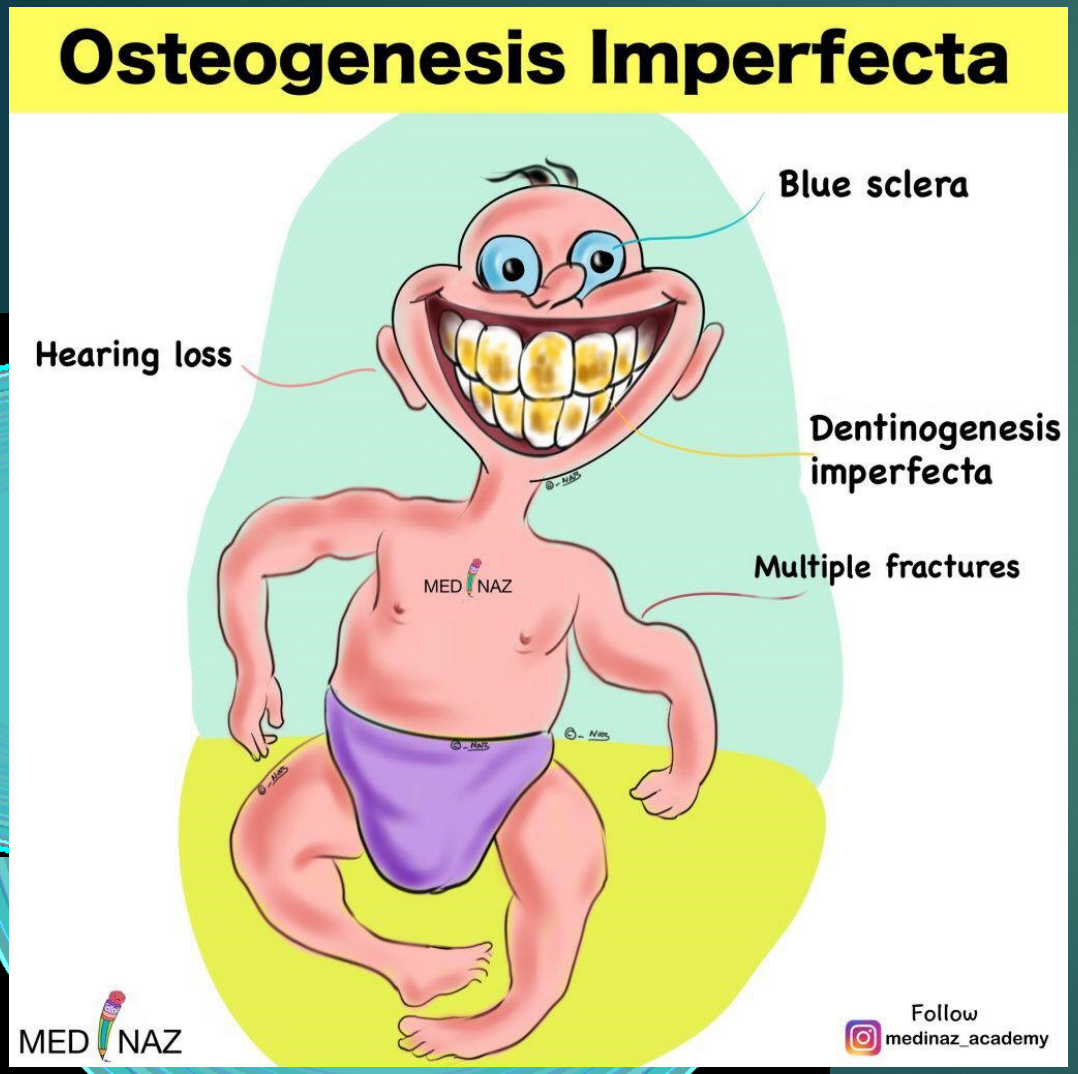
-Skin disorders

*Epidermolysis bullosa, Ichthyosis, Ectodermal dysplasia

Ichthyosis

Ehlers Danlos




Osteogenesis Imperfecta
Blue sclera
Hearing loss
Dentinogenesis imperfecta
Multiple fractures
MED NAZ
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ECTODERMAL DYSPLASIA
THIN SCALP HAIR
VISION DEFECTS
CLEFT LIP
ABNORMAL TEETH
INABILITY TO SWEAT
PEELING SKIN
DISCOLORED NAILS
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INDICATIONS OF PRENATAL DIAGNOSIS

1. Advanced maternal age.
2. Previous child with a chromosomal abnormality.
3. Family history of a chromosomal abnormality.
4. Family history of a single gene disorder.
5. Family history Neural Tube Defect.
6. Family history of other congenital structural abnormality.
7. Abnormalities identified in pregnancy.
8. Other risk factors(consanguinity , poor obs. History , maternal history)

METHODS OF PRENATAL DIAGNOSIS

NON INVASIVE TECHNIQUES

- Fetal visualization

1.ULTRASONOGRAPHY

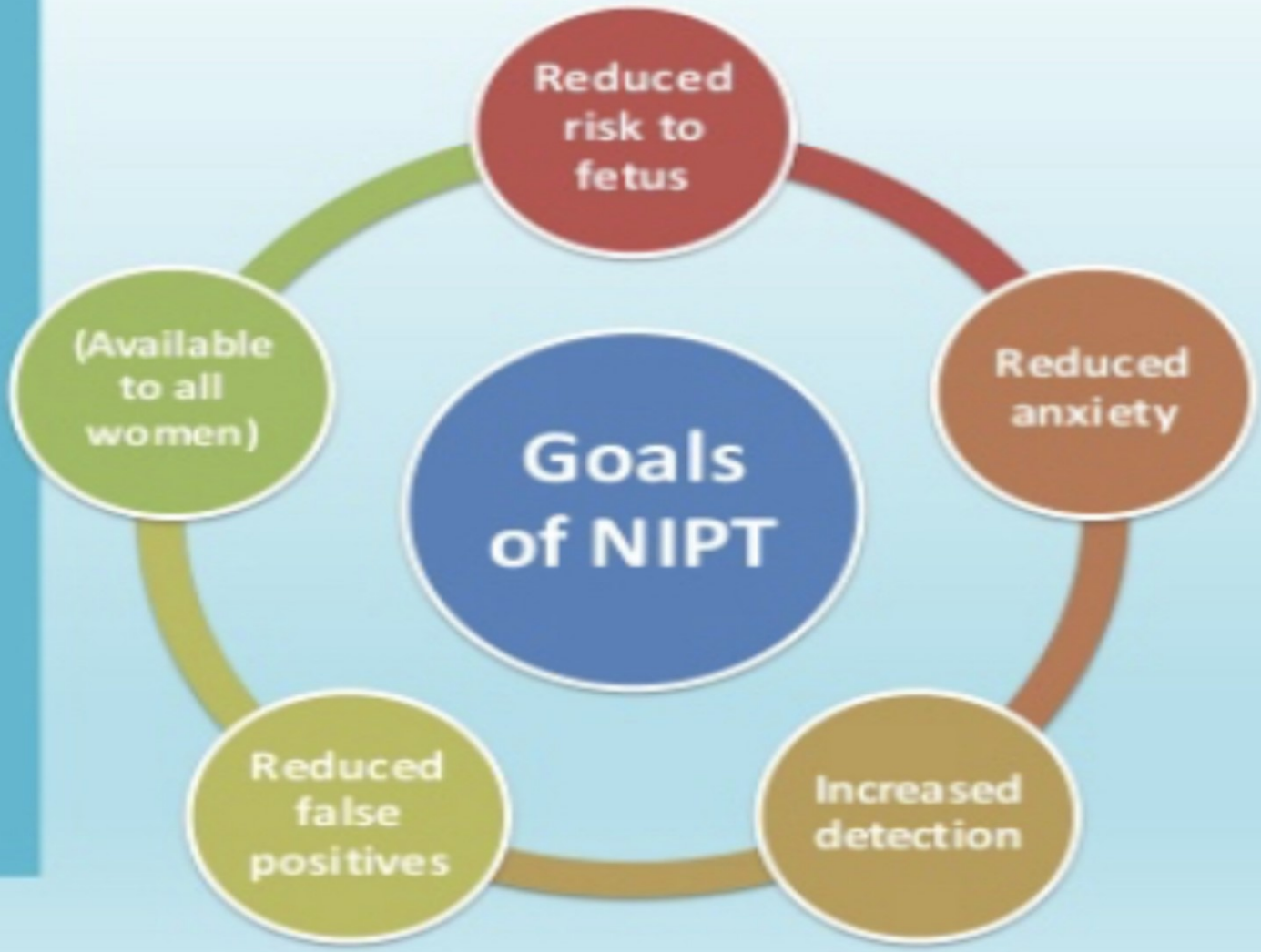
2.FETAL ECHOCARDIOGRAPHY

3.MAGNETIC RESONANCE IMAGING (MRI)

- Maternal serum screening
- Separation of fetal cells from the mother's blood

INVASIVE TECHNIQUES

- Fetal visualization
- Fetal tissue sampling
- Cytogenetics
- Molecular genetics


Goals of NIPT
Reduced risk to fetus
Reduced anxiety
Increased detection
Reduced false positives
(Available to all women)

FETAL VISUALISATION

1. ULTRASONOGRAPHY:

- The developing embryo can first be visualized at about 6 weeks gestation. Recognition of the major internal organs & extremities to determine if any are abnormal can best be accomplished between 16 to 20 weeks gestation

Ultrasound

- Screening (chromosome aberrations) and diagnostics (e.g. spina bifida) in one
- **Five US scan during pregnancy**
- 0. US: to diagnose pregnancy (embryonal heart function)
- 1st US scan: 12th week (NT<3 mm!)
- 2nd US scan: 17-21st week (detailed examination: diagnosis of congenital malformations)

- 3rd US scan: 28-32nd week (IUGR, flowmetry: placental circulation)
- 4th US scan: 36-38th week: fetal position, estimated weight, placental position, width of the scar)
- Fetal echocardiography: 18-22nd week: fetal heart anatomy and function

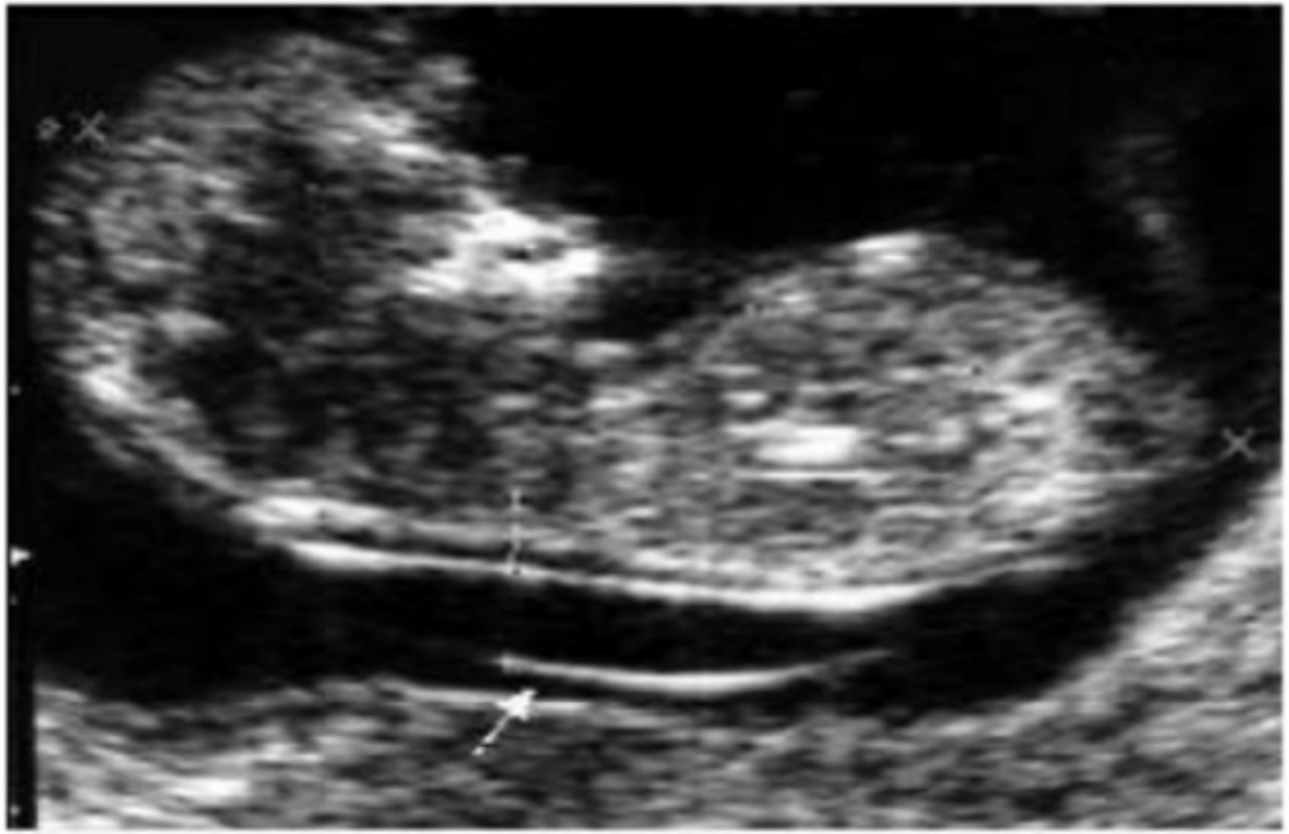
Nuchal Translucency

- Nuchal Translucency(NT) refers to the normal subcutaneous fluid filled space between the back of the neck and underlying skin.
- Single most powerful marker available today for differentiating Downs syndrome from euploid pregnancy in 1st trimester.
- NT is not specific for aneuploidy its also increased in

1. CHD
2. Genetic syndrome
3. Abnormal or delayed development of lymphatics.

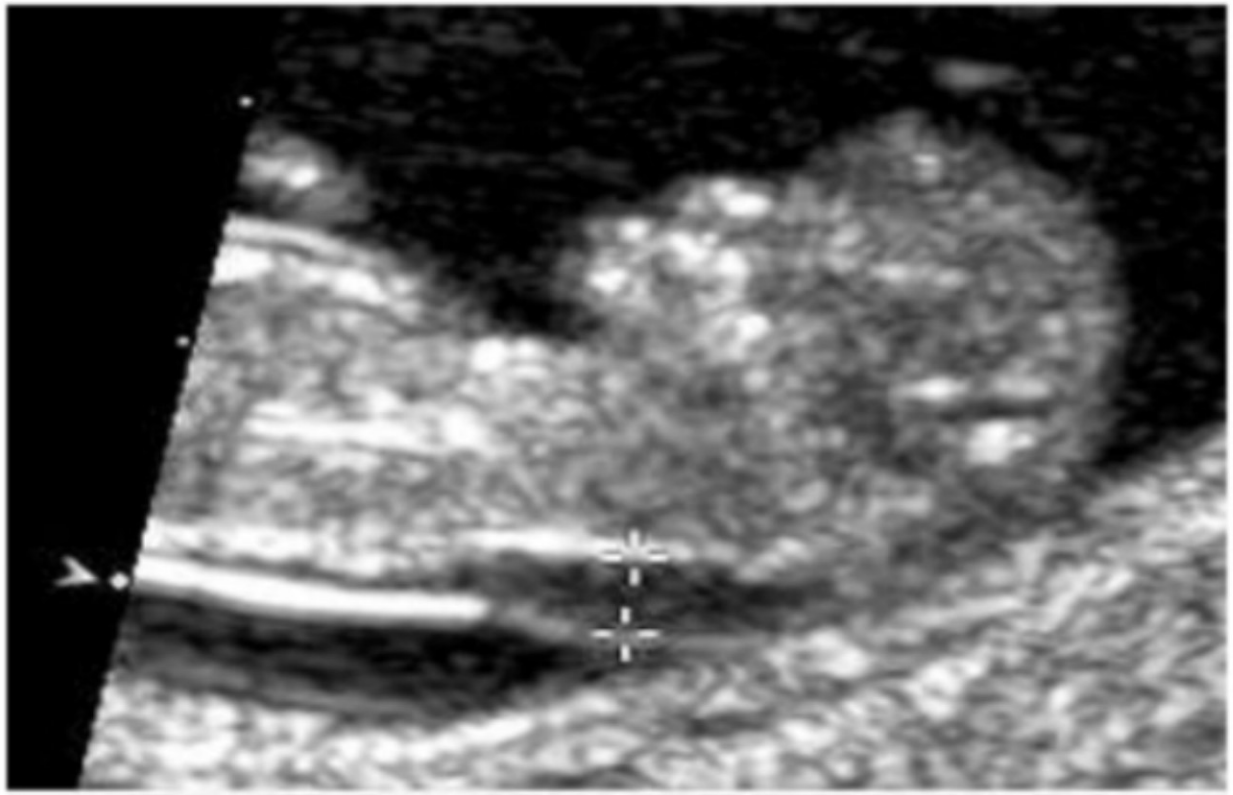
Time - 10 to 14 weeks (CRL of 36 to 84mm)

2D US



Normal Nuchal Lucency Thickening

2D US

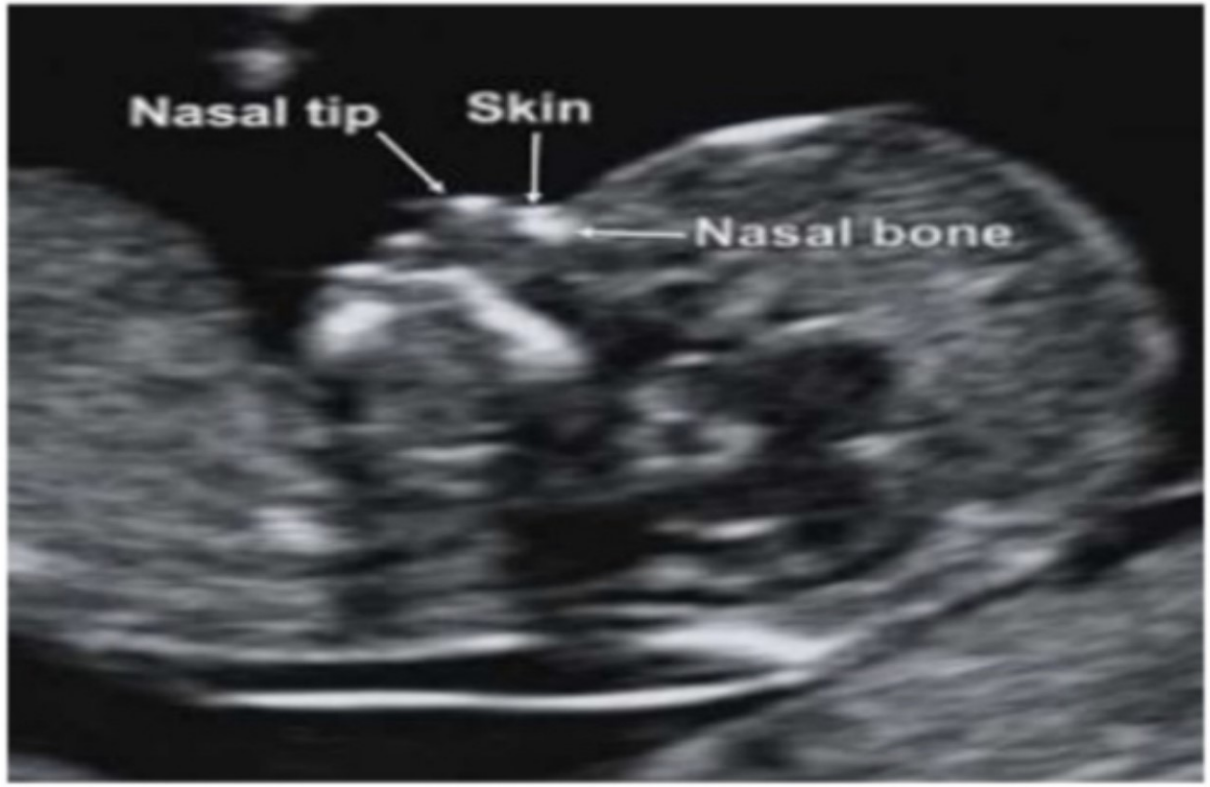


Abnormal Nuchal Lucency Thickening

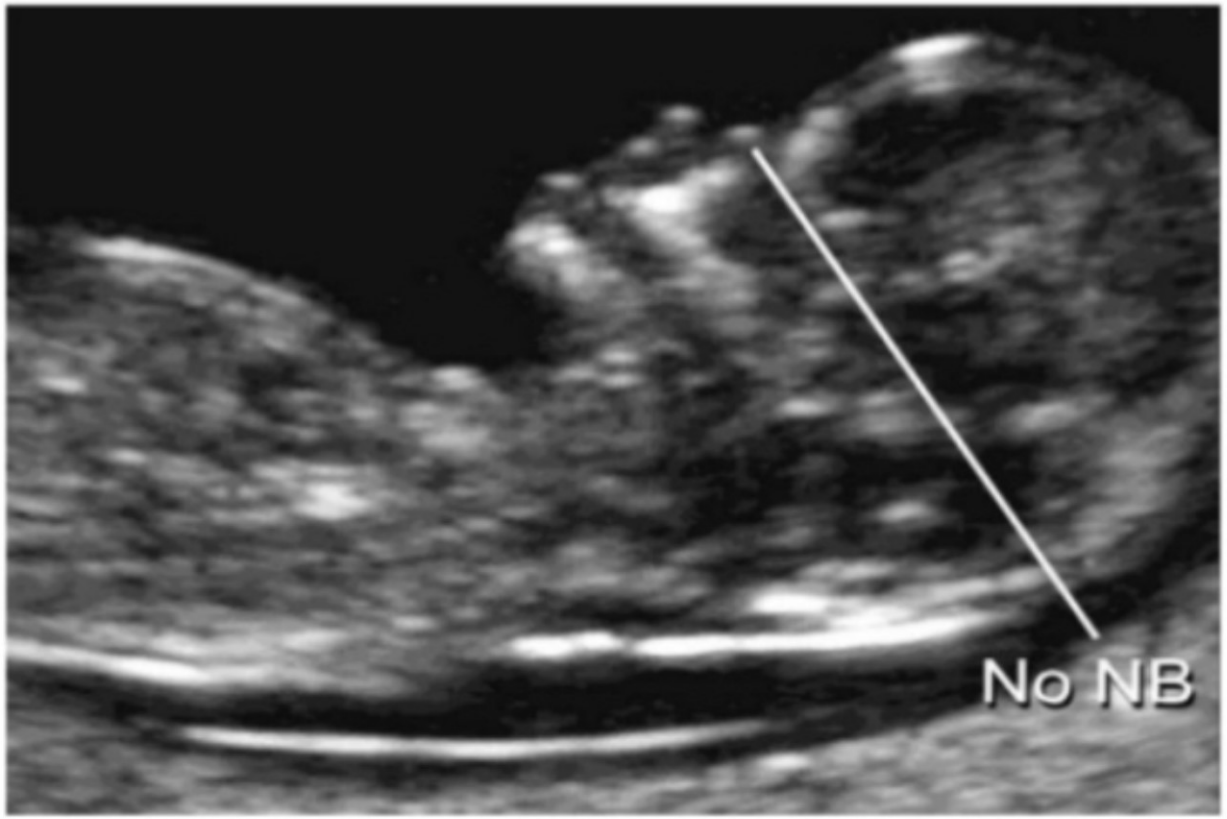
Nasal Bone (NB)

- Absent nasal bone on USG done at 11 to 13 weeks is another marker of Downs syndrome.
- Absence of Nasal bone is not related to NT and can be combined in one scan.
- Detection rate of Downs syndrome using absent nasal bone is 67%.
- When combined with NT detection rate is 90%

2D US



2D US



US markers of fetal congenital abnormalities or genetic syndromes found in first trimester scanning [at 11-13weeks' gestation]

- nuchal translucency> 3 mm: trisomy 21, Turner and other syndromes; heart and great vessels defects
- absence of the fetal nasal bone: trisomy 21
- hypoplastic maxilla: about 50% of fetuses with trisomy 21
- abnormal blood flow velocity in the fetal ductus venosus: in 80% of fetuses with trisomy 21
- omphalocele: trisomy 18
- hypoplastic bladder: trisomy 18 and 13
- single umbilical artery: trisomy 18

3D & 4D US

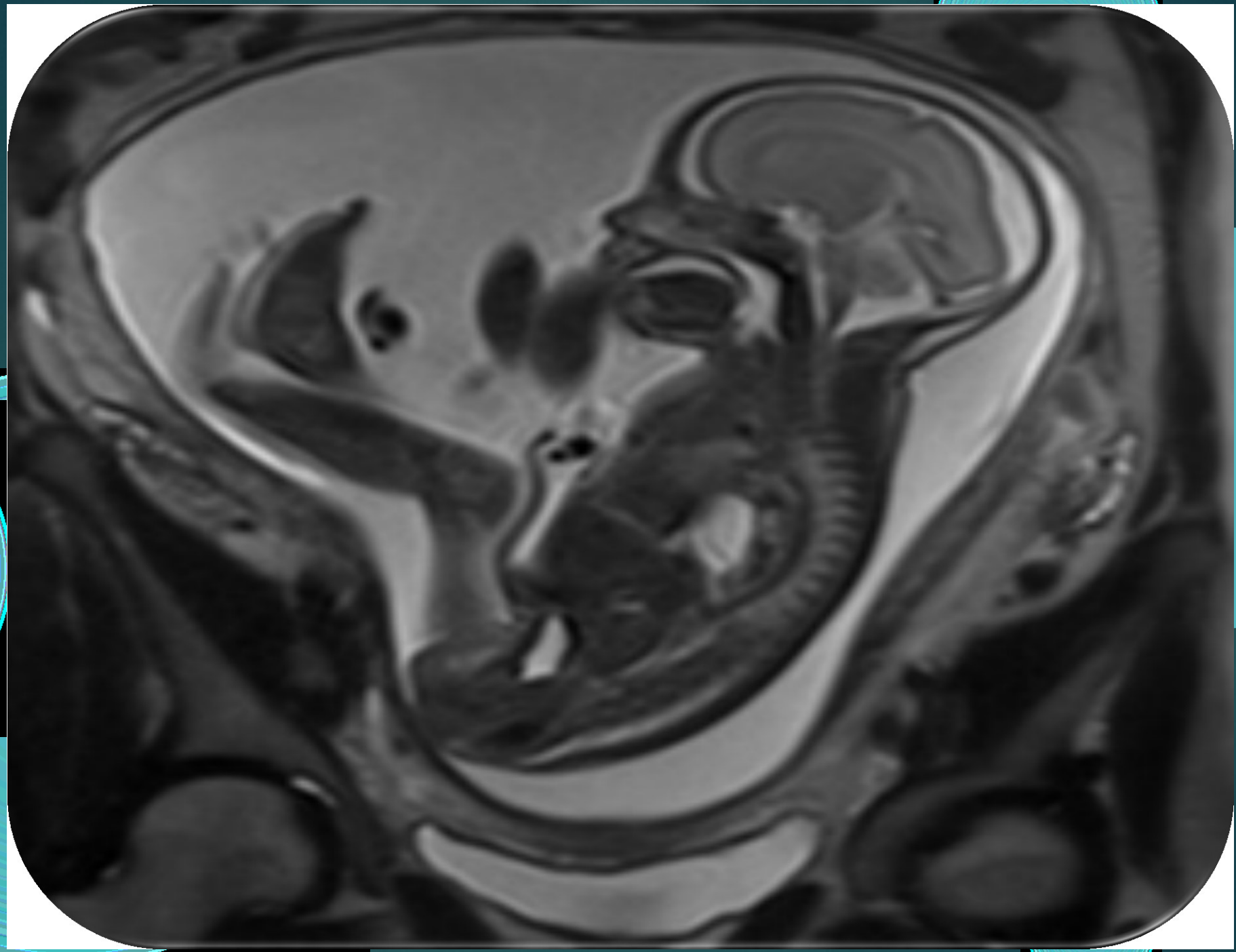
In recent years three-dimensional ultrasound (3D) & four-dimensional ultrasound (4D) have started to play an increasing role in prenatal diagnosis. They can be applied in assessing facial features, central nervous system abnormalities and skeletal defects

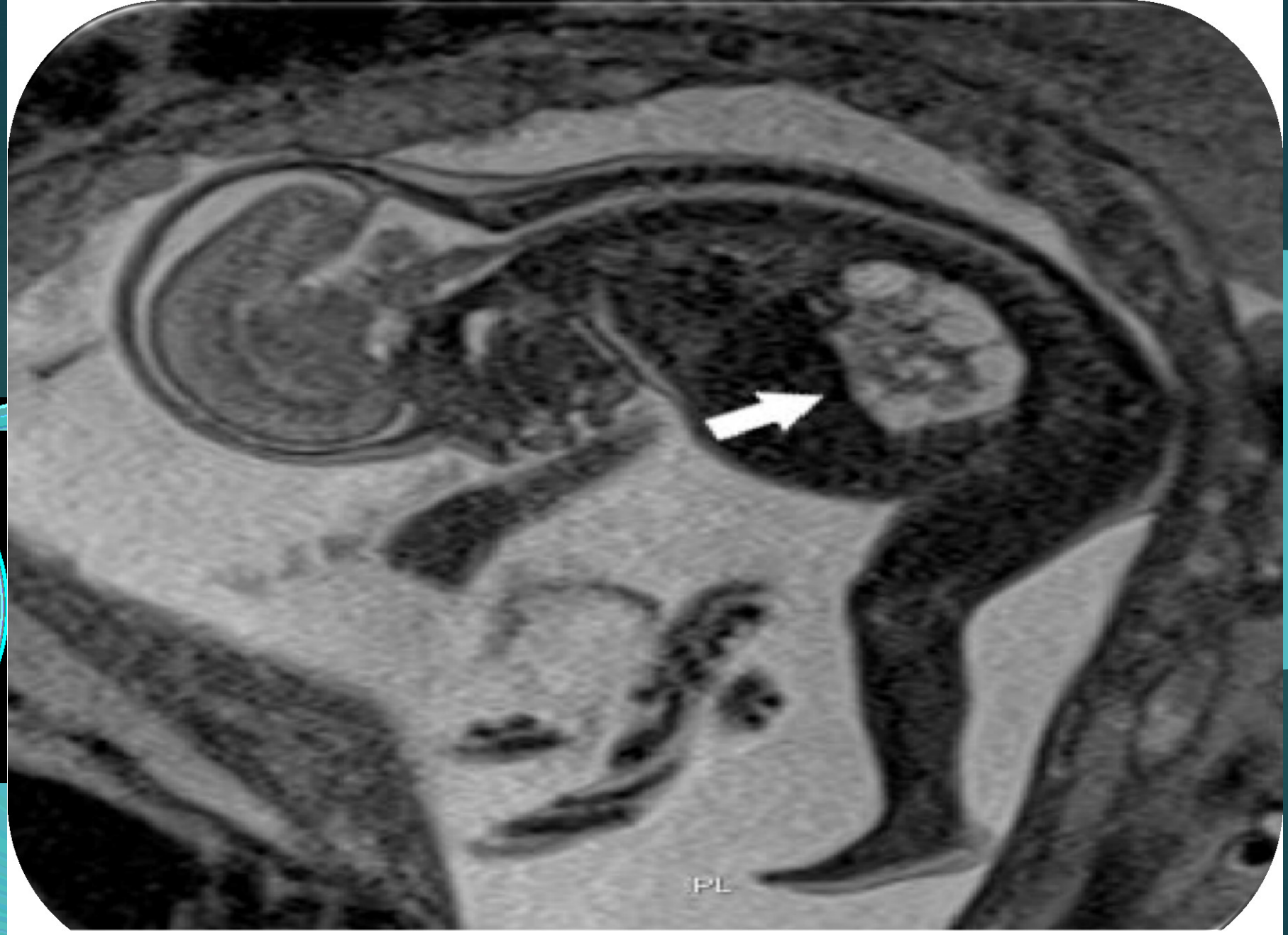
- Fetal MRI: an adjunct tool to US in fetal screening
- Images to be obtained from any directions
- Excellent soft tissue contrast
- Substituting US in case of oligohydramnios or obesity

Fetal MRI

INDICATIONS

- Central nervous system malformations (NTD, holoprosencephaly, hydranencephaly, AV malformations, intracranial hemorrhage)
- Cervical teratoma (relationship to vessels and airways)
- Chest masses (diaphragmatic hernia, CAM)
- Adrenal neuroblastoma





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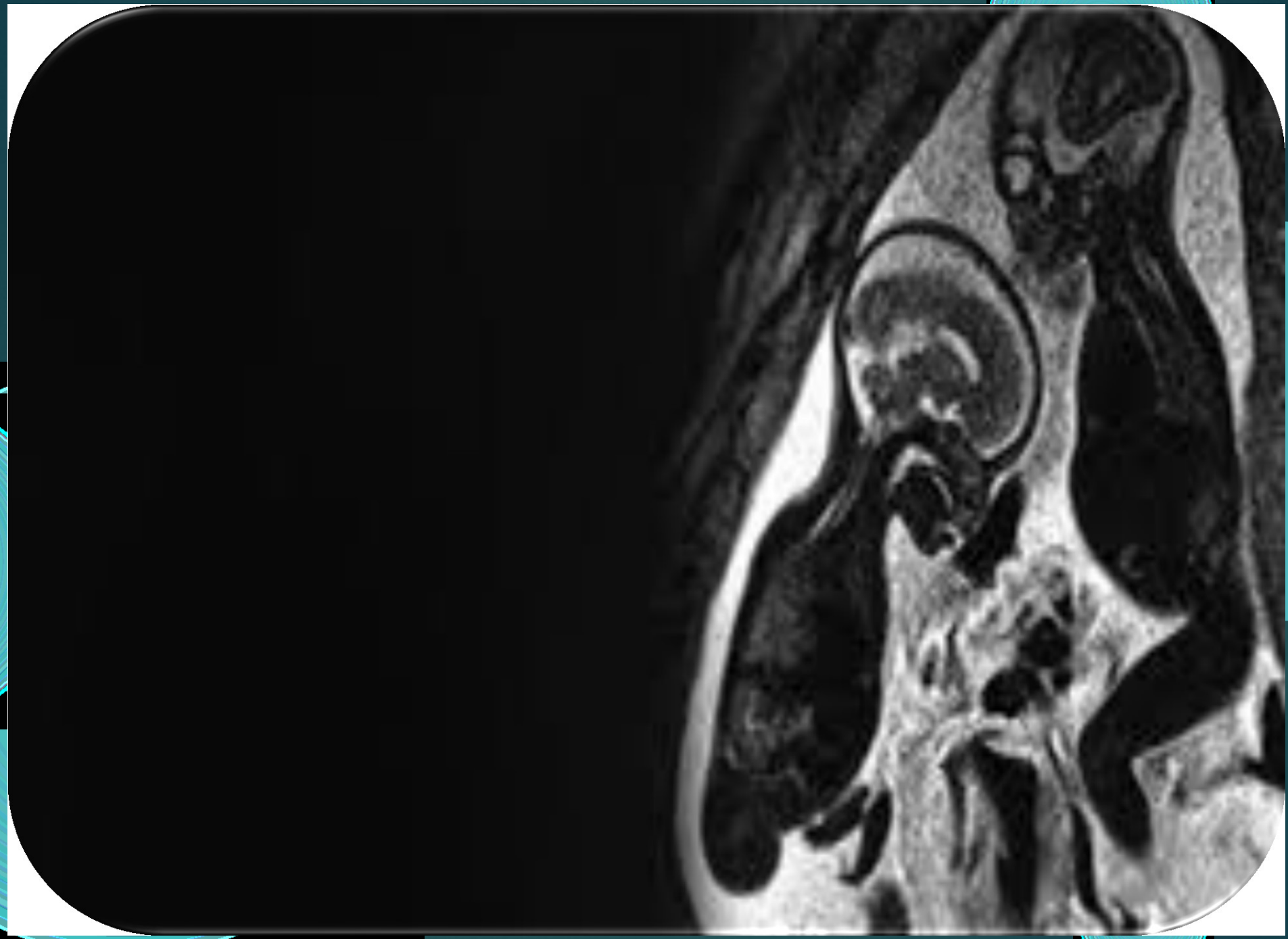
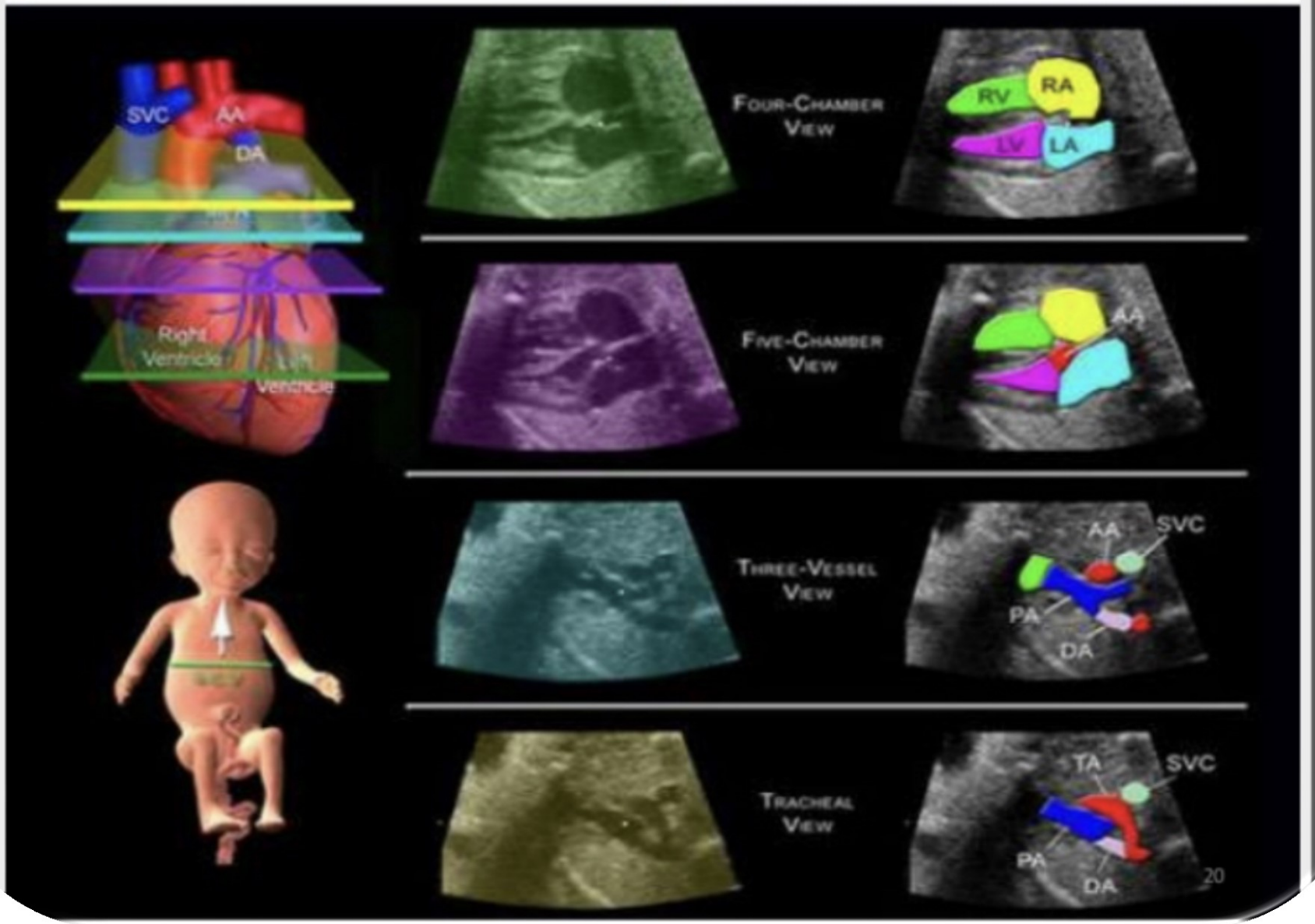


FETAL ECHOCARDIOGRAPHY

- Fetal echocardiography is capable of diagnosing most significant congenital heart lesions as early as 17-19 wk of gestation.
- When this technique is used with duplex or color flow Doppler, it can identify a number of major structural cardiac defects & -59% to 93% of aneuploid fetus has abnormal ductus venosus rhythm. flow.
- Abnormal tricuspid regurgitation in 1st trimester is associated with fetal aneuploidy.
- TR is considered to be present if a regurgitant jet of at least 60cm/sec is noted extending to over half of systole
- Down syndrome detection rate during 11 to 13 weeks is 68% alone

FETAL ECHOCARDIOGRAPHY : cont…



☞MATERNAL SERUM SCREENING

✓ **Downs syndrome :**

1st Trimester Screening Tests

- *Maternal Serum Markers*
  - -Preg. asso. Placental Protein A (PAPP-A)
  - -Free ß hCG

  } 65%

- Fetal Marker- Nuchal thickness } 60-75%

} 85%

2nd Trimester Screening Tests

- *Maternal Serum Markers*
  - -AFP
  - -E3
  - -hCG

  } ***Triple test 70%***

  -Inhibin A

} **76%** ***Quadruple test***

} 94%

MATERNAL SERUM SCREENING

- Neural Tube Defects & Abdominal Wall Defects:
- AFP is produced by the yolk sac & later by the liver; it enters the amniotic fluid & then the maternal serum via fetal urine.
- In the condition of an open NTD (eg , anencephaly, spina bifida) & abdominal wall defects in the fetus, AFP diffuses rapidly from exposed fetal tissues into amniotic fluid, and the MSAFP level rises.
- Also, a NTD can be distinguished from other fetal defects, such as abdominal wall defects, by the use of an **Acetylcholinesterase test** carried out on amniotic fluid. If the level of acetylcholinesterase rises along with AFP, it is suspected as a condition of a NTD.

Conditions associated with abnormal level of MSAFP

ELEVATED LEVELS

- underestimation of gestation age.
- Multifetal gestation.
- IUD •
- NTD •
- Gastroschisis and omphalocele •
- Low maternal weight •
- Pilonidal sinus
- Esophageal or intestinal obstruction.
- sacrococcygeal teratoma.
- Urinary obstruction •
- Renal anomalies- Polycystic kidneys, renal agenesis, congenital nephrosis. (as high as > 10 MOM)
- Congenital skin defects
- Cloacal extrophy
- Chorioangioma of placenta
- Placental abruption
- Oligohydramnios
- Preeclampsia
- Low birth weight
- Maternal Hepatoma or teratoma

LOW LEVELS

- Obesity
- Diabetes
- Chromosomal Trisomy.
- GTD
- Fetal death
- overestimated gestational age

Combined 1st and 2nd trimester screening

1) **Integrated screening:**

- combines both 1st and 2nd trimester screening test into single risk.
- Highest downs syndrome detection rate- 90% to 96%.
- Disadvantage being test results are not available until the second trimester test has been complete

2) Sequential screening:

- This test obviate some of the disadvantages seen in integrated test. in this test, result of 1st trimester screening are disclosed to women at highest risk, thus allowing them the option of earlier invasive testing and those at lowest risk can still take advantage of higher detection rate achieved with second trimester screening.
- There are two testing strategies

1) Stepwise sequential screening-
2) Contingent sequential screening-

Stepwise sequential screening:

- In this strategy women determined to be at high risk (Downs syndrome risk above predetermined cutoff) after 1° trimester screening are offered genetic counseling and option of diagnostic testing, those at low risk offered 2nd trimester screening and both 1st and 2nd trimester results are used for final risk thus increasing detection rate in low risk women and allows early confirmation in high risk women.
- Detection rate is 95%.

Contingent sequential screening:

- This strategy classifies women as having High Low and Intermediate risk based on 1st trimester screening.
- High risk-CVS
- Low risk-No further screening
- Intermediate risk-2nd trimester screening.
- Detection rate is 88 to 94%

Triple test

- Triple test screens for following fetal disorders.

Disorders	MSAFP	uE3	Beta hCG	**Inhibin A**
Open NTD	increased	No change	No change	No change
Downs syndrome	decreased	decreased	increased	Increased
Trisomy 18	decreased	decreased	No change	No change

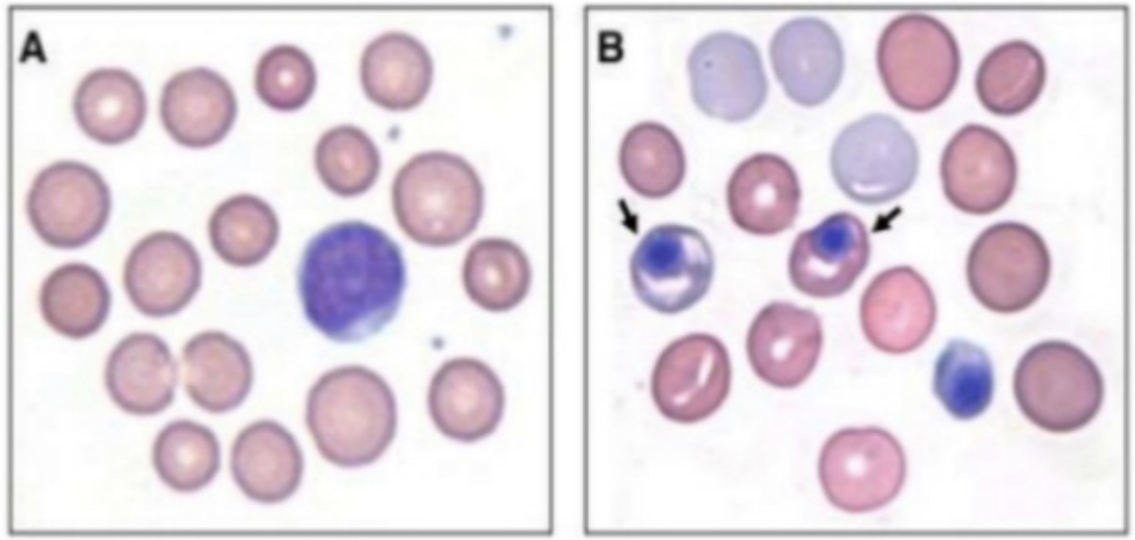
Separation of fetal cells from the mother's blood

✓ A technique currently being developed for clinical use involves isolating fetal cells from maternal blood to analyse fetal chromosomes and/or DNA. Ordinarily, only a very small number of fetal cells enter the maternal circulation; but once they enter, can be readily identified.

✓ These cells can be collected safely from approximately 12- 18 weeks' gestation onward.

✓ Nucleated fetal red blood cells (erythroblasts) are currently the ideal candidates for analysis, although leucocytes & trophoblast cells may also be identified

- ✓ Fetal blood cells can then be analyzed for the diagnosis of genetic disorders using FISH, PCR etc.
- ✓ Fetal cells separated from a mother's blood have been successfully used in the diagnosis of cystic fibrosis, sickle cell anemia, and thalassemia in a fetus.



A. Maternal RBCs

B. Fetal RBCs (nucleated)

Cell-free fetal DNA in maternal blood

- Both the mother and fetus produce cell-free DNA from apoptotic cells
- maternal DNA originates in bone marrow
- fetal DNA originates in placenta

- 150-200bp .

-Adequate amounts for clinical testing after 10 weeks (detected after 32 days gestation) .

- Can also be used in the third trimester

-Undetectable after 2 hours postpartum

An average maternal plasma sample contains

- 90% maternal-cell-free DNA
- -10% fetal cell-free DNA

Overall performance of NIPT for aneuploidy

- Sensitivity for detection of trisomy 21 is >99%
- False positive rates are extremely low (-0,5%)

-May have a biological basis.

- False negative rates are even lower (but not 0%)

-Mostly due to low fetal cell-free DNA fraction

INVASIVE TECHNIQUES

Pre procedure genetic counseling:

- Pre procedure counseling is necessary as it will allow patients to understand their situation allow them to give consent (or withhold) for the procedure.

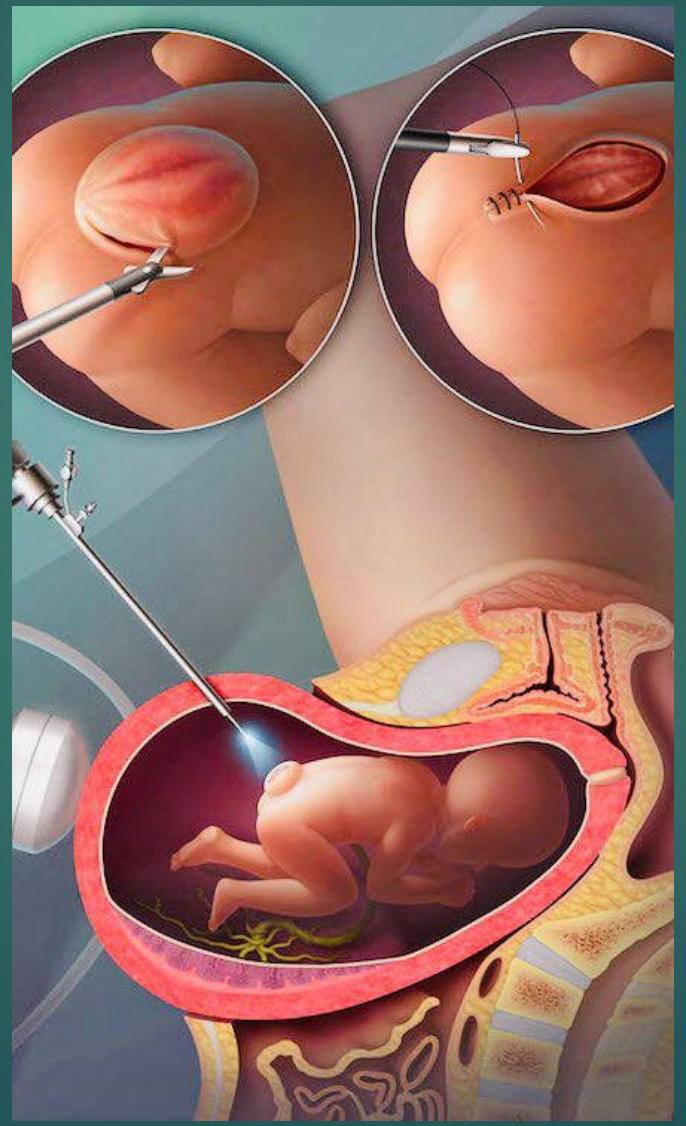
Following points to be explained:

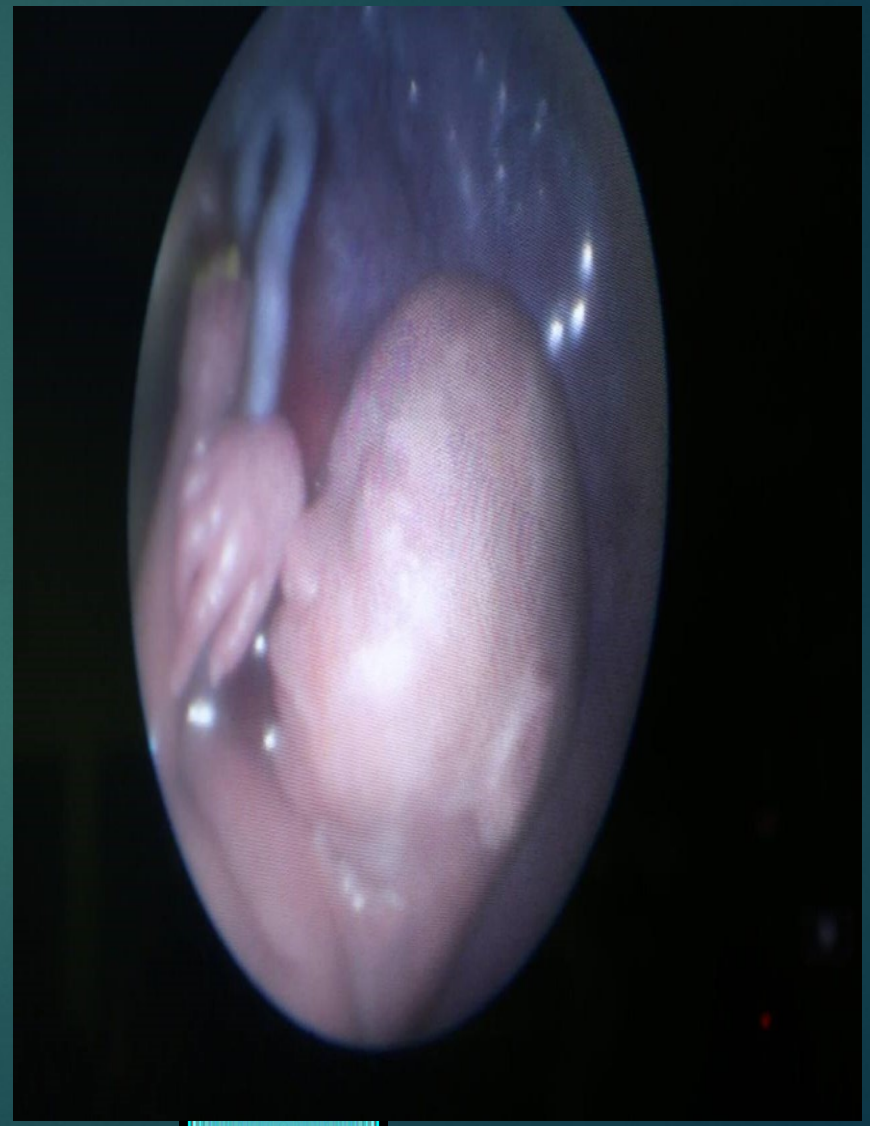
1. The chance that fetus will be affected.
2. Nature and consequences of disorder.
3. Risks and limitation of procedure.
4. Time required for reporting.
5. Possibility of complications of the procedure such as procedure related fetal loss, failed attempt to obtain a testable sample

INVASIVE TECHNIQUES

Fetal visualization:

1. Embryoscopy
2. Fetoscopy





Fetal Tissue Sampling:

- Amniocentesis
- Chorionic villus sampling (CVS)
- Percutaneous umbilical blood sampling (PUBS)
- Percutaneous skin biopsy
- Other organ biopsies, including muscle & liver biopsy

Fetal Tissue Sampling

Chorionic villus sampling (CVS):

✓ Under USG guidance, a sample of placental tissue is obtained- through a catheter places either transcervically or transabdominally.

✓ Performed at or after 10 wks' gestation, CVS provides the earliest possible detection of a genetically abnormal fetus through analysis of trophoblast cells.

✓ Transabdominal CVS can also be used as late as the 3rd trimester when amniotic fluid is not available or when fetal blood sampling cannot be performed.

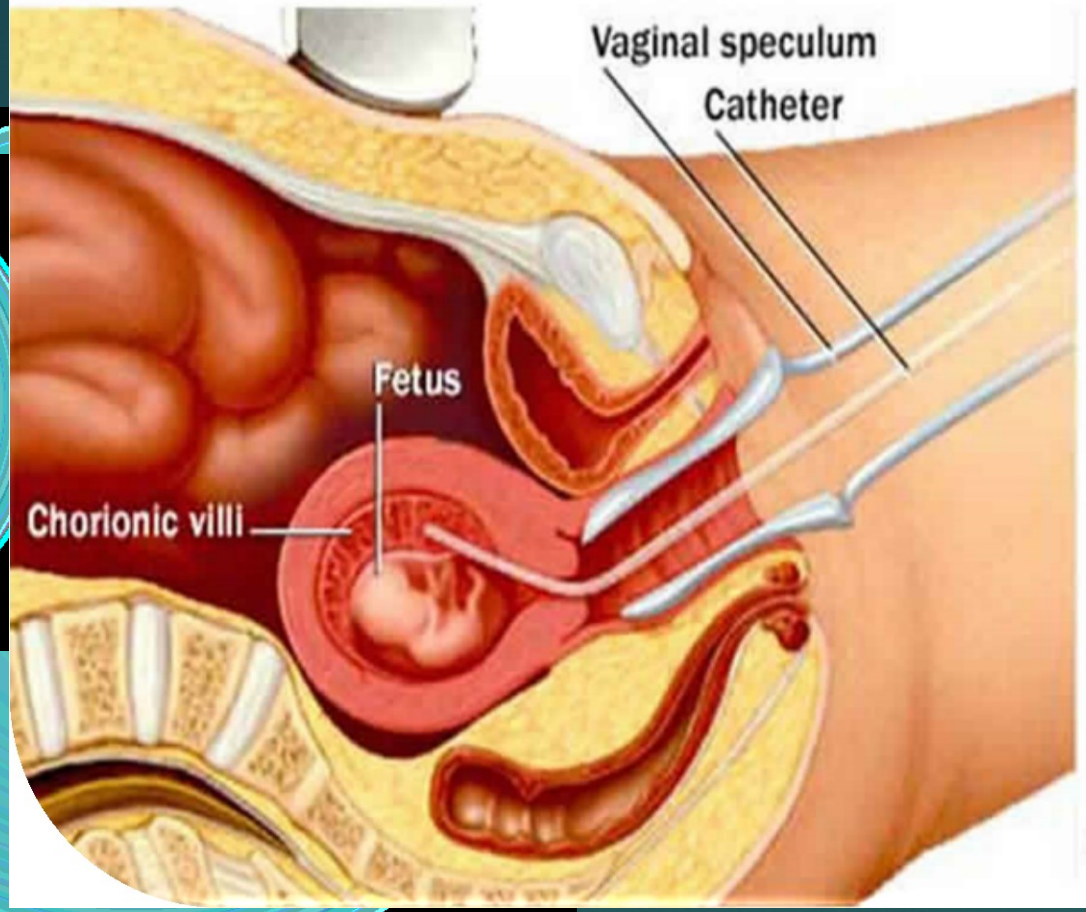
✓ CVS, if preformed before 10 wks' gestation, can be ass. with an increased risk of fetal limb reduction defects & oromandibular malformations.

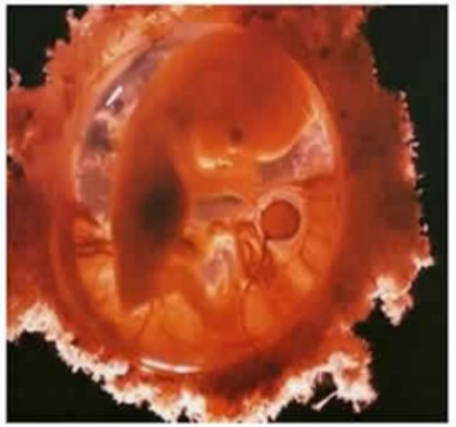
Chorionic villus sampling (CVS):

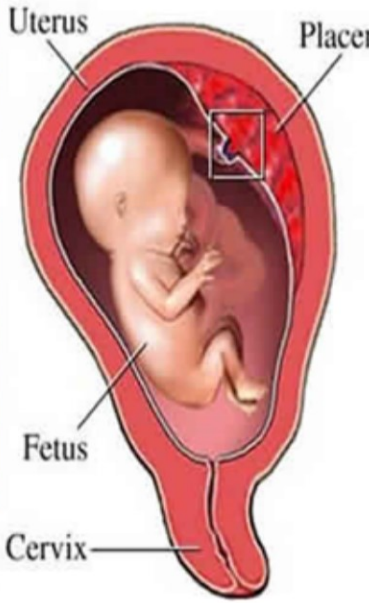
- Direct preparations of rapidly dividing cytotrophoblasts can be prepared, making a full karyotype analysis available in 2 days. Although direct preparation minimize maternal cell contamination, most centers also analyse cultured trophoblast cells, which are embryologically closer to the fetus.This procedure takes 8-12 days.
- In approximately 2% of CVS samples, both karyotypically normal & abnormal cells are identified. Because CVS acquired cells reflect placental constitution, in these cases, amniocentesis is typically performed as a followup study to analyze fetal cells. Approximatally 1/3rd of CVS mosaicisms are confirmed in the fetus through amniocentesis.

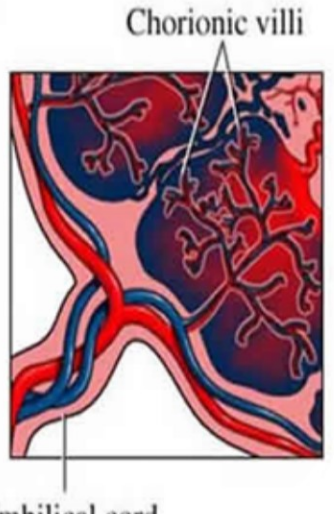
Chorionic Villus Sampling

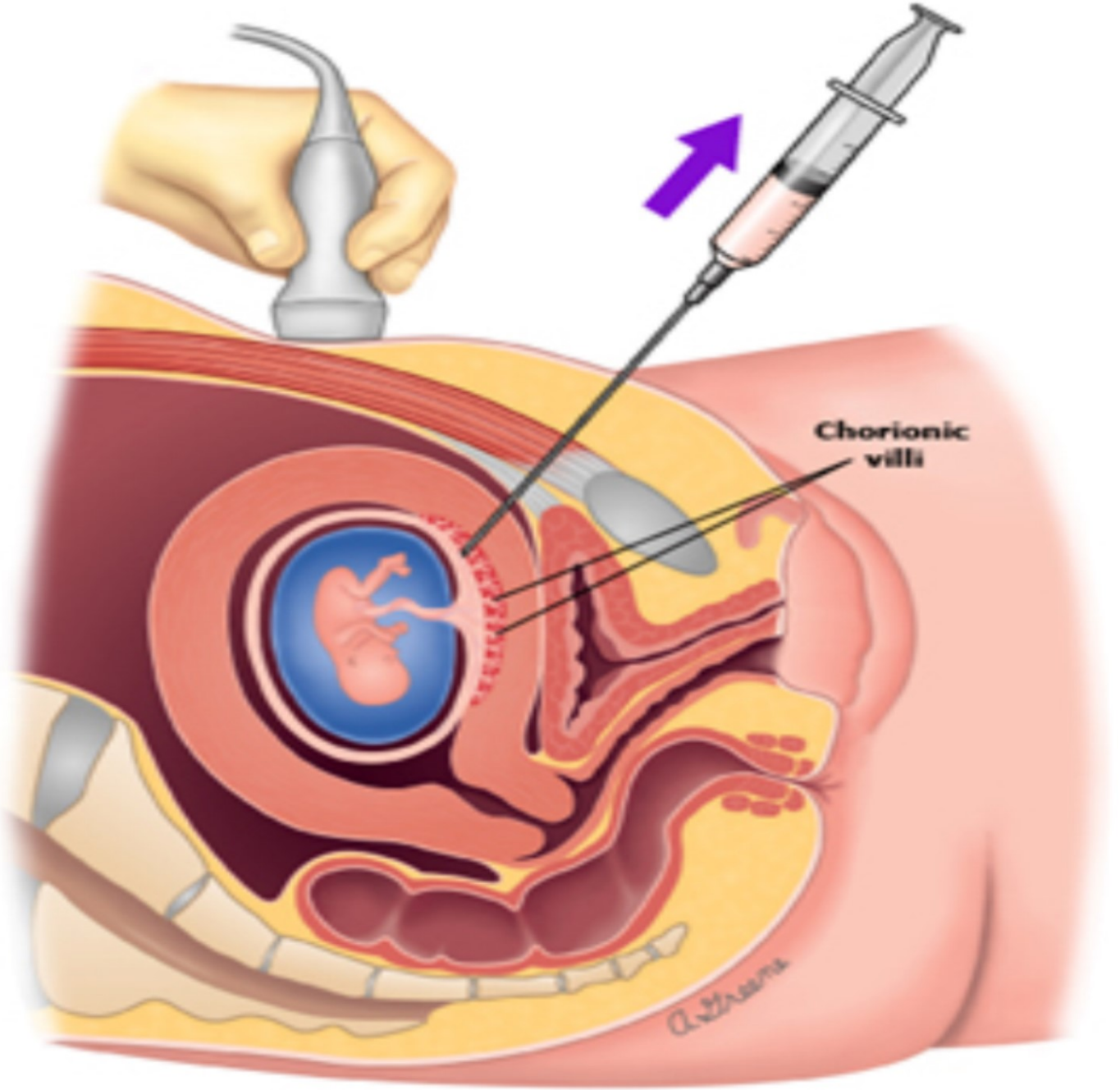
- catheter inserted through vagina into uterus to sample villi of placenta
- more fetal cells, earlier in pregancy










Chorionic
villi

Amniocentesis vs. CVS

	AMNIOCENTESIS	CVS
Procedure	AF removed by needle	CV removed by catheter (TC) or needle (TA)
Timing	16-20th week	10-12th week
Fetal malform. risk	-	1:3000 vascular limb malformation
Pregnancy loss	0.5-1%	2-3%
Time required for cytogenetic dg.	2-3 weeks	1 week
Accuracy	Highly accurate	Highly accurate
		Risk of placental mosaicism

Fetal Tissue Sampling

- **Percutaneous umbilical blood sampling (PUBS) (cordocentesis)**

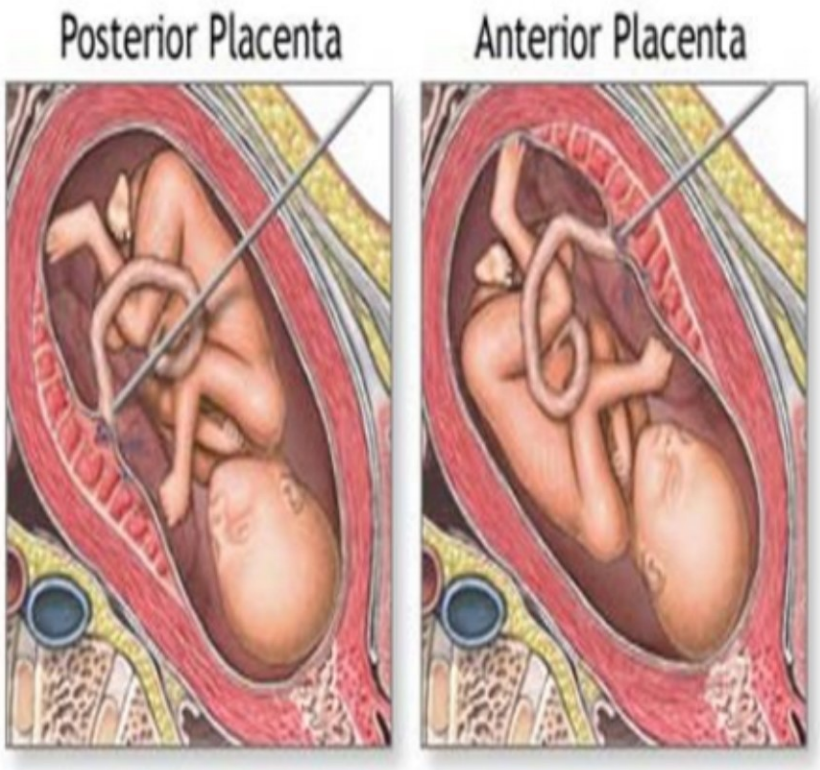
✓ PUBS is preformed under USG guidance from the 2nd trimester until term.

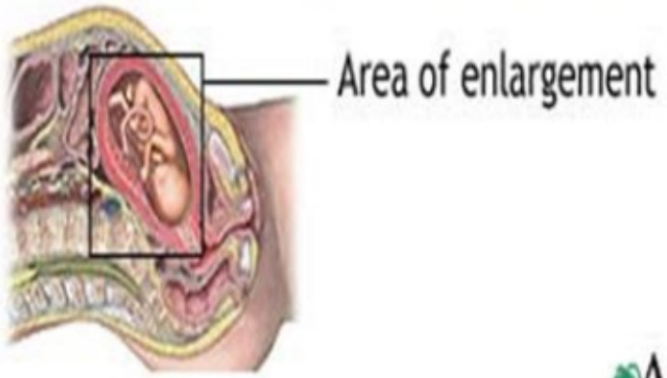
✓ PUBS can provide diagnostic samples for cytogenetic, hematologic, immunologic, or DNA studies: it can also provide access for treatment in utero.

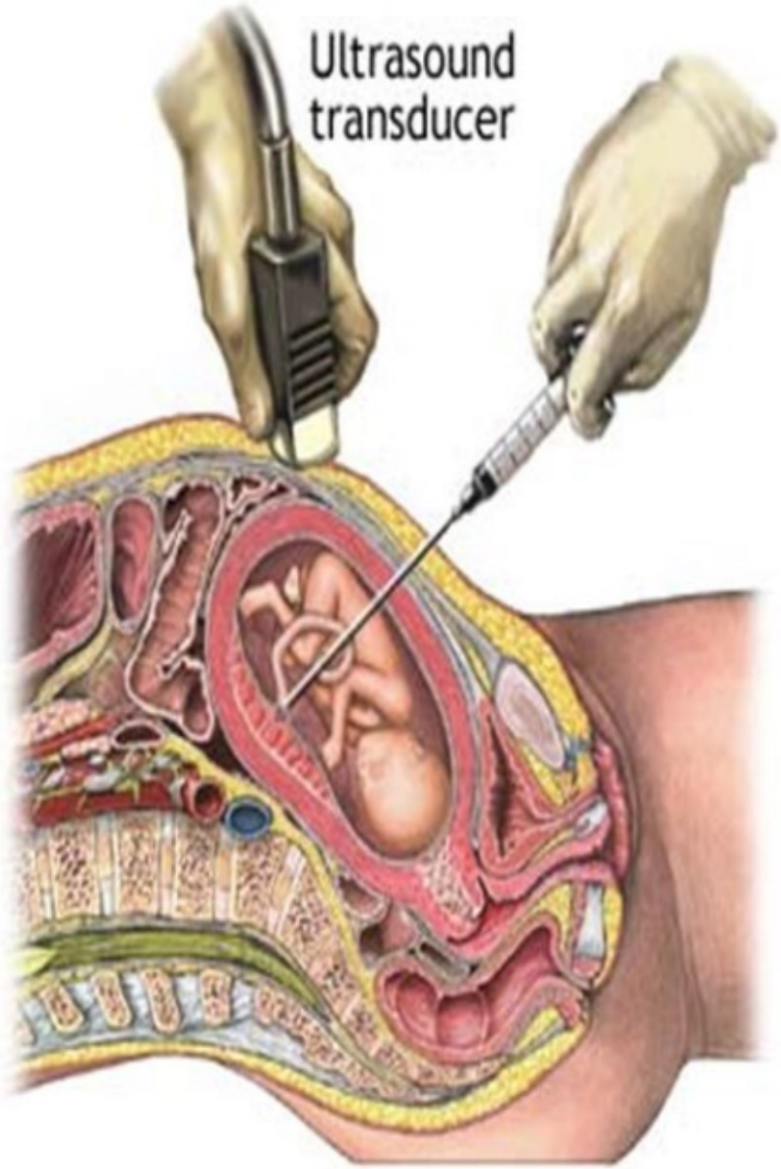
✓ An anterior placenta facilitates obtaining a sample close to the cord insertion site at the placenta.

✓ PUBS has a 1% - 2% risk of fetal loss, along with complication that can lead to a preterm delivery in another 5%

Percutaneous Umbilical Blood







Fetal Tissue Sampling

- **"Preimplantation Biopsy or Preimplantation Genetic Diagnosis:**

✓ The most frequent candidates are parents with family histories of serious monogenic disorders & translocations, who are therefore at increased risk for transmitting these conditions to future generations.

✓ Polar body & blastomere testing are the two primary methods.

✓ In Polar Body Testing, positive test results in two polar bodies ensure that the egg itself is unaffected - therefore, the mutation has segregated to the polar body, not to the developing ovum. Once an egg is found to be unaffected, it is fertilized via traditional in vitro fertilization (IVF) & implanted into the uterus.

Blastomere PGD first requires traditional in vitro fertilization, after which cells are grown to the 8-cell stage. One or two cells are harvested & anlaysed, & an unaffected blastocyst is implanted into the uterus.

✓ An advantage to preconception testing over traditional post conception prenatal diagnosis is that it allows parents to avoid the possibility of receiving abnormal prenatal diagnosis results, & thus the difficult decisions associated with pregnancy management and/or maintenance.

✓ PGD can be laborious, time-consuming & expensive. Complicating factors include a high rate of polyspermia, & a small amount of DNA in polar bodies (making it difficult to amplify) which can produce less definitive test results.

Termination of pregnancy (TOP)

- TOP may be permitted at any time when serious disease threatens the mother's life (e.g. heart failure, obstetric complication)
- TOP may be permitted at any time when fetal disease is incompatible with postpartum life (e.g. anencephaly)
- TOP up to the 12th gestational week is permitted, when the risk of genetic disorder or teratogenic damage to the fetus exceeds 10%
- TOP is possible until the 24th gestational week, when the risk of a severe, difficultly curable or incurable fetal disease is 50-100% (e.g. Down syndrome)

Termination of pregnancy (TOP)

Fetal indications:

- The probability of a severe AD disorder is 50% (Huntington disease)
- Mother carrying an XR-gene is pregnant with a male fetus
- Severe CNS malformation
- Severe bilateral kidney disease
- Severe chromosome aberration

Do you have any question

THANK YOU