

Chromosome Abnormalities in Prenatal Diagnosis

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Abstract

Objective: To investigate antenatal detection the chromosome abnormalities in high risk pregnancies and correlation between karyotype analysis and FISH (Fluorescent In Situ Hybridization).

Method: Were analyzed cytogenetic results from a total of 594 cases between 2008-2009. Amniotic fluid karyotyping and FISH have been offered to pregnant women with genetic risk, using the standard method and GTG banding techniques.

Results: Were found 22 abnormal karyotypes: 13 cases with numerical abnormalities (13 homogenous aneuploidies: trisomies - 3 cases of 47,XX+21, 3 cases of 47,XY+21; 2 cases of 47,XY+18, 1 case of 47,XXY, 2 cases of 47,XXX and monosomies - 1 case of 45,X0; 1 triploidy - 69 XXX), 1 structural abnormality, one case of 46,XY, der(14;21)(q10;q10) +21) and 8 normal variants (3 cases of 46, XX inv(9)(p11;q13); 1 case with 46,XY inv (3)(p11;q11.2); 2 cases with 46,XX inv(3)(p11; q11.2), 1 case 46,XY inv(3)(p11;q11.2), and 1 case of 46,XY inv (3)(p11;q11.2) inv(9)(p11;q13)). The numerical abnormalities have been further verified by FISH analysis. There was a 100% correlation between the results obtained.

Conclusion: This report confirms the importance of karyotyping and FISH in prenatal diagnosis, FISH being much more important for prenatal diagnosis due to the short time of results which is very important for the anxiety and management of the patients in due time.

Keywords: fluorescence in situ hybridization (FISH), aneuploidy, amniocentesis

Introduction

Aneuploidy is a common event in pregnancy with a wide spectrum of medical consequences ranging from the lethal to the benign^[1].

Trisomy 21 (Down Syndrome). The incidence of Down syndrome is estimated at one per 800 to one per 1,000 births^[2]. In 2006, the Centers for Disease Control and Prevention estimated the rate as one per 733 live births in the United States (5429 new cases per year)^[3].

Trisomy 18 (Edwards Syndrome). Trisomy 18 is the second most frequent autosomal chromosome abnormality and occurs in about 1 in 5,000 births. It is named after John H. Edwards, who first described the syndrome in 1960^[4]. The syndrome has a very low rate of survival. It is impossible to predict the exact prognosis of an Edwards Syndrome child during pregnancy or the neonatal period^[5]. The median life span is five to fifteen days^[6,7].

Trisomy X. Triple X syndrome occurs in around 1 in 1,000 girls. On average, five to ten girls with triple X syndrome are born in the United States each day^[8].

Trisomy XXY. Klinefelter's Syndrome is the most common sex chromosome disorder^[9]. The condition exists in roughly 1 out of every 1,000 males. One in every 500 males have an extra x chromosome but do not have the syndrome^[10].

Triploidy is a devastating condition caused by having a full extra set of chromosomes. Triploidy affects 2% of all recognized conceptions, but only a very small proportion of them are live born (0.1% of triploid conceptions or 1 in 50,000 live births).

Monosomy (Turner syndrome). Most monosomies are embryologically lethal, the only exception known in humans is monosomy X (45,X). The 45,X genotype, complete or mosaic, is found in 1 per 2,500 female births with a downward trend in frequency with increasing maternal age.

The aneuploidies presented above can account for up to 95% of live born chromosomal abnormalities^[11]. Diagnosis of chromosomal abnormalities in fetus is one of the most important challenges in modern perinatology.

Method

Amniocentesis is an ultrasound-guided invasive prenatal diagnosis procedure usually performed after 14 weeks gestational age for determination of fetal karyotype, molecular, and biochemical abnormalities.

The time of hospitalization of patients who underwent amniocentesis was at least one day. After the procedure all women were observed for the eventual occurrence of

amniotic fluid leakage, fetal loss (is estimated to be one in every 100 to 200 procedures above the background loss rate^[12,13]), bleeding, abdominal pain and symptoms of infection. The risk of infection introduced at the time of the amniocentesis is estimated to be one to two in 3,000 procedures^[14]. Recent information indicates that approximately 10 to 50 percent of post-amniocentesis losses have evidence of low grade infections at the time of the procedure with increased cytokine levels in the amniotic fluid^[15,16].

Investigation for chromosomal anomalies was routinely performed by cytogenetic analysis and FISH. The traditional "gold standard" for prenatal diagnosis of chromosome abnormalities is metaphase analysis by G-banding. The primary advantages of standard cytogenetic analysis are the ability to detect aneuploidies as well as structural chromosomal aberrations with great accuracy (>99.5%)^[17].

Traditional karyotyping, however, requires isolation of metaphase chromosomes from cultured fetal cells and therefore is time consuming. Although reporting time has decreased dramatically during the last 3 decades, conventional karyotyping still requires 7-12 days of which culture time is the most time consuming^[18].

Fluorescence in situ hybridization (FISH) introduced more than a decade ago, as a potentially powerful tool in clinical cytogenetics^[19] can provide a rapid and relatively reliable detection of aneuploidy of these chromosomes^[20].

The advantage of FISH technique is that it takes only 24-48 hours, costs about half of the conventional cytogenetic technique, and offers an opportunity to reduce anxiety through early decision making process.

FISH uses a fluorescently labeled probe targeted to a unique sequence of DNA where it selectively binds^[21]. For prenatal samples, FISH is done on uncultured, interphase cells. For purposes of RAD (rapid aneuploidy detection), the probes used are specific for chromosomes 13, 18, 21, X, and Y. Samples are visualized using a microscope; the number of fluorescent signals per cell indicates the number of copies of the targeted chromosome.

The FISH protocol that we used:

For each amniotic fluid sample, usually 5-7 ml of clear amniotic fluid sample was centrifuged for 10 min at 1200 rpm. The pellet was resuspended in 5 ml of trypsin-EDTA, gently vortexed, and incubated at 37°C for 30

min. After centrifugation at 1200 rpm for 10 min was performed. The pellet was resuspended by slowly adding 5-7 ml of prewarmed (37°C) hypotonic solution (0,625% sodium citrate). The tube was then placed in a 37°C incubator for 30 min followed by centrifugation at 1200 rpm for 10 min. The supernatant was removed and the pellet was resuspended by adding 5 ml of fixative (3:1 methanol and acetic acid) and gentle mixing. The suspension was kept in a refrigerator (2-8°C) for at least 30 min. Another centrifugation at 1,200 rpm for 10 min. Pellet was resuspended in 2 ml of fresh fixative.

Slide preparation for FISH analysis: 1-2 drops of the above cell suspension was dropped on clean, dry slides placed on a slide warmer at 40-42°C and allowed to dry for 30min.

Commercially available FISH probes specific for aneuploidies of the chromosomes 13, 18, 21, X and Y, common in prenatal samples, were used for the present study. We have been using Vysis Aneuvysion kit which contains three centromeric probes: CEP 18 (for chromosome 18), CEP X (for chromosome X) and CEP Y (for chromosome Y) and two locus specific probes LSI 13 (for chromosome 13), LSI 21 (for chromosome 21).

Two hybridisation areas were made for each sample, one area for chromosomes X, Y and 18 and another for chromosomes 13 and 21.

Denaturation and hybridization of the specimen DNA and the probe was performed according to the manufacturer's instruction. Slides were observed under a fluorescence microscope using appropriate filters (green for chromosomes 13 and X, red for chromosomes Y and 21, blue for chromosome 18). For result interpretation a minimum count for 100 nucleus was scored and results were interpreted following manufacturer's instructions.

Results and discussion

The present study for prenatal detection of chromosomal abnormalities in high risk pregnancies was performed using two approaches - FISH and conventional cytogenetics and was conducted among 594 women who underwent amniocentesis between August 2008 and June 2009 at Life Memorial Hospital Medlife, Bucharest. All cases were white Caucasians.

Of the 594 samples, 34,7 % had a maternal age between 31 and 35 years, which was the most common age group, followed by age 36-40 (183, 30,7%), 26-30

Probe Name	Probe Location	Fluorophore
Vysis CEP 18	18p11.1-q11.1 Alpha Satellite DNA	SpectrumAqua™
Vysis CEP X	Xp11.1-q11.1 Alpha Satellite DNA	SpectrumGreen™
Vysis CEP Y	Yp11.1-q11.1 Alpha Satellite DNA	SpectrumOrange™
Vysis LSI 13	13q14	SpectrumGreen™
Vysis LSI 21	21q22.13-q22.2	SpectrumOrange™

(143, 24.1%), 21-25 (35, 5.9%) older than 41-45 (24, 4.0%) and younger than 20 (3, 0.5%).graphic 1 The aneuploidies were most frequently detected in age 39-40 (5 cases- trisomy 21, and 1 case -trisomy XXY,).

Gestational age at the time of the procedure varied from 13 to 24 (amniocentesis was done at 18 gestatio-

nal weeks in 23.4% of cases, 17 in 22.2%, 19 in 15.7%, 16 in 11.6%, 20 in 9.4%, 21 in 6.6%, 22 in 3.0%, 23 in 3.0%, 24 in 2.0%, 15 in 1.9%,14 in 0.3% and less than 13 in 0.8% (graphic 2).

The aneuploidies were most frequently detected in gestational weeks 17 (6 cases, 46.1%)

Figure 1.
Age distribu-
tion of the
population
screened

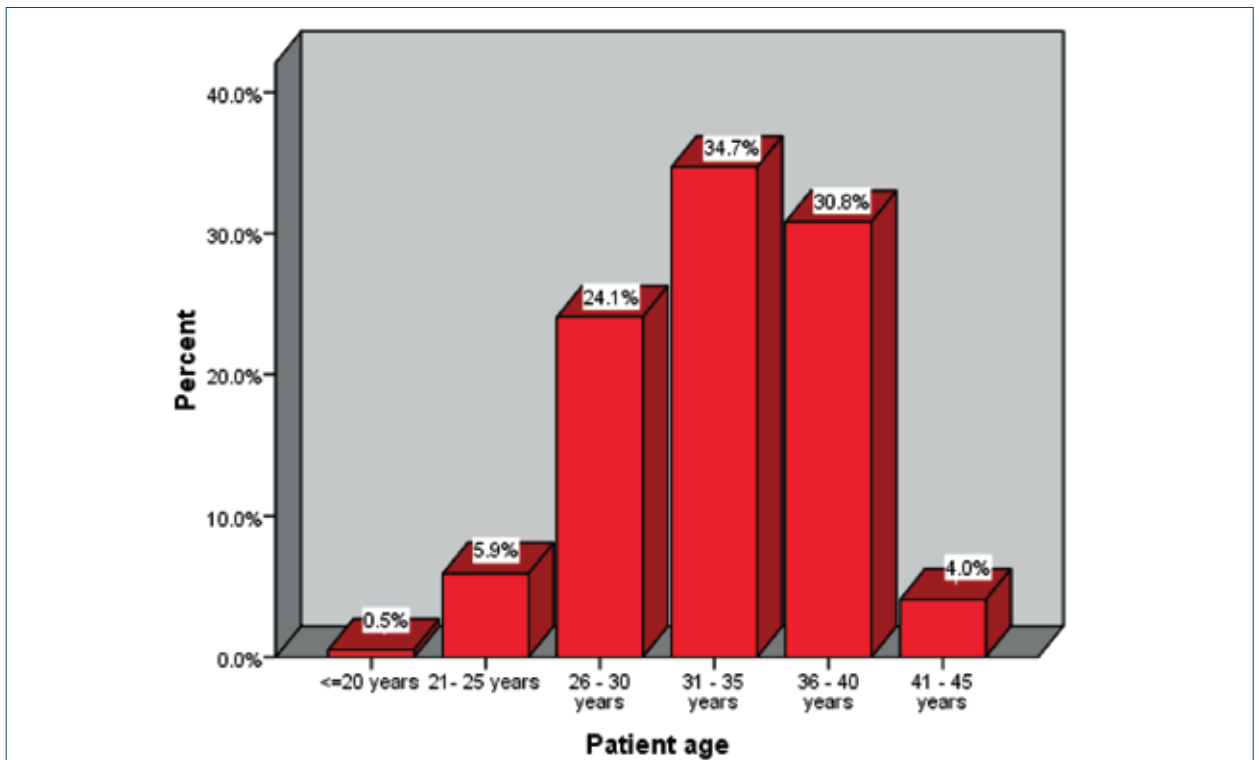
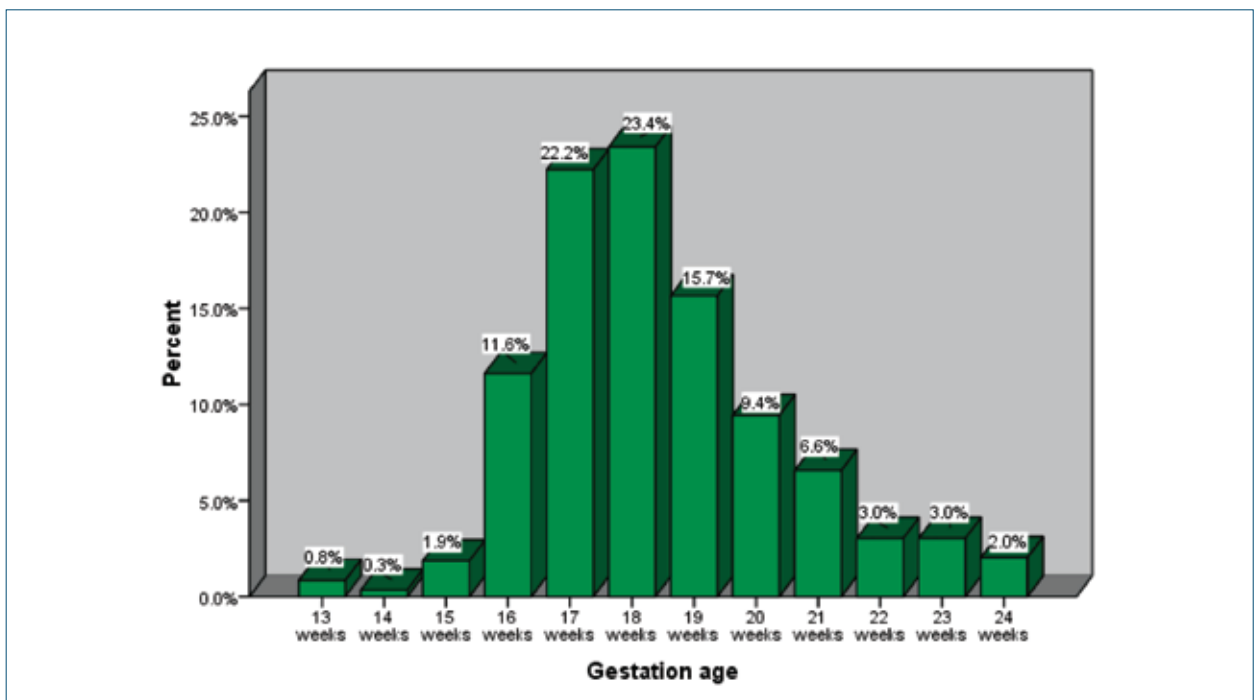


Figure 2.
Gestational
age distribu-
tion of the
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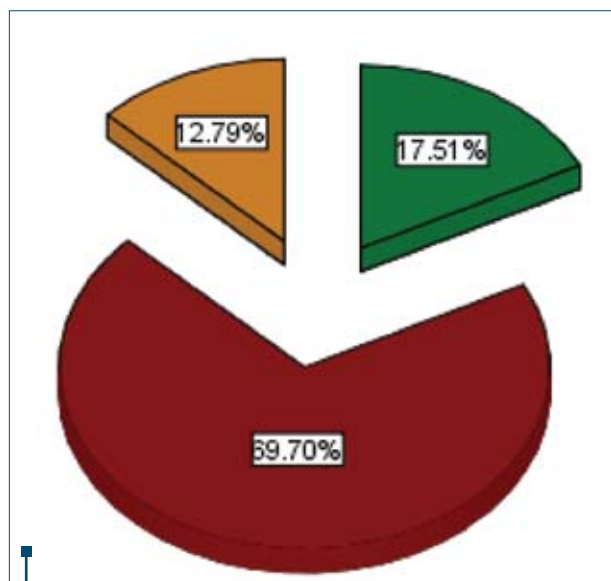


Figure 3. Abnormal results of double or triple test

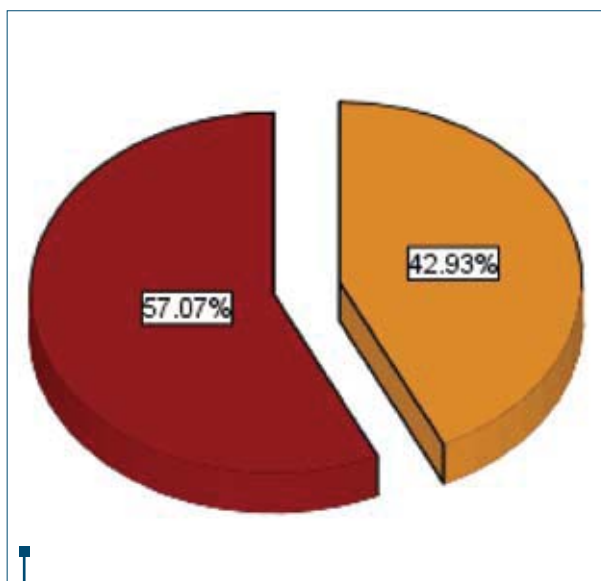


Figure 4. Advanced maternal age

The most frequent indications for amniocentesis were:

a) abnormal results of double or triple test (69.70% of patients had high risk, 12.79% low risk, 17.51% have not performed any of the two screening tests). Biochemical screening of specific markers in maternal serum is very important for risk stratification as it is increasingly employed to detect additional pregnancies in the low-risk population that need fetal karyotype evaluation. Low levels of alpha-fetoprotein (AFP) and unconjugated estriol and high levels of HCG in maternal serum are associated with increased risk of a chromosomally abnormal pregnancy^[1] (figure 3).

b) advanced maternal age (42.93 % had increased age risk and 57.07% had low age risk). The association of maternal age over 35 years with an increased risk of chromosomally abnormal conceptions is well documented. Maternal age influences the chances of conceiving a baby with Down syndrome. At maternal age 20 to 24, the probability is one in 1562; at age 35 to 39 the probability is one in 214, and above age 45 the probability is one in 19^[22]. Although the probability increases with maternal age, 80% of children with Down syndrome are born to women under the age of 35^[23] (figure 4).

c) fetal malformations found during ultrasound examination (43.77% of patients had no ultrasound reports for pregnancy in their discussion with genetic counselor, so ultrasound evolution of those fetuses is unknown, 9.43% had ultrasound malformations and 46.80% have no changes). Ultrasonography now has a considerable role in prenatal diagnosis. Certain major ultrasonographic defects are fairly specific: for example, holoprosencephaly predicts the likelihood of trisomy 13, fetal hydrops/cystic hygroma predicts monosomy X or trisomy 21, and an endocardial cushion defect or duodenal atresia predicts trisomy 21. The minor marker of increased nuchal translucency (actually, this separation of the skin from the under-

lying tissue can extend from as far as the occiput down to the lower back) is less specific. Cardiac malformations generally have a frequent association with fetal aneuploidy, as do certain renal defects^[24] reviewed 1800 cases in which an anomaly (an actual malformation, or a minor marker of aneuploidy) had been detected at ultrasonography, and assembled a table of risks of aneuploidy according to the findings (figure 5).

Indications for amniocentesis:

From these indications, 2 aneuploidies (trisomy 21, 1 case; trisomy 18, 1 case) were first indicated by abnormal results in maternal serum screening test, 3 (trisomy 18, 1 case; triploidy 1 case; monosomy 1 case) by abnormal ultrasound findings and 5 (trisomy 21, 5 cases) by abnormal results in maternal serum screening combined with advanced maternal age.

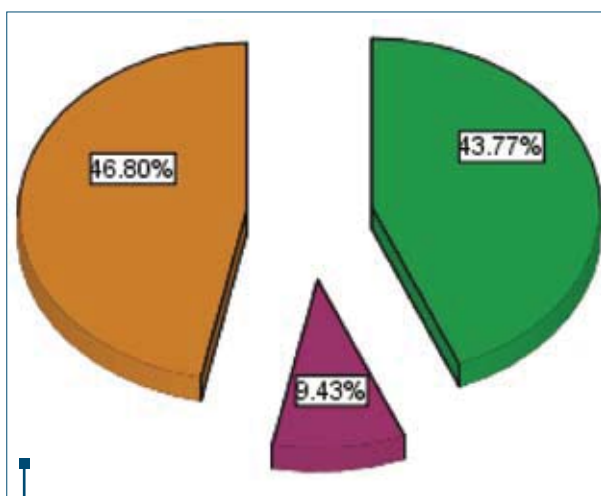


Figure 5. Ultrasound markers

Table 1 Chromosomal abnormality in cytogenetic study

Chromosomal abnormality	Disorders	Karyotype	Frequency	Number of cases
Numerical	Trisomy 21	47,XX+21	0,58%	3
		47,XY+21	0,58%	3
	Trisomy 18	47,XY+18	0,20%	1
	Trisomy X	47,XXX	0,39%	2
	Trisomy XXY	47,XXY	0,20%	1
	Triploidy	69,XXX	0,20%	1
Structural	Inversions	46,XX,inv(9)(p11q13)	0,58%	3
		46,XY,inv(9)(p11q13)	0,20%	1
		46,XY,inv(3)(p11q11.2)	0,39%	2
		46,XX,inv(3)(p11q11.2)	0,20%	1
		46,XY,inv(3)(p11q11.2)(9)(p11q13)	0,20%	1
	Translocations	46,XY,der(14;21)(q10q10)	0,20%	1
Total	-	-	3,92%	20

Of the total 594 patients, 510 opted for fetal karyotyping, 515 opted for FISH and 440 opted for both analyses.

Karyotyping results were categorized into normal karyotypes (96.08% of patients) and abnormal karyotypes (3.92% of patients). Abnormal karyotypes were divided into common aneuploidies of chromosomes (21, 18, X and Y) and other chromosomal abnormalities (inversions and translocations). One of the frequent occurrences in chromosome rearrangements is pericentric inversion of the chromosome 9 inv(9)(p11q13), which some scientists consider as a variant of normal karyotype. Although it seems not to correlate with abnormal phenotypes there have been many controversial reports in-

dicating that it may lead to abnormal clinical conditions such as infertility and recurrent pregnancy loss^[25,27]. The incidence of pericentric inversion of the chromosome 9, inv(9)(p11q13), is said to be about 1% to 1.65% in the general population^[26]. Table 1 shows the details of the 20 cases with chromosomal abnormalities.

Since the 1970s, karyotyping of fetal cells cultured from amniotic fluid has been the gold standard technique for the prenatal diagnosis of chromosomal disorders. Standard cytogenetic analysis performed on fetal cell samples identifies chromosome aneuploidies and rearrangements with approximately 99.5% accuracy. However, a significant limitation of this technique is that cells have to be cultured, leading to a delayed re-

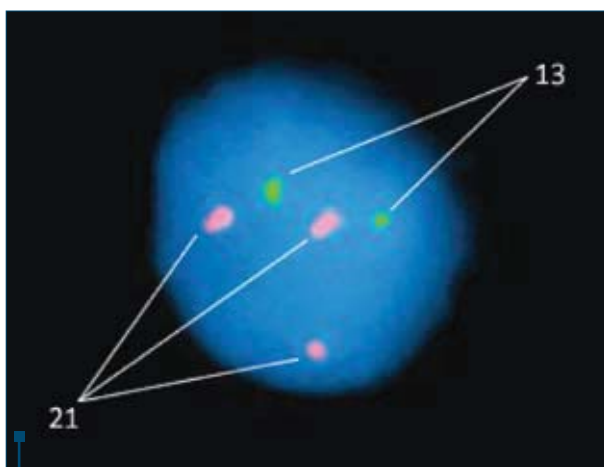


Figure 6. Interphase nucleus from uncultured amniocytes by FISH shows two green (13 chromosome) and three red (21 chromosome) - trisomy 21

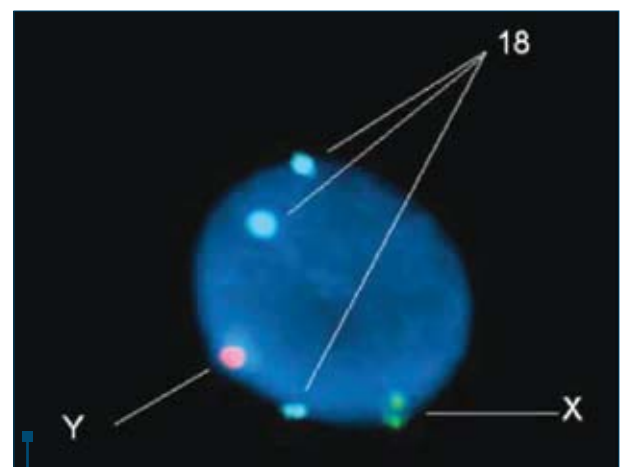


Figure 7. Interphase nucleus from uncultured amniocytes by FISH shows one green (X chromosome), one red (Y chromosome) and three blue (18 chromosome) - trisomy 18

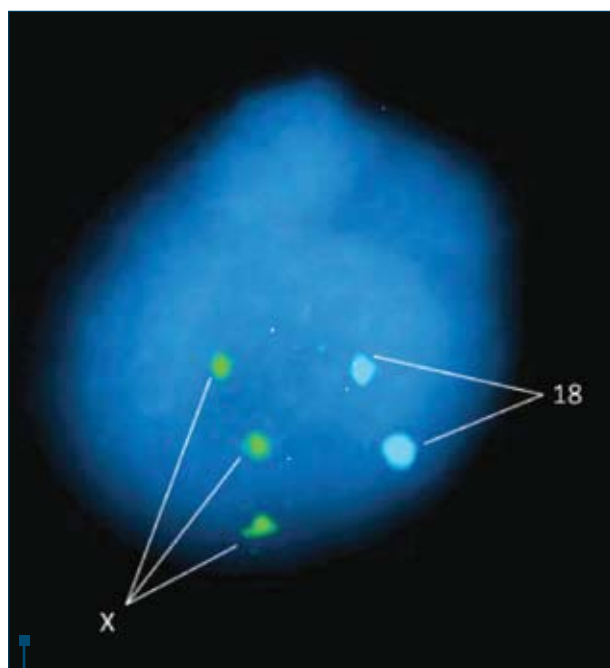


Figure 8. Interphase nucleus from uncultured amniocytes by FISH shows three green (X chromosome) and two blue (18 chromosome) - triple X

sult (commonly between 14 and 21 days). Waiting for chromosome analysis can be especially stressful for the patients^[28].

FISH results were categorized into normal FISH (97.87% of patients) and abnormal FISH (2.13% of patients). In 0,97 % it was trisomy 21 (fig.6) , in 0.19 % monosomy 45 X (figure 10), in 0.39% trisomy X,(figure 8) in 0.19% trisomy XXY (figure 9) and 0.39% trisomy 18 (figure 7).

Compared to cytogenetic studies, the sensitivity of the test FISH to detect aneuploidies in this study was 100%. Chromosomal abnormality, such as 46,XX,inv(9)(p11q13) and 46,XY,inv(3)(p11q11.2) cannot be detected by interphase FISH analysis. Table 2 shows the details of the 11 cases with FISH abnormalities.

Several studies have reported successful application of FISH on interphase cells for rapid prenatal diagnosis. Rapid detection of prenatal aneuploidy using interphase FISH on a large scale were successfully initiated^[29]. Their studies formed the basis of the clinical protocols for the application of FISH to prenatal diagnosis.

FISH analysis of uncultured amniocytes offers an informative result, in most cases, in 24-48 h. Rapid results may be crucial for important clinical decision-making in some cases and are helpful in decreasing the anxiety level in most patients with an abnormal maternal serum screening and increased risk for trisomy. However, it has been demonstrated that in case of not performing karyotype analyses this will lead to a significant number of false negative results related to other unbalanced abnormalities^[30].

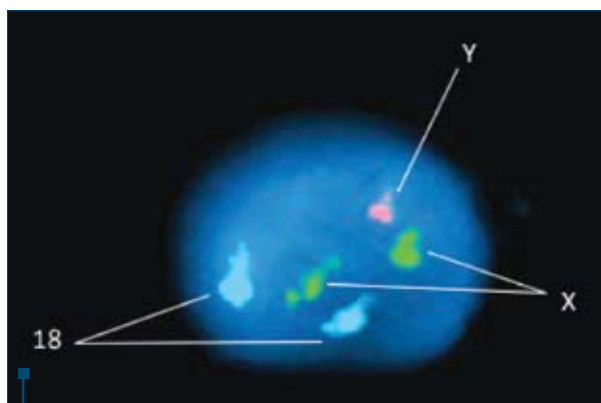


Figure 9. Interphase nucleus from uncultured amniocytes by FISH shows two green (X chromosome), one red (Y chromosome) and two blue (18 chromosome) - Klinefelter syndrome

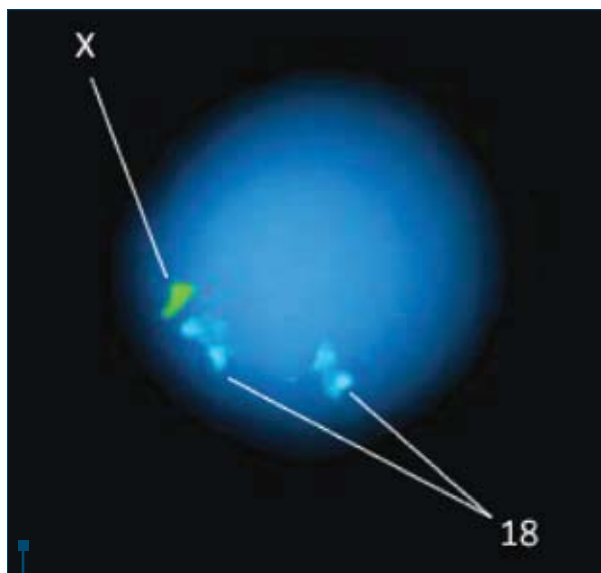


Figure 10. Interphase nucleus from uncultured amniocytes by FISH shows one green (X chromosome) and two blue (18 chromosome) - Turner syndrome

Conclusions

All chromosomal aneuploidies and the majority of structural chromosomal abnormalities (deletion, translocation) can be prenatally detected by conventional karyotype.

FISH analysis performed on uncultured amniocytes is important due to the fact that results generally can be obtained in the first 48 hours from amniocentesis.

In this study we have investigated the correlation between karyotype analysis and FISH and concluded that there was a 100% correlation between the results obtained. In one case which was identified as trisomy of the chromosome 21, karyotyping identi-

Table 2 Chromosomal abnormalities detectable by FISH

Chromosomal abnormality	Disorders	Frequency	Number of cases
Autosomal abnormalities	Trisomy 21	0,97 %	5
	Trisomy 18	0,39%	2
Sex chromosome abnormalities	Trisomy X	0,39%	2
	Monosomy X0	0,19%	1
	Trisomy XXY	0,19%	1
Total	-	2,13%	11

fied a derivative chromosome 14 - 46,XY,der(14;21)(q10q10).

FISH analysis identified this abnormality as trisomy because the probe used is specific for the region which was translocated to chromosome 14 and actually this aberration has a phenotypic expression as chromosome 21 trisomy. FISH limitation in detecting structural abnormal-

ities is one major point which makes it a good complementary technique and not the standard analysis.

Consequently, aneuploidy screening of uncultured amniotic cells with direct FISH is important for prenatal diagnosis due to short time of result delivery which is very important for the anxiety management of the patients. ■

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