Prenatal Diagnosis

Introduction

I- Families at risk

I- 1. Advanced maternal age
I- 2. Recurrence of numerical and structural chromosomal anomalies
I- 3. Fragile X syndrome and mental retardation
I- 4. Chromosomal instability
I- 5. Hereditary metabolic diseases
I- 6. Neural tube defects

II- Foetal Ultrasonography

II- 1. Ultrasonography
II- 2. Cardiac ultrasonography
II- 3. Markers suggesting the presence of a birth defect
II- 4. Foetal defects detected by ultrasound during the second trimester of pregnancy

II- 4.1. Nervous system anomalies
II- 4.2. Cardiovascular defects
II- 4.3. Thoracic anomalies
II- 4.4. Gastro intestinal malformations
II- 4.5. Urogenital malformations
II- 4.6. Musculo skeletal malformations
II- 4.7. Other anomalies

III- Techniques to obtain foetal tissues

III- 1. Amniocentesis
III- 2. Chorionic villus sampling (CVS)
III- 3. Cordocentesis
III- 4. Foetoscopy
III- 5. Fetal cells in maternal circulation
Introduction
Prenatal diagnosis answers the need to detect early in pregnancy a number of foetal anomalies and genetic diseases. The prenatal diagnosis of genetic diseases has become widely available for pregnancies at risk in the last three decades. In 1976 results of three multicentric studies, realized in America and Europe, confirmed that the tests performed on amniotic fluid cells (amniocytes) were reliable and that the amniocentesis done during the second trimester was a low risk procedure both for the mother and her foetus.

Approximately 3% of viable foetuses would be born with a severe anomaly. We now feel the impact of prenatal diagnosis on the incidence of severe defects at birth since a number of them will be diagnosed as early as the end of the first trimester. If a woman is known to be at risk to conceive a child with a genetic disease a prenatal diagnosis could be indicated. Knowing the nature of the anomaly to be detected, this diagnosis can be realized in ultrasonography, with or without the study of amniotic fluid cells or other foetal tissues. Before any intervention or use of an invasive procedure is attempted, the sine qua non rule of prenatal diagnosis is to make sure that the genetic disease, likely to be present, is detectable and that there is a possibility to show or exclude this defect by testing the foetal tissues. Prenatal diagnosis allows couples at risk to envisage a pregnancy since an alternative is now offered to them.

I- Families at risk
1- Advanced maternal age
Women who are 35 or more at delivery have a higher risk of giving birth to an infant with a chromosomal defect due to a non disjunction. This increased risk is due in part to ovum aging. This risk increases with age (fig1) and frequently involves a trisomy 21 (Down syndrome) that is the most frequent autosomal anomaly. Trisomies 13 and 18 and sex chromosome defects, XXY and XXX are frequently observed in children...
born to mothers in this age group. Among countries where prenatal diagnosis is available selection criteria for the availability of prenatal diagnosis is variable and amniocentesis will generally be offered to pregnant women aged 35 or more at the time of delivery. The availability of preventive medical measures for pregnant women may include a screening test for trisomy 21 by testing for maternal serum markers (see serological markers). This screening test, to some extent, may allow the identification of women more at risk of bearing a trisomic child and, if the test was negative, reassure those who would elect not to have an amniocentesis.

![Figure 1 - Maternal age and incidence of trisomy 21 at birth](image)

**Figure 1 - Maternal age and incidence of trisomy 21 at birth**

**I- 2. Recurrence of numerical and structural chromosomal anomalies**

All chromosomal defects (see also: Chromosomes abnormalities) resulting from a division error, or non disjunction, can reoccur in more than 1% of cases in a subsequent pregnancy and this risk can be very high for a woman in the 30 years or less age group. At the age of 20 the risk of trisomy 21 is approximately 1/2000, 1/1200 at 25, 1/900 at 30, 1/400 at 35, 1/100 at 40 and 1/40 at 45 years of age. This phenomenon also implies that the aneuploidy can involve chromosomes other than the one diagnosed initially. For instance a woman who had conceived a child with a trisomy 21 could, in a subsequent pregnancy, bear a child with a trisomy 13 or 18, or even a sex chromosome anomaly XXX or XXY. It is also possible that a trisomy involving other autosomes be non viable and result in an early miscarriage.

Familial chromosomal translocation: one of the parents is carrier of a balanced chromosomal translocation and according to: 1- the type of translocation, 2- the importance of the segments involved, 3- the segregation of chromosomes in meiosis, there is variable risk frequency:

- of spontaneous miscarriage due to a major imbalance of the chromosomal complement
- to give birth to an affected infant with a viable chromosomal defect
- that the foetus be a normal carrier like one of his parents
and the foetus may have a normal karyotype

When an individual is found to have a structural aberration the rule is to obtain the karyotype of his parents and if necessary of the siblings: first to find the origin of the defect and second to inform family members of the reproduction risk if themselves are normal physically but carriers of a translocation (fig 2). We also suggest to karyotype couples who have a personal or familial history of repeated foetal losses or birth of children with mental retardation with or without malformations or dysmorphism.

![Figure 2 - Partial family tree of a translocation 4;18](image)

Individuals with a Fragile X syndrome (see also: Dysgonosomies and related syndromes) have a peculiar facies and mental retardation of variable severity. The chromosomal study reveals a reduced density of the chromatin in region Xq28. The genetic defect was identified as an abnormal CGG triplet amplification (>60) at the Xq27.3 locus. If a child is affected, his mother can be a carrier (we then speak of pre-mutation) of the syndrome and she is expected to have an amplification of triplet CGG in the range of 60 to 200. The amplification inhibits the expression of gene FMR-1. Individuals of both sexes can be affected but males are generally more severely affected.

If there is a positive family history of X linked mental retardation a molecular study may allow the detection of a Fra X syndrome and if indicated a prenatal diagnosis could be offered.
I- 4. Chromosomal instability
Some syndromes, called chromosome instability syndromes manifest an unstable chromosomal structure. We cite here as examples Fanconi disease characterized by anemia, growth delay, skeletal anomalies and Bloom syndrome characterized by anemia, dwarfism and light hypersensitivity. Both have increased chromosomal breakages and affected individuals are predisposed to cancer. These diseases have an autosomal recessive mode of inheritance and the recurrence risk is 25% after the birth of an affected child. Chromosomal exchanges are frequent in Fanconi disease while sister chromatid exchanges are observed in Bloom syndrome. Using appropriate techniques for the culture of foetal cells and special staining techniques for the chromosomes, these syndromes can be diagnosed prenatally when the parents have been shown to be heterozygous or normal carriers. However the demonstration of specific mutations in those rare diseases is not always feasible. If a prenatal diagnosis is requested one can count on the molecular study of foetal cells only if parental mutations have been identified prior to the procedure.

I- 5. Hereditary metabolic diseases
a-metabolic disease diagnosed in a child and finding of an enzyme deficiency or a mutation that could be detected in a subsequent pregnancy.
b-known metabolic disease: the study of the parents show that they are both carriers (heterozygous) and that they have a 25% risk of conceiving an affected child like in cystic fibrosis. Due to the high gene frequency of this disease screening programs to detect carriers are now being offered in some high risk populations.

I- 6. Neural tube defects
Neural tube defects have a multifactorial etiology and their incidence is widely variable. They used to be more frequent in the British Isles, Canada, China and other countries like Hungary with an incidence of 5/1000 births and a recurrence risk of 5%. In France and United States the incidence was more like 1/1000 with a low recurrence risk.
It has been shown, first in Great Britain, that the intake of folic acid may help the closure of the neural tube and lower the recurrence frequency in high risk pregnancies. Closure of the neural tube takes place during the first 4 weeks of embryogenesis. The intake of folic acid as soon as a pregnancy is planned, and for the first two months has reduced the incidence and recurrence of the neural tube defects.
It is highly recommended when there is a family history of neural tube defect to monitor a pregnancy in ultrasonography to make sure that both foetal cranium and rachis are normal. In families at risk it is very important to encourage the intake of folic acid as soon as a woman plans a pregnancy or cease all means of contraception.
Spina bifida aperta: opening of the tissues over the bone defect with or without extrusion of the meninges; it is occulta if the skin is normal.
Anencephaly: closure defect of the cranial vault. The skull defect may be limited to a region of the cranium and be variable in size, we then refer to an encephalocele.
Open lesions of the neural tube and cranium induce an elevation of alpha-foetoproteins in amniotic fluid and subsequently in maternal serum.
If there is a history of previous or familial microcephaly a family study will help determining if this is a hereditary defect: autosomal dominant or recessive.
Ultrasound examinations of the developing foetus may facilitate the follow up of the head bi-parietal diameter and circumference, but only severe growth deficiencies will likely be detected.
II- Foetal Ultrasonography

II- 1. Ultrasonography (Fig 3) makes use of ultrasounds to study tissues and organs. It is applied from the first trimester but it is only during the second trimester that one can best evaluate foetal morphology and preferably around the 18th week of gestation.

II- 2. Cardiac ultrasonography, that allows examination of great vessels and heart chambers, is done usually around the 20th week of pregnancy.

II- 3. Markers suggesting the presence of a birth defect.

Ultrasonographic markers are variations observed during the ultrasound session that will alert the examiner to the possibility of an abnormal foetal development or a genetic disease. Those markers can reveal for example the possibility of a chromosomal anomaly such as a trisomy 21, 13, 18, or a chondrodysplasia.

ULTRASOUND MARKERS SUGGESTING THE PRESENCE OF A FOETAL ANOMALY

- Abdominal calcifications (meconial peritonitis)
- Bladder hypertrophy (urethral valve)
- Bone hypodensity (hypophosphatasia)
- Cerebral ventricles increased (hydrocephaly)
- Cono-truncal defect or defect of the heart common trunk, manifested as a tetralogy of Fallot or a vascular defect (Di George, and velo-cardio-facial syndromes secondary to a deletion — del 22q11-)
- Cranial vault ossification defect (anencephaly)
- Cystic hygroma (Tumer syndrome, 45X)
- Double buble in the gastric region (duodenal atresia)
- Endocardial cushion defect, or of the primary cardiac septum, described as an atrium septal defect (trisomy 21)
- Facial hypoplasia and cleft lip (trisomy 13 - holoprosencephaly)
Fractures (osteogenesis imperfecta)
Increased number of choroidal cysts (trisomy)
Increased volume of cerebral ventricles (hydrocephaly)
Lemon sign, lemon shape head (spina bifida)
Long bones shortening (bone dysplasia)
Nucal skin folds increased (trisomy 21)
Persistent flexed fingers (trisomy 18 - arthrogryposis)
Polydactyly (trisomy 13; Ellis Van-Creveld syndrome)
Ptterygium colli (Turner syndrome; pterygium multiple)
Stomach unseen (oesophageal atresia)
Thoracic deformity (skeletal dysplasia)

II- 4. Foetal defects detected by ultrasound during the second trimester of pregnancy
II- 4.1 Nervous system anomalies
  Anencephaly
  Cyst of the posterior fossa
  Encephalocele
  Facial dysplasia
  Holoprosencephaly (cerebral ventricle and facial anomalies)
  Hydrocephaly
  Microcephaly
  Myelomeningocele
  Porencephaly (cystic lesions of the brain)
  Rachischisis (significant vertebral closure defect)
  Spina-bifida

II- 4.2 Cardiovascular defects
  Arythmia
  Pericardial fluid collection
  Septal defect
  Situs inversus
  Valvular defect
  Vascular anomalies
  Ventricular hyperplasia
  Ventricular hypoplasia

II- 4.3 Thoracic anomalies
  Atresia of the oesophagus
  Diaphragmatic hernia
  Pleural effusion
  Intrathoracic cysts

II- 4.4 Gastro intestinal malformations
  Absence of abdominal muscles
  Ascites
  Cystic lymphangioma
  Diaphragmatic hernia
  Intestinal atresia
  Laparoschisis (para-umbilical extrusion of abdominal viscerae)
  Mesenteric cyst
  Omphalocele (umbilical hernia of abdominal viscerae)
  Umbilical cord tumor (chorioangioma)

II- 4.5 Urogenital malformations
Hydronephrosis
Hydroureter
Polycystic kidneys
Renal agenesis
Teratoma
Urethral valve

II- 4.6 Musculo skeletal malformations
Arthrogryposis
Bone dysplasias
Club foot
Fractures
Limb palsy
Limb reduction defect
Mineralization defect
Pterygium colli,
Pterygium multiple,

II- 4.7 Other anomalies
Acardiac monster
Amniotic band
Cystic lesions
Siamese twins
Teratomas
Tumors

III- Techniques to obtain foetal tissues
III- 1. Amniocentesis
The amniocentesis (fig 4) is early if done around the 12th week of gestation. Today several prenatal diagnostic clinics perform amniocenteses between the 14th and 16th week of pregnancy. Studies have shown an increased loss of amniotic fluid if the amniocentesis done before the 12th week and there is a risk of skeletal anomalies in particular of club feet secondary to oligoamnios. According to the age of pregnancy from 10 to 30 ml of fluid are obtained during the procedure ( fig 5). Foetal cells from the upper digestive system, urinary tract, skin and membranes are found in the fluid and recuperated by centrifugation of the specimen. They are then kept in culture for a period of 5 to 10 days in a culture medium to which calf serum has been added. Cellular multiplication is then sufficient and allows the preparation of microscopic slides allowing the numerical and structural studies of the metaphasic chromosomes. Treatment of chromosomes during the slide preparation reveals segments of different intensity or banding patterns. Those bands reflect a variable ratio of AT;GC nucleotides on the chromatids and help to identify chromosome pairs.
III- 2. Chorionic villus sampling (CVS)

The biopsy or aspiration of chorionic villi by the vaginal route (fig 6) yields foetal cells, several of which are in the process of dividing and can be analysed during the hours following the procedure. There is a risk of miscarriage and maternal cell contamination of the specimen thus leading a number of clinicians to abandon this procedure done before the 12th week of pregnancy. Reduction limb defects have been reported if the CVS is done towards the end of the first trimester. In special circumstances when the risk of genetic disease is high as for instance in hereditary metabolic diseases or if one of the parents is carrier of a balanced chromosomal translocation, this technique has the advantage of reaching a diagnosis around the 11th or 12th week of gestation.
Blood can be obtained from the foetal cord under ultrasound guidance. If at the end of second trimester there is an urgent need to confirm a diagnosis or to avoid extraordinary measures if there is a threat of premature labor and the foetus is found to be abnormal. This procedure will allow a short-term chromosomal analysis from lymphocytes or an enzyme study. The rapid cytogenetic study could also confirm or exclude a chromosomal defect previously found in the amniocytes. This approach can also be useful to delineate mosaicism like, for instance, a trisomy 20 which usually has a favourable outcome or identify a chromosomal marker confined to annexial tissues. Cordocentesis has been used to study severe immunological disorders by measuring adenosine deaminase and doing T cells analysis.

Foetoscopy is a technique that allows to visualize the foetus around the end of the second trimester, by introducing a tube with optic fibres through the abdomen and the uterus allowing to biopsy the foetus or proceed to surgical interventions. For security reasons this invasive technique is not a routine procedure and is rarely utilized except in development programs.

The presence of foetal cells in the maternal blood stream could give us some information of the chromosomal complement and the foetal genotype. Research in this direction has been initiated several years ago but up to date has yielded meagre results on the efficacy of this non-invasive procedure. Several difficulties are encountered: the low frequency of nucleated cells, means to isolate them, their identification and genetic analysis. The venue of FISH technique and other means to identify abnormal chromosomal complements, and the PCR (polymerase chain reaction) for molecular analysis have recently convince researchers not to abandon this avenue which could be a great asset to the prenatal diagnosis of genetic disease.
Several congenital malformations have a hereditary origin and their mode of transmission can be autosomal dominant, recessive or X linked. Some syndromes could be identified during the second trimester of pregnancy if a major anomaly is detected at ultrasound (like bone dysplasias). However if there is a history of congenital anomaly of unknown etiology but seen in a previous pregnancy, the mother could be reassured at ultrasound if she is pregnant again. This measure applies for instance in cases of omphalocele and laparoschisis for which the risk of recurrence is low.

V- Maternal serum markers
Serological markers are normal proteins found in the maternal circulation and if their level is abnormal it will allow the detection of some foetal pathologies early in pregnancy. The screening efficacy is increased if both procedures foetal ultrasonography and marker studies are completed simultaneously.

V- 1. Neural tube defects
Alpha-1 foetoprotein (AFP) constitute 20% of circulating foetal proteins and its level varies with foetal age at the time it is measured (fig 7). An increase of AFP in the amniotic fluid will reflect on the maternal serum level and may alert to the presence of an open neural tube defect. A higher than normal level in the amniotic fluid may be due to a skin lesion unseen at ultrasound or may be due to foetal demise.

![Figure 6 - Amniotic fluid AFP values during the second trimester](image)

V- 2. Trisomy 21 or Down syndrome
AFP may also lead to suspecting a foetal trisomy 21 if the maternal level is low according to the maternal age around the 16th week of pregnancy

a **triple test** is the combination of three maternal serum markers: human chorionic gonadotrophin (hCG), oestriol (uE3) and AFP. The measure of these proteins at a given maternal and gestational ages, may give an approximate risk figure that the foetus is trisomic (21). The sensibility of this test is 60% and more based on criteria used for the evaluation.

an early screening procedure based on the nucal translucency, hCG and a placental protein PAPP-A, is available in some clinics from the end of the first
trimester to the 13th week of pregnancy. Nuchal translucency corresponds to the nuchal tissue thickness. A measure over 3 mm is considered suspicious at 12 weeks of pregnancy. This screening procedure has a 80% sensitivity for trisomy 21 and can suggest the presence of other pathologies. An abnormal result will be validated by a chromosomal study.

VI- Fluorescence in situ hybridization (FISH)
Fluorescence in situ hybridization makes use of molecular probes marked in fluorescence that correspond to a gene or DNA sequence and showing a bright signal under UV microscope at a specific locus on a chromosome. First the FISH technique can apply to interphasic cells readily obtained at amniocentesis and confirm the presence of an euploid or aneuploid complement like for chromosomes X, Y, 13, 15, 18, 21. This technique also applies to chromosomal markers that can be detected in amniocytes with the help of FISH. The technique can also be used to identify the origin of supernumerary segments or confirm the loss of specific sequences or a deletion on a given chromosome.

VII- Future perspectives
VII- 1. In utero treatment
The in utero treatment is limited to fluid aspiration for instance in bladder hypertrophy secondary to an urethral valve, and in some thoracic or abdominal fluid collections. Other surgical procedures have been completed like the insertion of a catheter in the bladder, correction of certain heart defects, to name the most common. The finding of an abdominal wall defect (ex: omphalocele) allows time for planning the mode of delivery and alert neonate specialists and pediatric surgeons to an immediate intervention at birth to prevent systemic complications. As part of indications for medical foetal therapy, foetal arrhythmia can be corrected via a maternal medication approach. Finally a restrictive phenylalanine diet, prescribed as soon as a phenylketonuric woman plans a pregnancy, will prevent foetal complications and especially a microcephaly and severe mental retardation in the child.

The prenatal diagnosis of some metabolic diseases like galactosemia or leucinosis will allow to plan ahead if the foetus is sick and prescribe a restrictive metabolic milk for the infant to prevent early complications secondary to abnormal metabolites.

VII- 2. Preimplantation diagnosis
Preimplantation diagnosis is defined as the analysis of a cell taken from a fertilized egg at, for instance, the eight cell stage. It was introduced as an assisted procreation technique in 1989 but it still considered a research and development procedure. We do not know yet if it is a secure and reliable procedure although to date but according to literature reports a few dozens infants born after this technique seem to have a normal development. A limited number of laboratories in Europe and America have the facilities and the knowledge to offer this test as a diagnostic procedure. To date this technique has been attempted in more than one hundred pregnancies at risk and especially in mucoviscidosis, Duchenne muscular dystrophy, hemophilia A, alpha and beta thalassemia, bulbar atrophy, Lesch Nyhan syndrome, incontinentia pigmenti, Huntington disease, myotonic dystrophy. For X linked diseases foetal sexing is recommended before proceeding to a molecular diagnosis.

II- 3. Preconception screening
Preconception screening is a recent avenue in the field of prevention. It implies the detection of gamete anomalies and more specifically at the ovum level. Recent publications refer to preconception diagnosis by studying the first polar body in
maturing ova: polar bodies reflect the chromosomal complement and genotype of the ovum that could be used in the in vitro fertilization. In a recent literature report, a normal ovum was selected from a mother known as a carrier of a dominant gene for a severe form of Alzheimer disease. It was reported that this ovum free of mutation was used for the in vitro fertilization and a normal infant was carried to term. The route is then traced for diagnostic interventions based on gamete studies, but ethic dilemmas will surge from any attempt to manipulate germinal cells.