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## Prenatal Biochemical and Ultrasound Markers in Chromosomal Anomalies

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Additional information is available at the end of the chapter

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### Abstract

The unbalanced chromosomal anomalies generate an abnormal pattern of development and usually determine miscarriage. The most frequent prenatal chromosomal anomalies are X monosomy, trisomies of chromosomes 21, 18, 13, 16, 8, triploidy and tetraploidy. Identification of chromosomal anomalies can be done by prenatal screening and diagnosis. Prenatal screening is biochemical, sonographic or molecular (detection of fetal DNA in maternal blood). Biochemical screening can be done in the first or second trimester. First-trimester screening is based on the detection in maternal serum of beta-hCG ( $\beta$ -hCG) and pregnancy-associated plasma protein-A (PAPP-A). Biochemical screening in the second trimester requires the detection of alpha-fetoprotein (aFP) hGC, unconjugated estriol ( $\mu$ E) and inhibin A. The sonographic examination can be used in the first or second trimesters. In the first trimester, an ultrasound can identify soft markers like nuchal translucency, nasal bone and ductus venous flow. In the second trimester the sonographic examination can identify congenital anomalies or different soft markers. Prenatal chromosomal diagnosis requires an invasive procedure to obtain embryonic or fetal material. Such procedures are represented by chorionic villus sampling amniocentesis or cordocentesis. The fetal cells are used for cell cultures (in cytogenetic methods) or for molecular analyses (FISH, QF-PCR, MLPA, array-CGH).

**Keywords:** chromosomal anomalies, chromosomal syndromes, prenatal screening, biochemical screening, ultra-sonographic screening

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## 1. Introduction

Chromosomal anomalies represent large genomic modifications that could be identified using light microscope. Apart from these characteristics, many chromosomal anomalies produce severe changes in the phenotypes of carriers that induce a high rate of miscarriage (>50% of spontaneous abortions are produced by a chromosomal anomaly) and chromosomal disorders in neonates and infants (global incidence in newborns is 1%). Early identification of chromosomal anomalies during the prenatal period has become the purpose of prenatal screening and diagnosis. Prenatal screening allows discovery of pregnancies at risk using non-invasive methods that do not harm the pregnant woman and fetus. An abnormal prenatal screening imposes the confirmation by using a prenatal diagnosis method. Prenatal screening methods could be biochemical, ultra-sonographic and genomic. Biochemical methods imply detection of some serum constituents in maternal blood like hCG,  $\beta$ -hCG, aFP,  $\mu$ E3, PAPP-A, and so on. Ultra-sonographic methods imply the use of ultrasound for the assessment of morphologic features of the fetus. Genomic prenatal screening allows the detection of free fetal DNA in maternal blood. Prenatal diagnosis imposes the use of the invasive methods that allow the harvesting of these fetal cells (trophoblastic cells, amniotic cells or blood cells). The major inconvenience of invasive methods is the risk of miscarriage or fetal damage. Fetal cells could be used for cytogenetic or molecular genetics investigations that allow a prenatal diagnosis [1].

## 2. Chromosomal anomalies

Chromosomal anomalies are produced by genomic or chromosomal mutations. Chromosomal anomalies could be numerical and structural. Numerical chromosomal anomalies produce an important genomic imbalance and the phenotype of carriers is severely affected. Numerical chromosomal anomalies are represented by aneuploidies (trisomies, monosomies, etc.) and polyploidies (triploidy and tetraploidy). These are generated by errors during meiosis, fertilization or first mitosis of embryo. The most common error is chromosomal non-disjunction during the first meiosis that generates an aneuploidy (trisomy or monosomy) and is associated with advanced maternal age. Structural chromosomal anomalies are generated by changes of structure of one or multiple chromosomes and could be divided into unbalanced and balanced anomalies. Unbalanced structural chromosomal anomalies are characterized by absence (partial monosomy) or supplementary (partial trisomy) chromosomal segment(s) and produce a genomic imbalance that induces an abnormal phenotype. Balanced chromosomal anomalies are characterized by changes in the position of some chromosomal segments, but the quantity of genetic material remains unchanged. The phenotype of carriers is usually normal, but they could present a malsegregation of derivative chromosomes during meiosis that generates formation of gametes with genomic imbalances. Fertilizing such gametes results in embryos with unbalanced chromosomal anomalies [1].

Unbalanced chromosomal anomalies produce a severe modification of phenotype and generate chromosomal diseases. The majority of such anomalies do not permit survival of the embryo and the pregnancy ends in miscarriage. Other anomalies—gonosomal trisomies (XXX, XXY, XYY), some autosomal trisomies (21, 18, 13), some cases with X monosomy, some partial monosomies or partial trisomies—are compatible with life, but children have a chromosomal disease [1].

The epidemiological studies showed that the frequency of chromosomal anomalies will be reduced during pregnancy, from 1/4 at conception to 1/100 at birth (**Table 1**). The aneuploidies are the most frequent of these anomalies. The most common chromosomal disorders are Down syndrome (trisomy 21—**Figure 1**, birth prevalence 1/700–1/800) [2, 3], Klinefelter syndrome (trisomy XXY—**Figure 2**, birth prevalence 1/1000), triplo X syndrome (trisomy X—**Figure 3**, birth prevalence 1/1000), Turner syndrome (monosomy X—**Figure 4**, birth prevalence 1/2000 girls), Edwards syndrome (trisomy 18—**Figure 5**, birth prevalence 1/6500) and Patau syndrome (trisomy 13—**Figure 6**, birth prevalence 1/12,500). The risk of a couple having an affected child with a major trisomy (13, 18 and 21) is associated with advancing maternal age. In the last decade, the age of women at first pregnancy increases such that the birth prevalence for trisomy 21 in the USA has increased from 1 in 740 in 1974 to 1 in 504 by 1997 [4, 5].

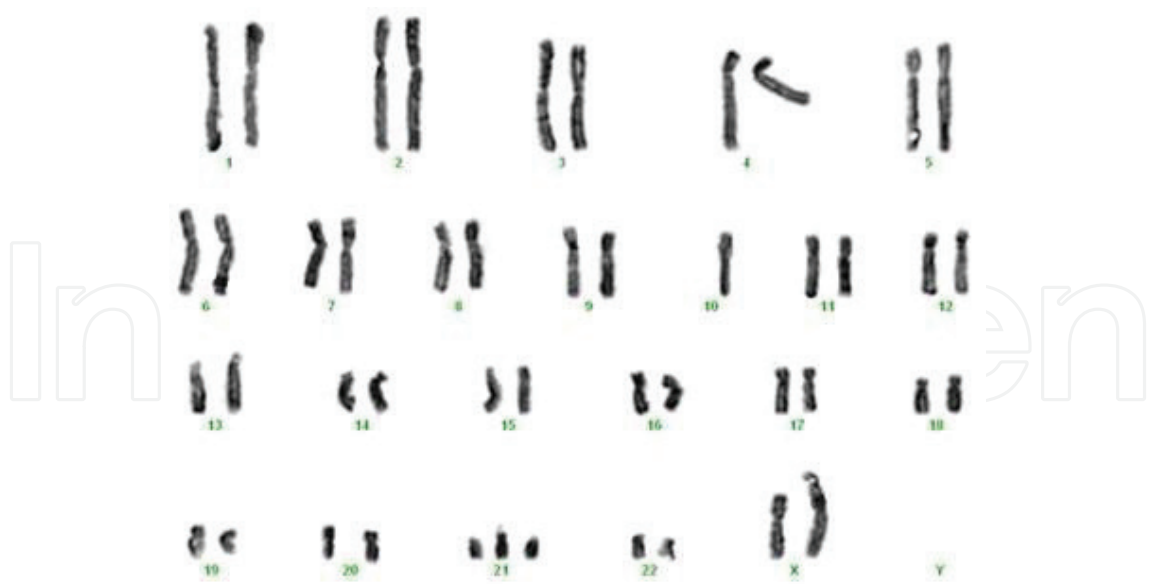
The majority of chromosomal disorders has a high lethality rate during pregnancy and thus in the first trimester there are a significant number of fetuses affected than at full term. For example, in the case of trisomy 21, there is a 40% fetal loss between 12 weeks and full term and a 30% fetal loss between 16 weeks and full term. For trisomies 13 or 18, the loss is more important with an 80% fetal loss between 12 weeks and full term and a 40% fetal loss between 16 weeks and full term [6].

The incidence of the main chromosomal disorders in neonates is present in **Table 2**.

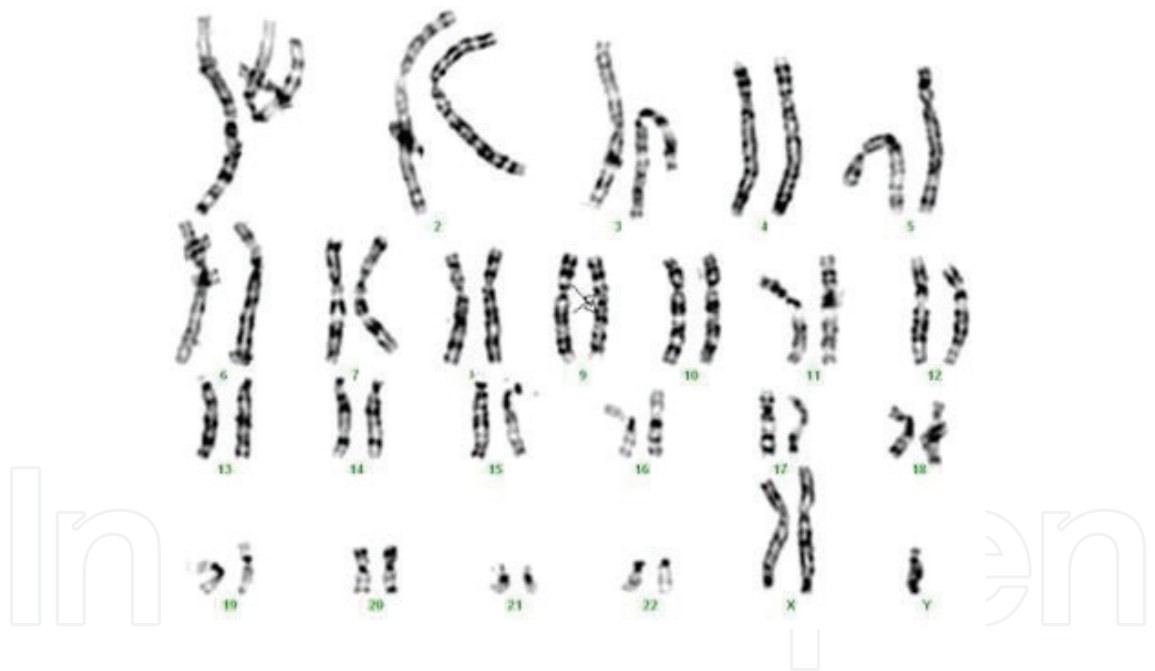
For trisomy 21, the risk at 12 weeks of gestation is 1/1000 for a woman aged 20 years and 1/250 for a woman aged 35 years. The risk of delivering an affected baby with Down syndrome is 1/1500 for a woman aged 20 years and 1/350 for a woman aged 35 years. For trisomy 18, the risk at 12 weeks of gestation is 1/2500 (for a woman aged 20 years) and 1/600 (for a woman aged 35 years) [7].

Ontogenetic period/ disorder	Incidence of chromosomal anomalies
1. gametes	10-25%
2. biochemical miscarriages	unknown, probably 33-67%
3. spontaneous miscarriages during first trimester of pregnancy	50-70%
4. intrauterine death	5% - 10%
5. newborns	1%
6. severe congenital anomalies	4%
7. plurimalformative syndromes	5.5%

**Table 1.** Incidence of chromosomal anomalies in prenatal and perinatal period (adapted from Gorduja [1]).



**Figure 1.** Trisomy 21 (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).



**Figure 2.** Trisomy XXY (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).

The risk of delivering an affected baby with Edwards syndrome is 1/18,000 for a woman aged 20 years and 1/4000 for a woman aged 35 years. For trisomy 13, the risk at 12 weeks of gestation is 1/8000 (for a woman aged 20 years) and 1/1800 (for a woman aged 35 years). The risk of delivering an affected baby with Patau syndrome is 1/42,000 for a woman aged 20 years and 1/10,000 for a woman aged 35 years [7].



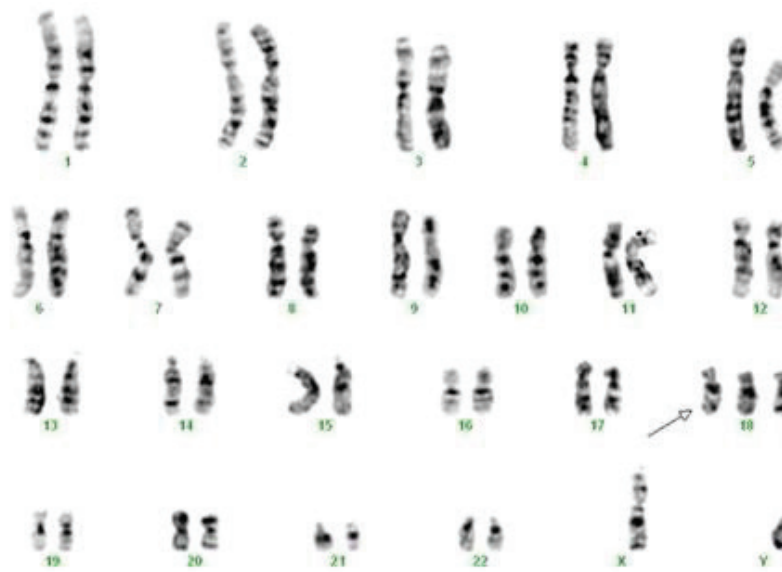
**Figure 3.** Trisomy X (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).



**Figure 4.** Monosomy X (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).

In the case of gonosomal aneuploidies the risks are not correlated with advanced maternal age, and with the exception of monosomy X, the chromosomal anomaly does not modify the viability of the fetus. In monosomy X, the prevalence of anomaly is about 1/1500 at 12 weeks of gestation and 1/4000 at 40 weeks. For the gonosomal trisomies (47,XXX, 47,XXY and 47,XYY), the overall prevalence of 1/500 does not decrease with gestation [7].





**Figure 5.** Trisomy 18 (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).



**Figure 6.** Trisomy 13 (collection of cytogenetic laboratory, “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania).

Triploidy is unrelated to maternal age and the prevalence is about 1/2000 at 12 weeks, but the majority of affected fetuses die by 20 weeks and the born babies are mosaics 46/69 [7].

The birth of a child is an important event in the life of every family and represents the end of a long period of uncertainty generated by fear that the future child will be abnormal. The high incidence of chromosomal anomalies during prenatal life and the severity of phenotype of chromosomal anomalies that allow survival, imposed the development of methods of prenatal

Autosomal trisomies	13 –Patau syndrome	0.08‰	Global 1.4‰
	18 –Edward syndrome	0.15‰	
	21 –Down syndrome	1.2‰	
Triploidies			0.02‰
Gonosomal aneuploidies in boys	47,XXY –Klinefelter syndrome	1‰	Global 2.75‰
	47,XYY –double Y syndrome	1‰	
	other	0.74‰	
Gonosomal aneuploidies in girls	monosomy X –Turner syndrome	0.3‰	Global 1.8‰
	47,XXX –triplo X syndrome	1.1‰	
	other	0.37‰	
Unbalanced structural chromosomal anomalies			0.7‰
Unbalanced chromosomal anomalies (numerical and structural)			4‰(1/250)
Balanced structural chromosomal anomalies	Reciprocal translocations	2.5‰	Global 4.3‰
	Robertsonian translocations	1‰	
	Inversions	0.8‰	

**Table 2.** Incidence of chromosomal anomalies in neonates (adapted from Gorduza [1]).

screening and diagnosis. Different such methods were developed during the last decades and now it is possible to discover the chromosomal pathology of the embryo in a couple at risk.

### 3. Methods of prenatal screening and diagnosis

The main procedures of prenatal screening are biochemical screening and ultrasound that allow the identification of pregnancies with increased genetic risk. The prenatal screening methods were introduced in medical practice in the 1980s, based on the association between incidence of trisomy 21 (Down syndrome) and advanced maternal age. A decade later both the maternal serum biochemistry and detailed ultra-sonographic examination in the second trimester were developed and allowed the identification of high-risk pregnancies. In the 1990s, the prenatal screening shifted to first trimester by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum-free  $\beta$ -hCG and PAPP-A. In the last 10 years, the prenatal screening methods changed again by the introduction of genomic screening that searches free fetal DNA in maternal blood [8].

The prenatal diagnosis imposes the obtaining of embryonic or fetal cells by using invasive methods like chorionic villus sampling (CVS), amniocentesis or cordocentesis. All these methods present risks for spontaneous miscarriages, obstetrical hemorrhages and fetal damages. The embryonic or fetal cells are used for cytogenetic or molecular diagnosis.

#### 3.1. Biochemical screening

Biochemical screening is based on the determination of maternal serum markers that are associated with an increased risk of chromosomal diseases. Biochemical screening could be



applied during the first or the second trimester of pregnancy. The presence of a fetal aneuploidy is associated with changes of maternal serum concentrations of some fetoplacental products: aFP, free  $\beta$ -hCG, inhibin A,  $\mu$ E3 and PAPP-A. In normal pregnancies, aFP concentration in maternal serum increases from 11.3 ng/ml in the 8th week of gestation to 250 ng/ml in the 32nd week of pregnancy. After that, it then declines slightly until term. In normal pregnancies, hCG in the maternal serum increases in first trimester of pregnancy and reaches a peak in weeks 7–9 (100.000 IU/ml). After that, it reduces continuously until around 20 weeks of pregnancies, when reduction is stopped and plasma levels remain constant until term. In normal pregnancies, inhibin A increases from the 6th week of pregnancy to the 9th week of pregnancy and reaches a peak (~550 pg/ml). After that, the values decline continuously to the 14th week of pregnancy. In normal pregnancies,  $\mu$ E3 is first detectable at 9 weeks of gestation (0.05 ng/ml) and after that it increases continuously to about 30 ng/ml at term. In normal pregnancies, PAPP-A is first detected in maternal serum after 4 weeks of pregnancies. PAPP-A concentration increases exponentially in the first trimester. After that, the rise occurs slowly, but it continues until delivery [9–12].

In biochemical screening, the measured concentration of the markers is converted into a multiple of the median (MoM) of unaffected pregnancies at the same gestation. The Gaussian distributions of  $\log_{10}$  (MoM) in trisomy 21 and unaffected pregnancies are then derived, and the ratio of the heights of the distributions at a particular MoM represents the likelihood ratio for a trisomy [7].

### 3.1.1. First-trimester prenatal screening

During the first trimester of pregnancy, different serological components present variations, but only free  $\beta$ -hCG and PAPP-A were associated with the presence of a trisomy 21. Other serological marker could be ADAM-12.

#### 3.1.1.1. Human chorionic gonadotropin

The first attempts of using the hCG in detection of trisomy 21 in the first trimester of pregnancy gave controversial results. Use of total hCG in the first-trimester screening is inadequate because this marker becomes elevated only after 11 weeks of gestation [13]. In opposition,  $\beta$ -hCG is substantially elevated at 8–14 weeks of gestation in trisomy 21 pregnancies [14]. At a 5% false positive rate, the association between  $\beta$ -hCG and maternal age allows the detection of 42–46% of cases with trisomy 21 [15, 16].

#### 3.1.1.2. Pregnancy-associated plasma protein-a

Pregnancy-associated plasma protein-A (PAPP-A) is produced by placental trophoblasts, but its function still unclear. The level of maternal serum PAPP-A is low during the first trimester in pregnancies with trisomy 21 and thus this marker could be used in prenatal screening of first trimester [17]. By using a protocol that associates PAPP-A in combination with maternal age, at a 5% false positive rate, the detection rate of cases with trisomy 21 ranges from 48 to 52%. After 15 weeks of gestation, the efficacy of this marker is low and its use in second-trimester screening is without benefits [15, 16].

#### 3.1.1.3. Other biochemical markers

The serum marker used in second-trimester screening (aFP,  $\mu$ E3 and inhibin-A [INH-A]) shows minimal differences in trisomy 21 pregnancies and thus could not be used in first-trimester screening [14].

ADAM 12 is a glycoprotein synthesized by the placenta and secreted through pregnancy. ADAM 12 presents proteolytic functions and has a low level in first-trimester cases with trisomy 21 or trisomy 18. This reduction is more pronounced in earlier gestation, with best results at around 8–10 weeks. ADAM 12 in combination with PAPP-A (both measured at 8–9 weeks), nuchal translucency (NT) and free  $\beta$ -hCG measured at 12 weeks allow a detection rate of 97% at a 5% false positive rate and thus could be the best protocol for prenatal screening in the first trimester of pregnancy [18, 19].

#### 3.1.1.4. Screening of other aneuploidies

The screening of other aneuploidies in the first trimester of pregnancy is also possible and has a good rate of detection. Low maternal serum PAPP-A was identified in trisomy 18, trisomy 13, triploidy and monosomy X. Low levels of free  $\beta$ -hCG were discovered in trisomy 18, trisomy 13 and in some cases of triploidy [20–23]. However, all these disorders have a high rate of spontaneous miscarriages (minimum 80%) and any conversion of the observed detection rates to true detection rates is, therefore, associated with a substantial degree of uncertainty [13].

#### 3.1.2. Second-trimester prenatal screening

Second-trimester serum markers are represented by alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol and inhibin-A.

Screening for aneuploidies was initially focused on the second trimester of pregnancy and demonstrated a substantial improvement in detection rates of trisomy 21, compared with screening using only maternal age. At a false positive rate of 5%, the detection rate improves from 30% in screening by maternal age alone to 60–65% by combining maternal age with serum AFP and free  $\beta$ -hCG (double test), 65–70% with the addition of  $\mu$ E (triple test) and 70–75% with the addition of inhibin A (quadruple test). In the case of hCG, it is better to search for free  $\beta$ -hCG than total hCG [24–27].

##### 3.1.2.1. Alpha-fetoprotein

The first report concerning the association between low level of maternal serum alpha-fetoprotein and fetal trisomy 21 was made in 1984, by Merkatz et al. [9]. At a risk cut-off of 1:270 for trisomy 21 (equivalent to the maternal age of 35), using this parameter alone would allow the detection of 55% of cases with trisomy 21 [28, 29].

The aFP is produced by fetal liver, but its biological functions and the reason why the aFP level is lower in Down syndrome pregnancies remain unclear. Placentas of affected pregnancies show a high level of aFP suggesting a defect in the secretion of AFP into the maternal circulation [30].

### 3.1.2.2. Human chorionic gonadotropin

In 1987, Bogart et al. showed that human chorionic gonadotropin (hCG) levels are generally higher in the maternal serum of women with Down syndrome pregnancies. They noted that hCG appeared to be superior to aFP in detecting fetal chromosome abnormalities, and association of maternal age with hCG as the screening method allows the identification of about 60% of pregnancies with trisomy 21 [31].

hCG is a glycoprotein produced by the placenta, composed of two subunits:  $\alpha$  and  $\beta$ . Maternal serum contains intact hCG but also free  $\alpha$ , free  $\beta$  and degradation products. At 8–10 weeks of gestation, intact hCG and free  $\beta$ -hCG show peak concentrations. Similar high levels of these compounds are found during the second trimester of pregnancy [32].

The anomalies of the placenta, characterized by disturbance in fluid homeostasis, like fetal hydrops and/or a cystic placenta, are associated with high levels of hCG in maternal serum. Such anomalies are present in hydropic Down syndrome, triploidy, Turner syndrome or other causes of hydrops fetalis. Even in the absence of hydrops, in cases with trisomy 21, there is a fluid accumulation that causes enlarged nuchal translucency and thickening and this could be related to the increase of hCG [33–35].

### 3.1.2.3. Unconjugated estriol

The presence of a low level of estriol in maternal urine in the case of a pregnancy with trisomy 21 was first reported by Jørgansen and Trolle [36]. Other studies confirmed this particularity and  $\mu$ E3 could be used as a marker in the prenatal screening of Down syndrome [37, 38].

The placenta uses 16 alpha-hydroxydehydroepiandrosterone sulfate (DHEAS) as the precursor of  $\mu$ E3. In Down syndrome pregnancies, both  $\mu$ E3 and DHEAS present lower levels in different tissues, including placenta [39]. During the second trimester the concentration of  $\mu$ E3 rises quickly and this marker can identify pregnancies with a small or underdeveloped fetus at the time of screening [27].

### 3.1.2.4. Inhibin-A

The possible application of inhibin in Down syndrome prenatal screening was suggested by Van Lith et al. [10]. Inhibin is a glycoprotein synthesized by gonads and placenta. The functional protein is dimeric with two subunits,  $\alpha$  and  $\beta$ . The subunit  $\alpha$  could be coupled with two different  $\beta$  subunits ( $\beta$ A or  $\beta$ B) and forms inhibin-A or inhibin-B. Inhibin-A (INH-A) is increased in pregnancies with trisomy 21 and presents an interdependent secretion with hCG. INH-A allows a good distinction between affected and unaffected pregnancies, alone or in combination with hCG [40–42]. INH-A has not got variations in relation with gestational age and thus the accuracy of testing is better by comparison with other serological markers [43].

### 3.1.2.5. Multiple marker screening in the second trimester of pregnancy

Second-trimester serum screening could be applied between 14 and 22 weeks of gestation, but usually it is carried out at 15–18 weeks of pregnancy. The serum markers could be combined in different ways but the most commonly used tests are double test (association between aFP

and hCG), triple test (association between aFP,  $\mu$ E3 and hCG), and quadruple test (association between aFP,  $\mu$ E3, hCG and INH-A) [42, 44, 45]. The values obtained by these tests are combined based on a multivariate Gaussian model and finally a risk algorithm is obtained [46].

A very important parameter is the age of gestation and this must be established using an ultrasound determination of gestational age (best results with crown-rump measurement between 8 and 11 weeks) [47]. The risk algorithm must include other variables like maternal weight, ethnicity, maternal diseases (diabetes, systemic lupus erythematosus), multiple gestation, smoking, in vitro fertilization, sex of the fetus or maternal rhesus blood type [48–56]. In unaffected twins' pregnancy, the concentrations of maternal serum markers are twofold higher than that seen in unaffected singleton pregnancies and thus the algorithm needs an adjustment by using a "pseudo-risk" [51].

### 3.1.2.6. Screening of other aneuploidies

In case of a pregnancy with trisomy 18, all serum markers (aFP,  $\mu$ E3, hCG and INH-A) for the second trimester are characterized by low concentrations [57]. The risks are calculated using a multivariate normal model and the detection rates for this aneuploidy are similar to those of trisomy 21 screening [58]. For other autosomal trisomies (13, 16, 20) and for triploidy different protocols for the second-trimester biochemical screening were tried but without specific features [27].

Among the sex chromosome aneuploidies, only one presents a bad prognosis: monosomy X with fetal hydrops. In this case, the biochemical prenatal screening shows an association between low aFP and  $\mu$ E3 and elevated hCG and INH-A. In pregnancies with monosomy X without hydrops, all serological markers are lower than normal concentrations [59].

## 3.2. Ultrasound screening

Ultrasound examination represents a good tool for the detection of morphological abnormalities in fetuses with chromosomal aberrations. In aneuploidies we could identify structural defects and non-structural findings (sonographic markers). Sonographic markers of fetal aneuploidy (SMFAs) are insignificant by themselves because they are nonspecific and often transient [60]. The sensitivity of sonography for detecting these abnormalities varies with a number of factors: type of chromosomal abnormality, gestational age, quality of the sonography and the experience of the sonographer. During the first trimester only the SMFA could be identified in pregnancies with aneuploid fetuses. During the second trimester, major/structural abnormalities could be observed in 20% of fetuses with trisomy 21 and in the majority of fetuses with trisomies 18 and 13. By combining SMFA and structural defects, the sonography allows the identification of 50% of fetuses with trisomy 21, 80% of fetuses with trisomy 18 and 90% of fetuses with trisomy 13 [61, 62].

### 3.2.1. First-trimester screening

During the first trimester of pregnancy many sonographic markers of fetal aneuploidy were described, but the most used are nuchal translucency and nasal bone (NB). Also, some major congenital anomalies could be identified but usually such changes are diagnosed during the second trimester of pregnancy.



### 3.2.1.1. Nuchal translucency

Nuchal translucency (NT) was introduced in medical practice in the 1990s and it is the sonographic appearance of a subcutaneous collection of fluid in the region of fetal neck [63]. Using a fixed cut-off for the NT measurement of 3 mm, it was possible to identify 64% of chromosomally abnormal cases, while only 4.1% of normal fetuses showed similar NT values [64]. It was proved that cut-off value was variable depending on gestational age, and it developed a Gaussian model for the NT variable that allowed this test to be readily combined with other markers [65].

The measurement of fetal NT thickness is done at the 11–14th week of gestation. This sonographic scan has been combined with maternal age to provide an effective method of screening for trisomy 21—at 5% false positive rate, about 75% of trisomic pregnancies can be identified by this method [63].

The explanations of NT are multiple but the most plausible are cardiovascular defects which cause over-perfusion of the head and neck and abnormal or delayed development of the lymphatic system. Thus, NT marker could be associated also with other chromosomal anomalies that produce fluid accumulation in the neck region. Also, NT can be a transient phenomenon that appears in a normal fetus and it spontaneously resolves in the second trimester [66].

### 3.2.1.2. Nasal bone

Cicero et al. were the first who evaluated the absence of the nasal bone (NB) in pregnancies and showed that it was present in 73% of trisomy 21 fetuses versus 0.5% of unaffected fetuses. They concluded that in trisomy 21, the absence of the nasal bone is not related to the nuchal translucency thickness, and thus both sonographic markers could be combined to provide an effective method of early screening for trisomy 21 [67]. This combined screening allows an increase of sensitivity from about 57 to 86% at a fixed false positive rate of about 1%. If the biochemical screening in the first trimester is added, a sensitivity of more than 90% could probably be achieved at a false positive rate of 1% [5, 67]. The analysis of nasal bone in aneuploidy versus euploidy, made by Kagan et al., showed that the nasal bone was absent in 2.6% of the euploid fetuses, in 59.8% with trisomy 21, 52.8% with trisomy 18, 45.0% with trisomy 13 and in none of the fetuses with Turner syndrome [68]. In contrast, Cicero et al. indicated that trisomy 21 was associated with the absence of nasal bone in 68.8% of cases, trisomy 18 in 54.8% of cases, trisomy 13 in 34.2% of cases, monosomy X in 10.9% of cases, gonosomal aneuploidies (XXX, XXY, XYY) in 5% of cases and other types of autosomal aneuploidies in 16.7% of cases, but none of the 19 cases with triploidy presented the absence of nasal bone [69].

### 3.2.1.3. Other sonographic markers in the first trimester

Another potential marker for trisomy 21 in the first trimester of pregnancy is tricuspid regurgitation (TR) observed by pulsed wave Doppler ultra-sonography. Falcon et al. indicated a tricuspid regurgitation in 67.5% of fetuses with trisomy 21, 33.3% of fetuses with trisomy 18 and only 4.4% of euploid fetuses [70]. Kagan et al. reported this anomaly in 55.5% of fetuses with trisomy 21, 33.3% of fetuses with trisomy 18, 30% of fetuses with trisomy 13, 37.5% of fetuses with monosomy X and only 0.9% of euploid fetuses. The free  $\beta$ -hCG and PAPP-A present



an independent variation in relation with tricuspid regurgitation and by combining all these parameters it would be expected to achieve a detection rate of 95% at a 5% false positive rate or 90% at a 2% false positive rate [71].

Borrell et al. measured the pulsatility index for veins and found an abnormal blood flow through the ductus venosus (DV) in the Down fetus. \*\*\*They indicated that with a 4–5% false positive rate, the detection rate for trisomy 21 is 65–75%. The use of this marker in association with NT increased the detection rate to 75–80%. Combining these two markers with serum biochemical markers measured at 10 weeks provided a detection rate of 92% at a 5% false positive rate [72].

Abele et al. analyzed retrospectively the NB, TR and DV. For normal children NB was identified in 2.0% of cases, TR was identified in 1.7% of cases and DV was identified in 3.5% of cases. In opposition, in cases with trisomy 21, NB was identified in 61.3% of cases, TR was identified in 61.3% of cases and DV was identified in 60.2% of cases. The normal children presented at least one of the markers in 5.9% of cases while more than 95% of the fetuses with trisomy 21 presented minimum 1 of these markers. The use of such combined prenatal screening (maternal age + NT + NB + TR + DV) during the first trimester could offer a detection rate of 95% with 1.5% false positive rate at a risk cut-off of 1:50 [73].

The assessment of each of these ultrasound markers can be incorporated into first-trimester combined screening by maternal age, fetal NT and serum-free  $\beta$ -hCG and PAPP-A, resulting in the improvement of the performance of screening with an increase in detection rate from 93 to 96% and a decrease in false positive rate to 2.5% [68, 71, 74].

### *3.2.2. Second-trimester screening*

During the second trimester of pregnancy different sonographic markers could be identified, as well as major congenital anomalies. The most common markers in the second trimester are nuchal thickening, hyperechoic bowel, shortened extremities, renal pyelectasis, echogenic intracardiac foci (EIF) and choroid plexus cysts. The discovery of such a marker is important especially in cases with trisomy 21 because many cases with Down syndrome do not present major congenital anomalies. In other aneuploidies and also in triploidy, usually the visceral anomalies are common, and identification of a congenital defect imposes an invasive procedure, followed by a chromosomal diagnosis [62].

#### *3.2.2.1. Trisomy 13*

Trisomy 13 is a severe disorder, characterized by the presence of different malformations in the brain (holoprosencephaly, agenesis of the corpus callosum, Dandy-Walker malformation, vermian agenesis and neural tube defects), face (cyclopia, hypotelorism, cleft lip and palate), kidneys (renal cystic dysplasia and hydronephrosis) and heart (arterial septal defect, ventricular septal defect and patent ductus arteriosus). Also, cystic hygroma, polydactyly and club or rocker-bottom feet could be identified. All these anomalies can be observed easily by ultrasound examination in the second trimester of pregnancy. Also in the cases of trisomy 13 some sonographic markers can be discovered, but they are nonspecific. Such markers

are intrauterine growth restriction (IUGR), mild dilatation of the lateral cerebral ventricles, hyperechoic bowel and echogenic intracardiac foci. The most commonly identified marker is echogenic intracardiac foci. A specific association that can be considered is the one between echogenic intracardiac foci and the hypoplastic left side of the heart [62].

#### 3.2.2.2. *Trisomy 18*

Trisomy 18 is a severe disorder characterized by a lot of congenital anomalies that could be identified by ultrasound examination during the second trimester of pregnancy. These anomalies involve the central nervous system (hydrocephalus, spina bifida, vermian agenesis), lymphatic system (cystic hygroma, nonimmune hydrops), cardiovascular malformations (ventricular and atrial septal defects, patent ductus arteriosus and polyvalvular disease), thorax and abdomen (diaphragmatic hernia, tracheoesophageal fistula, omphalocele), genitourinary system and skeletal system (clenched hands, club feet, radial aplasia, limb shortening). Also, the ultrasound examination shows an intrauterine growth restriction associated with polyhydramnios [75, 76].

Some subtle anomalies discovered by ultrasound examination were considered markers for trisomy 18. Such anomalies are choroid plexus cysts, brachycephaly, “strawberry-shaped” head and single umbilical artery [62]. Choroid plexus cysts are a relatively common variant during the second trimester. It is transient, and if the karyotype is normal it does not have an adverse outcome. The median prevalence of choroid plexus cysts in the general population has been reported as 1–2%. The importance of this marker is limited. Snijders et al. discovered only two cases of chromosomal anomalies in a cohort of 107 fetuses with isolated choroid plexus cysts who had karyotyping. On the other hand, the same authors found no chromosomal anomaly among the 174 children with choroid plexus cysts who did not have amniocentesis [77]. However, Nyberg et al. noted that choroid plexus cysts are observed in 30–40% of fetuses with trisomy 18 before 20 weeks, but usually this marker was associated with other major anomalies specific to trisomy 18 [62]. The presence of isolated choroid plexus cysts is associated with higher likelihood ratios for trisomy 18. Thus, Ghidini et al. [78] found a likelihood ratio of 7.1 and Yoder et al. [79] found a likelihood ratio of 13.8 for trisomy 18 in the presence of isolated choroid plexus cysts. Nyberg et al. concluded that fetal karyotyping should be offered only in cases when all the following conditions are met: maternal age at delivery is over 36 years, the biochemical risk for trisomy 18 is more than 1/3000 and choroid plexus cysts are large. However, the detection of isolated choroid plexus cysts imposes an ultrasound follow-up that can show other abnormalities that were previously missed [62].

#### 3.2.2.3. *Trisomy 21*

During the second trimester of pregnancy, congenital anomalies in trisomy 21 fetuses are not frequently discovered. Before 20 weeks, the detection of such anomalies in trisomy 21 is around 16% and this value reflects the low sensitivity of sonography for detection of cardiac defects. The most frequent anomalies are cardiac defects, hydrops, cystic hygroma and duodenal atresia associated with esophageal atresia [62]. However, a sonographic scan at

24 weeks of gestation performed by a competent specialist could discover around 50% of congenital anomalies (including subtle ventricular septal defects) in trisomy 21 fetuses [80].

The markers for trisomy 21 in the second trimester of gestation are nuchal thickening, hyper-echoic bowel, shortened limbs, pyelectasis, echogenic intracardiac foci, widened pelvic angle, shortened frontal lobes, clinodactyly, pericardial effusion, right-left disproportion of the heart and small ears [81, 82].

Each marker taken alone has a low sensitivity, but the presence of more than one marker is detected in minimum half of the pregnancies with trisomy 21. The presence of only one marker is discovered in around 22.6% of fetuses with trisomy 21 but also in 11% of normal fetuses. In opposition, identification of more than two markers is present in one-third of pregnancies with trisomy 21, while only 2% of normal pregnancies show such a characteristic. The risk for trisomy 21 is twofold higher if one marker is detected, but the risk becomes 10-fold higher when two markers are present and gets more than 100-fold when three markers are identified. The search of these markers is widely referred to as a “genetic sonogram”. The results of a genetic sonogram depend on gestational age, quality of the apparatus and competence of the sonographer and the type of marker, but the minimum sensitivity of this method is around 60% [62]. The big inconvenience of this screening is the high false positive rate associated with the use of multiple sonographic markers that generates anxiety among low-risk patients and imposes an invasive procedure to deny the presence of trisomy 21 [83].

To improve the use of sonographic markers in detection of trisomy 21, Bromley et al. proposed a scoring index, and amniocentesis is offered only to those with a score of 2 or higher. A major congenital anomaly, nuchal thickening and an age bigger than 40 years are noted with 2 points each. Hyperechoic bowel, shortened femur, shortened humerus, pyelectasis, echogenic intracardiac foci and age between 35 and 40 years are noted with 1 point. The authors showed that a score  $\geq 2$  is associated with 75.5% of 21 trisomic pregnancies, while in normal pregnancies such a score is discovered in only 5.7% of cases [84]. An improvement of sonographic screening is the combination of this with biochemical screening, because the sonographic features are independent of biochemical analytes [85].

#### 3.2.2.4. *Nuchal thickening*

Redundant skin on the neck is a clinical feature of infants with trisomy 21 and it was first reported by Benacerraf et al. as nuchal thickening during the second trimester [86]. Nuchal thickening is one of the most important markers of trisomy 21 during the second trimester. The sensitivity of this criterion is 20–40%. First, a threshold  $\geq 6$  mm after 15 weeks of pregnancy was associated with a high risk of trisomy 21, but now a nuchal thickening of greater than 5 mm up to 20 weeks is considered an adequate value [87]. The normal nuchal thickness varies with gestational age. Thus, the better choice is the use of multiple-of-the-median data, comparing the nuchal measurement with the expected measurement. In these conditions, the calculation of likelihood ratios would permit integration with maternal serum biochemical markers for a combined risk [88].

#### 3.2.2.5. *Hyperechoic bowel*

Hyperechoic bowel is detected with increased frequency in cases with aneuploidy (including trisomy 21) but it is nonspecific and could be observed also in about 0.5% of normal fetuses. In addition, this marker was identified also in other disorders like bowel atresia, congenital infection, meconium ileus secondary and cystic fibrosis. The presence of hyperechoic bowel also represents a risk factor for intrauterine growth retardation, fetal death and placenta-related complications [62]. To standardize this feature, a scale with three values was proposed: grade 1—mildly diffuse echogenic, grade 2—moderately focal echogenic and grade 3—very echogenic (like in a bone). The echogenicity of normal bowel increases with transducer frequency, and to minimize the subjectivity, is preferable to take in consideration only the cases with moderate and markedly hyperechoic bowel. In these situations, the risk for fetal aneuploidy is high, but the sensitivity remains acceptable [89].

#### 3.2.2.6. *Skeletal abnormalities*

Skeletal abnormalities in trisomy 21 that could be identified in the second trimester of gestation are limb shortening, clinodactyly and widened pelvic angle. The last two are difficult to detect and for this reason are not commonly used in screening protocols [62]. A slightly rhizomelic short stature is characteristic for trisomy 21, with both shortening of femur and shortening of humerus. These features can be detected in some cases with trisomy 21 during the second trimester [90], but a shortened humerus is a slightly more specific indicator than a shortened femur. These markers vary with gestational age, ethnic group and fetal gender. The practical use of these parameters is based on the comparison of measurement of humerus and femur length with expected length of these bones. Optimal results were obtained by using a multiple-of-the median data and corresponding likelihood ratios rather than a single cut-off. However, in a simplified manner a single cut-off of 0.91 multiples of the median for a short femur and 0.89 for a short humerus could be used [62, 90].

#### 3.2.2.7. *Renal pyelectasis*

Mild pyelectasis (hydronephrosis) is associated with high risk for aneuploidy (especially for trisomy 21). This marker was also detected in about 3% of normal fetuses. The interpretation of this feature is very subjective because its prevalence varies with gestational age and it is influenced also by maternal hydration and degree of fetal bladder distension. Renal pyelectasis is measured as the fluid-filled renal pelvis in an anterior–posterior dimension. The threshold for a positive finding is a dimension  $\geq 3$ . At this threshold, the risk of trisomy 21 is 1.6-fold over the baseline risk [62, 91].

#### 3.2.2.8. *Echogenic Intracardiac foci*

Echogenic intracardiac foci (EIF) are marker that are found in 3–4% of normal fetuses, with a three times bigger prevalence in Asian populations [92]. The evaluation of EIF is very subjective and depends on resolution of the sonographic equipment, technique, thoroughness of the examination, the sonographer's experience and fetal position. In normal fetuses, it typically



disappears during the third trimester of pregnancy [93]. The EIF is not an efficient marker for detection of trisomy 21 because it occurs only in 16% of fetuses with this anomaly and the false positive rate is 17%. In contrast, EIF is present in 29% of those with trisomy 13% [94, 95]. The likelihood ratio of EIF in trisomy 21 has been estimated in the range of 1.8–4.2 [62]. Usually, the EIF is detected in the left ventricle, but right-sided or bilateral EIF had an approximately twofold greater risk of aneuploidy compared with left-sided foci [96].

#### 3.2.2.9. Mild ventricular dilatation

The lateral cerebral ventricles normally show a mean diameter of  $6.1 \pm 1.3$  mm. The presence of ventriculomegaly is suspected when diameter reaches 10 mm and such change has been associated with trisomy 21 (and also with other aneuploidies) [97]. The prevalence of this marker in trisomy 21 fetuses varies between 2.8 and 5% [98–100]. On the other hand, van der Hof et al. showed that mild ventriculomegaly is present in 0.15% of euploid fetuses compared with 1.4% of the fetuses with trisomy 21, with a likelihood of aneuploidy 9 times greater in aneuploid fetuses versus normal fetuses [101].

### 3.3. Prenatal diagnosis methods

The prenatal diagnosis is based on the chromosomal or molecular analyses of embryonic or fetal cells obtained using an invasive method.

#### 3.3.1. Invasive methods to obtain embryo-fetal cells

The methods for obtaining embryonic or fetal cells are chorionic villus sampling, amniocentesis and cordocentesis.

**Chorionic villus sampling** (CVS) is a method that allows prenatal diagnosis in the first trimester of pregnancy, typically at 10–12 weeks of gestation. It is done transabdominal or transcervical, under ultrasound guidance. The samples obtained contain trophoblast cells (derived from fetus) and maternal decidua cells. The last must be removed before analysis. The major advantages of this technique are reduced maternal risk generated by termination of pregnancy and a limited emotional trauma on the patient. The risks of this method are injury of the embryo (especially limb abnormalities in early procedures), miscarriage, bleeding or embryonic infection [102, 103]. The risk of miscarriage after performing chorionic villus sampling was initially estimated at 2–3%. Recent data disproves this risk, showing that the real risk is about 0.22% [104].

**Amniocentesis** is performed from the 16th week of gestation (rarely sooner) and involves the transabdominal extraction of 10–20 ml of amniotic fluid, under ultrasound guidance. The method has a low risk of miscarriage and other incidents. The risk of miscarriage was initially estimated at 1%, but recent data disproves this risk, showing that the real risk is about 0.11%. There is also a risk of amniotic fluid leakage and some rare complications (placental hemorrhage, intra-amniotic infection, abdominal wall hematoma and fetal lesion). A major disadvantage of this method is that it provides the final results of prenatal diagnosis in the second half of the pregnancy [103–105].



**Cordocentesis** is a method that consists of transabdominal or transvaginal puncture of the umbilical cord under ultrasound guidance in order to obtain small amounts of fetal blood. The method is performed after week 20 of pregnancy and has a reduced applicability in the diagnosis of chromosomal diseases. It is usually used to diagnose fetal blood diseases. Risk of fetal loss is high, about 3%. The major advantage of the diagnosis of chromosomal disorders is associated with the best results of blood cells culture in comparison with amniotic or chorionic cells [106].

### 3.3.2. *Methods of prenatal diagnosis*

The biological material obtained via invasive prenatal diagnosis methods can be used for cytogenetic or molecular assays.

#### 3.3.2.1. *Cytogenetic techniques*

Cytogenetic techniques require dividing cells. They can be applied directly or after a cell culture.

##### 3.3.2.1.1. *First trimester cytogenetic analyses*

In the case of trophoblast cells, obtained by chorionic villi sampling, the analysis can be done directly because the rate of division of placental tissue is high. However, even in the case of chorionic tissue, the cell culture is preferable because after harvest the quality is better and the cell number is high. Direct analysis of dividing cytotrophoblast cells is provided after uniform staining or after a chromosome banding procedure. The major advantages of this method are rapid final results (2/3 days) and absence of maternal cell contamination [103, 107].

The culture of chorionic villus cells is done in a special medium, after fragmentation of trophoblast tissue. The culture allows the formation of cell colonies adhering to the surface of the flask culture. At the end of 12–14 days, the cell division is blocked and chromosomal preparations are made. The chromosomes are banded using an R banding protocol because it does not require an aging period. The metaphases are analyzed on an optical microscope in direct illumination, using an immersion objective [103, 107].

The main advantage of cytogenetic diagnosis in the first trimester of pregnancy is achieving final results quickly, which decreases the time of uncertainty, the psychological distress of the parents and allows the end of pregnancy in the first trimester, when methods are easier and less traumatic [103, 107].

The main disadvantages of the chromosomal analysis in cells obtained by CVS are reduced number of mitosis and poor quality of chromosomal preparations which reduces resolution, allowing only the identification of numeric chromosomal abnormalities and those structural abnormalities of large dimensions. Another inconvenience is the possible detection of chromosomal mosaics. This could be real or confined to placenta. The inconsistencies can be explained by possible contamination with maternal cells, a chromosomal abnormality that occurs during the culture or the real existence of a placental mosaicism. In these cases, the karyotype must be

repeated after amniocentesis or cordocentesis to determine real fetal chromosomal formula. Another problem associated with CVS is the failure of culture that requires the repeat of CVS or the application of amniocentesis in the second trimester of gestation [103, 107].

#### 3.3.2.1.2. *Second-trimester cytogenetic analyses*

Fetal karyotype analysis, using cells obtained by amniocentesis, requires a step of 10–14-day cell culture. Culture technique and processing steps are similar to those used in the case of trophoblastic cells. Amniotic cultures can be done in situ (allow differentiation between mosaics and pseudomosaics) or in culture flasks. In the last case, at the end of the cultures, the separation of cells from the vessel is obtained using an enzymatic treatment. The contamination with maternal cells is insignificant and the quality of prepared chromosomes is better in comparison with chorionic villus cells [103, 107].

#### 3.3.2.2. *Molecular techniques*

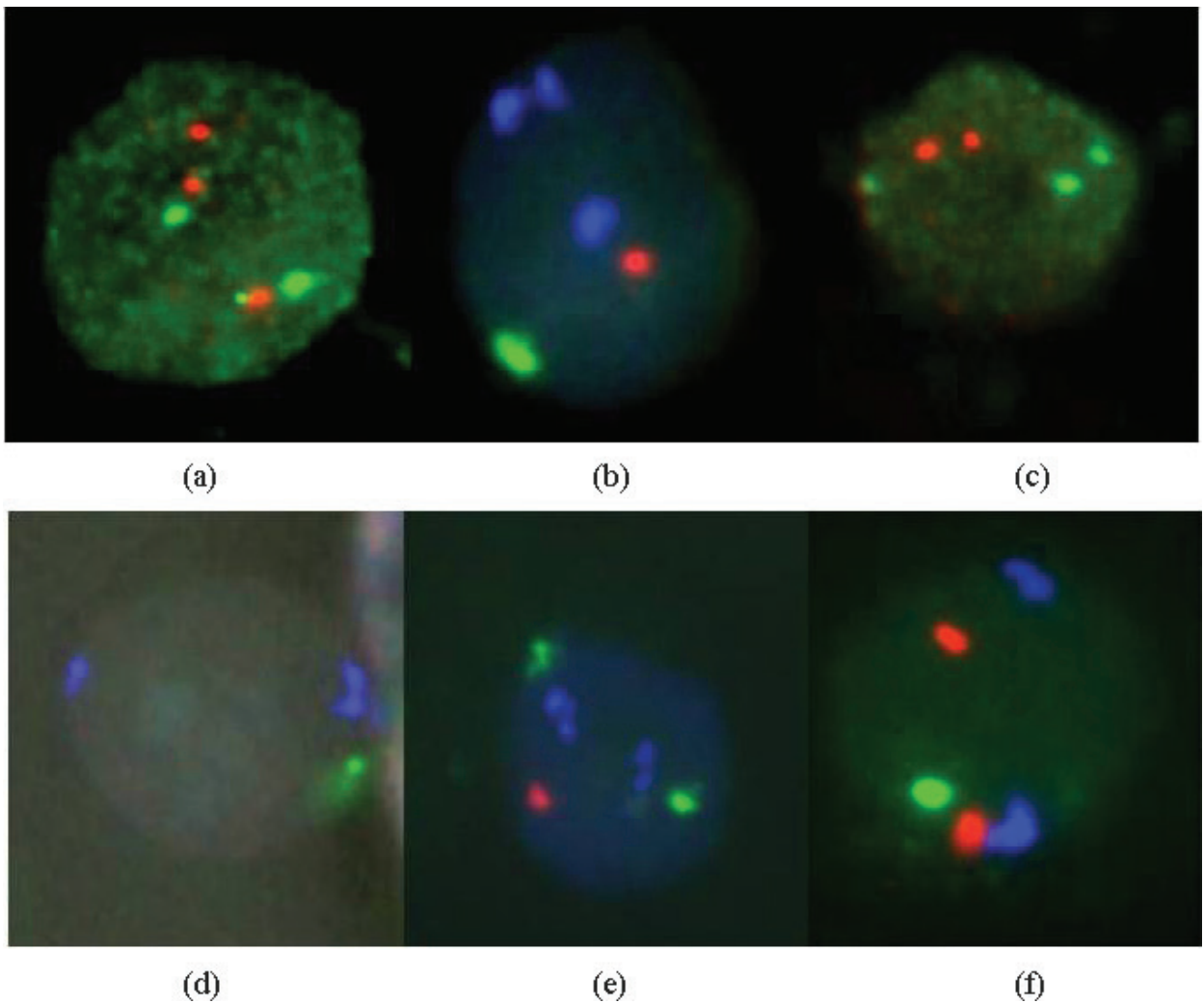
Molecular techniques for prenatal diagnosis of chromosomal disorders were introduced in the medical practice in the last 25 years with the aim of improving the resolution of chromosomal detection and to eliminate the major inconvenient of classic cytogenetic techniques—the requirement of cell culture. Such techniques are fluorescence in situ hybridization (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), multiplex ligation probe amplification (MLPA) and array-comparative genome hybridization (CHG).

##### 3.3.2.2.1. *Fish*

FISH technique allows a hybridization between a fluorescent probe and a complementary DNA sequence present on the target chromosome. In prenatal diagnosis this method has been developed in order to identify the aneuploidy of chromosomes 21, 18, 13, X and Y (the most common chromosomal abnormalities) (**Figure 7**). The probes for chromosomes 13 and 21 are locus specific while the rest are centromeric. The method is done on uncultivated interphasic amniocytes. The final results are obtained in 24–48 hours after amniocentesis which allows maternal anxiety relief [108–110]. The results are obtained after the evaluation of minimum 50 cells. The main advantages are faster results, high specificity and sensibility (close to 100%) and the absence of cell culture. The inconvenients are impossibility of detection of blood contamination, bad quantification of chromosomal mosaics and impossibility of detection of structural abnormalities of chromosomes investigated. FISH can be done also for the evaluation and elucidation of uncommon structural abnormalities: microdeletion syndromes, cryptic or subtle duplications and translocations, complex rearrangements and marker chromosomes [111].

##### 3.3.2.2.2. *QF-PCR*

QF-PCR method allows the detection of major prenatal numerical chromosome disorders within 24–48 hours. The method identifies polymorphic chromosomal specific repeat sequences (short tandem repeats—STRs) that are amplified by the PCR using fluorescent



**Figure 7.** Aneuploidies detected using FISH method. (a) Trisomy 21; (b) Trisomy 13; (c) Trisomy 18; (d) Monosomy X; (e) Trisomy XXY; (f) Trisomy XYY (collection of prenatal diagnosis laboratory of “Cuza Vodă” obstetrics and gynecology hospital, Iași, Romania).

primers. The amplification is observed and quantified using a genetic analyzer and appropriate software. In a normal case, two peaks of fluorescence activity were obtained that reflect a normal heterozygous fetus (1:1 diallelic normal ration). Trisomic samples demonstrate either three peaks with a ratio of 1:1:1 (trisomic triallelic) or two peaks with a ratio of 2:1 (trisomic diallelic) for each informative probe. For each chromosome four or more polymorphic STR markers are analyzed, and thus only few fetal samples will remain uninformative. By comparison with FISH, QF-PCR can detect mosaicisms that have a rate of 20–30% and also identify the maternal cell contamination. The main advantage of QF-PCR is that it is considerably more cost-effective particularly when larger sample numbers are processed [111]. The main limitation of assay is the impossibility to detect triploidy [112].

3.3.2.2.3. *MLPA*

MLPA is a PCR-based method used for quantification of the copy numbers of specific sequences of DNA. This method uses a two-part probe of unique length that, when hybridized

to adjacent target sequences on genomic DNA, can be joined together by the DNA ligase. This permits the amplification of all target sites, using a single primer pair that is complementary to the two free ends which are common to all probes. The PCR products are run on a capillary electrophoresis system and MLPA allows relative quantification of up to 50 different target sequences in one reaction. MLPA is a fast method (final results in 2–3 days) and it is less labor intensive and cheaper compared to karyotyping and FISH. The main domain of application of MLPA in prenatal diagnosis is the detection of most common aneuploidies (of chromosomes 21, 18, 13, X and Y) but this technique has some inconvenients associated with the impossibility of detection of triploidy, mosaicisms and maternal contamination. Other applications concern the cases with multiple congenital anomalies and intrauterine growth retardation detected by ultra-sonography. In these situations, using subtelomere probes or specific probes for some specific syndromes (velocardiofacial, Williams, Wolf-Hirschhorn, Prader-Willi, etc.), MLPA could confirm the presence of some subtle unbalanced structural chromosomal anomalies. MLPA can also be used to determine the origin of marker chromosomes, frequently discovered in chromosomal prenatal diagnosis [113, 114].

#### 3.3.2.2.4. Array-CGH

Array CGH is a method that can detect simultaneously sub-microscopic copy number changes across the whole genome, thus overcoming the limitations of karyotyping and locus-specific techniques. Array CGH has become an important tool for clinical diagnostics and gene-identification studies and is having a great impact on the understanding of pathologies, the counseling of families and patient management. Different types of array CGH platforms at an increasingly higher resolution have been developed, differing mainly in the type of the interrogating probes and in their coverage of the genome. The microarray consists of thousands of unlabeled different probes (particular to a specific DNA sequence) fixed on a glass slide or a silicon chip, arranged in orderly rows in the form of a network with a specific density ("DNA chip"). The two samples of DNA (genomic DNA extracted from patient and reference sample) are fragmented and labeled with different fluorochromes (Cy3—green for patient and Cy5—red for test sample), mixed in equal amounts, denatured and co-hybridized on the microarray. The chips are scanned with a microarray scanner and the images obtained are analyzed using a programme that determines the intensities of emissions of both red and green fluorochrome for each spot on the network and calculates their report. This ratio is in proportion to the number of copies of the patient's genome DNA and test sample. If the intensities of the two fluorochromes are equal to a spot (ratio  $Cy3/Cy5 = 1$ , or  $\log_2 = 0$ ), this region of the patient's DNA is interpreted as normal. If there is a deletion, the test sample hybridizes preferentially to DNA control, and the ratio  $Cy3/Cy5$  will be smaller than 1 (ex. 1:2,  $\log_2 = -1$ ). On the other hand, if there is a duplication, the patient DNA will hybridize preferentially, and the ratio  $Cy3/Cy5$  will be greater than 1 (ex. 3:2,  $\log_2 = 0.58$ .) [115].

The application of array CGH eliminates the majority of fetal chromosomal analysis inconveniences: the long period of waiting for the final result, the possible failures of culture, the poor quality of chromosomal preparations and the reduced number of chromosomal bands. The method uses genomic DNA from fetal cells and can be applied on cells in interphase or division. The sensitivity of method is higher than the standard karyotype, and thus array-CGH allows the detection of all unbalanced chromosomal abnormalities (excepting polyploidy), even the smallest



such subtelomeric rearrangements. In the prenatal diagnosis, “targeted” arrays are commonly used, containing genomic clones for subtelomeric regions and those that are frequently involved in microdeletion/microduplication syndromes. The major advantage of array-CGH is the very high resolution, this technique allowing the detection of genomic changes of 50–100 kb. The main inconvenient is the possibility of detecting copy number variants (CNVs) with unknown clinical consequences. In this case, with limited possibility of fetal phenotype investigation, the evaluation of functional consequences of genomic changes is very difficult. A CNV discovered in these conditions is most probably pathogen if it is *de novo*, has a size >1 Mb, contains a deletion rather than a duplication and involves a gene-rich area [116–118].

Nowadays, in developed countries, the array-CGH represents the first option in the prenatal genetic investigation of fetuses with multiple congenital anomalies detected by ultra-sonography.

#### **4. The combined use of biochemical and ultrasound prenatal screening**

Each method used in prenatal screening has some inconvenients that can be limited by using a combination of multiple assays. Biochemical and ultrasound screening can be done at the same time or at different times. The first method is called concurrent and the second sequential. The sequential protocol can offer the results when all analyses are complete—non-disclosure method—or at the time when each analysis is finished—step-wise method [119].

The biochemical screening in the first trimester is based on the detection of PAPP-A and  $\beta$ -hCG. The PAPP-A has a different discriminatory power in a different week of gestation with a decline from the 10th to 13th week. For a predicted detection rate with 5% false positive rate the combination of PAPP-A and  $\beta$ -hCG has a detection rate of 72% in 10th week, 65% in 11th week, 57% in 12th week and 51% in 13th week. By using a combination between double test (PAPP-A and  $\beta$ -hCG) with detection of aFP and  $\mu$ E during second trimester, the detection rate increases to 78% (with PAPP-A measured in the 10th week), 72% (with PAPP-A measured in the 11th week), 66% (with PAPP-A measured in the 12th week) and 61% (with PAPP-A measured in 13th week) [119].

Nuchal translucency (NT) is independent for gestational age and its screening can be done at 11–13 weeks, with a detection rate of 73% for a 5% false positive rate. By combining biochemical screening in the first trimester with sonographic examination, the detection rate can increase. The best solution is to apply the biochemical screening in weeks 10–12 and the sonographic examination 1 week later. Using this protocol, the detection rate is 92% with double test done in the 10th week, 89% with double test done in the 11th week and 87% with double test done in the 12th week [119].

The best results are obtained using a non-disclosure protocol that combined NT with double test in the first trimester and detection of aFP and  $\mu$ E in the second trimester. This test, called integrated test, generates a detection rate of 93% (with PAPP-A measured in the 10th week), 92% (with PAPP-A measured in the 11th week), 91% (with PAPP-A measured in the 12th week) and 90% (with PAPP-A measured in the 13th week) for a 5% false positive rate. The use of such an algorithm has the inconvenience of having to wait a long time because the final



results are done during the second trimester which increases the anxiety of the couple and limits its reproductive options [119, 120].

## 5. Conclusions

The chromosomal disorders have an important impact on the health of future infants. For this reason, in the last decade, important efforts were made to improve the prenatal screening and diagnosis. The prenatal screening uses non-invasive methods that allow the detection of pregnancies with risk of chromosomal anomalies. These methods can be done in the first or second trimesters of pregnancy. The first-trimester screening methods are biochemical and sonographic. Biochemical screening for first trimester uses the detection of PAPP-A and  $\beta$ -hCG in the maternal serum. Sonographic examination in the first trimester allows the detection of some markers—nuchal translucency, absence of the nasal bone, tricuspid regurgitation or abnormal blood flow through the ductus venosus—that are associated with high risk for aneuploidy. The prenatal screening during the second trimester of pregnancy can be done by biochemical or sonographic examinations as well. The biochemical screening is based on the detection of aFP, hCG (or  $\beta$ -hCG),  $\mu$ E and inhibin A in maternal serum. The sonographic examinations in the second trimester can identify some structural defects (cardiac, cerebral, renal, etc.) but more frequent are the sonographic markers like nuchal thickening, hyperechoic bowel, shortened limbs, pyelectasis, echogenic intracardiac foci, widened pelvic angle, and so on. The best choice for prenatal screening of aneuploidies is the use of combined biochemical (in the first and second trimesters) and sonographic examination. Such protocol has a detection rate higher than 90% at 5% false positive rate. The prenatal diagnosis requires an invasive procedure (chorionic villus sampling, amniocentesis, cordocentesis) to obtain fetal material. The fetal cells can be used for cytogenetic or molecular analyses. The cytogenetic analyses (fetal karyotype) require a long-time cell culture and have a limited resolution but have the advantage of diagnosis of all chromosomal anomalies. Some molecular analyses (FISH, QF-PCR, MLPA) are targeted methods and can identify only specific anomalies, such as aneuploidies of chromosomes 21, 18, 13 X and Y. Array-CGH (molecular karyotype) eliminates the major inconvenience of karyotype (long-time culture and limited resolution) but is expensive and thus its use remains prohibitive in countries with limited economical resources. However, the implementation of prenatal screening and diagnosis allows many couples the opportunity to take an informed decision in relation to their baby's future.

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