MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

PRENATAL DIAGNOSIS OF TRISOMY 21 MOSAICISM

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Abstract:
Aim: To summarize the experience of prenatal diagnosis of trisomy 21 mosaicism.
Methods: A retrospective study which includes seven prenatally detected cases of mosaic trisomy 21, routinely diagnosed among 5837 prenatal investigations performed during a 13-year period (2003-2015) in a single tertiary center.
Results: Mosaic trisomy 21 was detected in 0.1% of all prenatal karyotyping analyses performed. Six cases were revealed after amniocentesis and one after chorionic villus sampling. The mean maternal age was 33 years. The proportion of trisomy 21 cells ranged from 4% to 42%. Five out of seven cases (71%) had positive confirmatory studies. Abnormal ultrasound findings, bilateral pyelectasia and cystic hygroma were diagnosed in two cases confirmed to be mosaic for trisomy 21. Pregnancy outcome and postnatal follow-up in two cases with normal karyotypes observed after confirmatory studies were uneventful.
Conclusion: Confirmatory studies using amniocentesis and/or cordocentesis should be performed when mosaic trisomy 21 is disclosed, while FISH analysis on uncultured amniocytes is the method of choice in resolving low-level or cryptic mosaicisms. Although fetal outcome is not strictly dependent on the level of mosaicism, an increasing proportion of trisomic cells, and especially the presence of ultrasound anomalies, correlates with high risk of fetal abnormality.

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INTRODUCTION

Prenatal diagnosis of chromosomal abnormalities is routinely performed as part of obstetric management of pregnancies carrying a high risk of chromosomal defects. Non-invasive screening methods are used for risk assessment of affected pregnancies, while a definite diagnosis is made by cytogenetic analysis of cultured fetal cells obtained by one of the available invasive procedures, mainly chorionic villus sampling (CVS) or amniocentesis. Mosaicism, the presence of two or more cell lines with a distinct karyotype derived from a single zygote, is found in approximately 0.3% of amniotic fluid cell cultures and in 1-2% of chorionic villus samples.[1, 2] When discovered during prenatal diagnostics, it presents one of the major interpretative issues. The main difficulties concern the ability to differentiate pseudomosaicism, which delineates cultural artefacts, from true mosaicism; the possibility of misdiagnosis of cryptic or low-level mosaicisms; and the prediction of fetal outcome. Furthermore, when mosaicism is found in chorionic villi, additional caution regarding fetal involvement should be considered.
Trisomy 21, which is causative of Down syndrome, is one of the most common chromosomal abnormalities, occurring in approximately one in 200 of all clinically recognized pregnancies, and between one in 1000 and one in 700 live births.[3, 4] Approximately 95% of Down syndrome individuals have regular form or free trisomy 21, about 2-4% have Robertsonian translocation involving chromosome 21, while it is in only 1-2% of patients that a mosaicism comprising two

cell lines, one with a normal karyotype and other with trisomy 21, is present.[5] Mosaicism for trisomy 21 discovered during prenatal diagnosis is a rare finding, revealed in approximately 0.02% to 0.07% of all fetal karyotyping investigations performed. [2, 6]

The aim of this study is to summarize the experience of prenatally detected cases of mosaic trisomy 21, observed within a 13-year period in a single tertiary center.

MATERIAL AND METHODS

This retrospective study encompasses seven prenatally detected mosaicisms for trisomy 21, routinely obtained after CVS or amniocentesis during the period from 2003 to 2015 at our Department. Throughout the observed period, a total of 5837 invasive procedures were performed. The indications for prenatal diagnosis were advanced maternal age (Ó35 years), the presence of abnormal ultrasound findings, positive familial history for chromosomal diseases, or abnormal maternal serum screening (biochemical tests or non-invasive prenatal test ï NIPT).

Amniocentesis was performed at 15-19 weeks gestation, when 15 to 20 ml of amniotic fluid was obtained. The flask method was used for cell culturing according to standard protocols, and three independent cultures were set up for each sample. In order to obtain high-resolution chromosome preparations, cell cycle synchronization using 5-bromo-2ô-deoxyuridine (BrdU) and thymidine was applied to one primary culture. After 10 to 14 days, the cultures were harvested, followed by chromosome slide preparation.

CVS was carried out transabdominally between 11 and 14 weeks of gestation. At least 15-20 mg of chorionic villi were acquired, and both short-term cytotrophoblast and long-term mesenchymal stroma cultures were set up. For short-term culturing, the villi were cultivated over a 24 hour period, applying overnight sample incubation at 4°C as a method of culture synchronization. Trypsin-Giemsa-banding technique was used for chromosomal analysis, with the cytogenetic results obtained within 72 hours. The other part of the chorionic villus sample was disaggregated by mechanical and enzymatic treatment, and the long-term culture was performed using the flask method. Chromosomal preparations were available for analysis in 10 to 14 days.

Cytogenetic analysis was performed following the European Cytogeneticists Association guidelines and the recommendations for the management of suspected mosaicism by Hsu and Benn.[7, 8] At least 20 metaphases from three flasks were examined. In cases where trisomy 21 was found in single or multiple metaphases at initial flask, at least 20 metaphases from the other two flasks were analyzed. A mosaicism was reported when at least two cells with trisomy 21 from two independent flasks were found. In all cases of mosaicism, results were confirmed by a subsequent invasive procedure, i.e. amniocentesis or cordocentesis, or by analysis performed after termination of pregnancy (TOP). For fetal blood analysis short-term phytohemagglutinin-stimulated whole blood culturing was used. Cytogenetic analysis after TOP was performed on cultured skin fibroblasts.

Fluorescence in situ hybridization (FISH) was carried out with a commercially available 21q22.1 specific region probe (Kreatech FISH probes, Leica Biosystems, Nussloch, Germany), according to manufacturerôs protocols.

Table 1. Data on cytogenetic analysis and pregnancy outcome for seven detected cases of mosaic trisomy 21

Indication	*Maternal age*	*Weeks' gestation*	*Cytogenetic analysis*			*Confirmatory studies*			*Pregnancy outcome*
			Fetal sample	Percentage of trisomy 21 cells	No. of cells examined	Fetal sample	Percentage of trisomy 21 cells	No. of cells examined	
familial history	27	16	AF	42%	76	AF PUBS	0 0	72 130	NL
AMA, ultrasound anomaly	41	18	AF	38%	93	AF PUBS	31% 13%	63 100	TOP
maternal serum screening	27	16	AF	7.5%	40	AF PUBS	0 0, FISH ï 0	67 150, FISH ï 312	NL
AMA	40	17	AF	33%	9	PUBS	38%	50	TOP
AMA, ultrasound anomaly	37	12	CVS	25%	12	Fetal skin	20%	30	TOP
AMA, maternal serum screening	37	18	AF	4%	84	AF	9% FISH - 16,8%	58 FISH ï 310	TOP
AMA, NIPT	38	18	AF	38%	50	AF PUBS	23% 6%	133 123	TOP

Legend: AF- amniotic fluid; AMA - advanced maternal age; CVS - chorionic villus sample; FISH - fluorescence in situ hybridization; NIPT - noninvasive prenatal test; NL - normal liveborn; PUBS - percutaneous umbilical blood sampling; TOP - termination of pregnancy

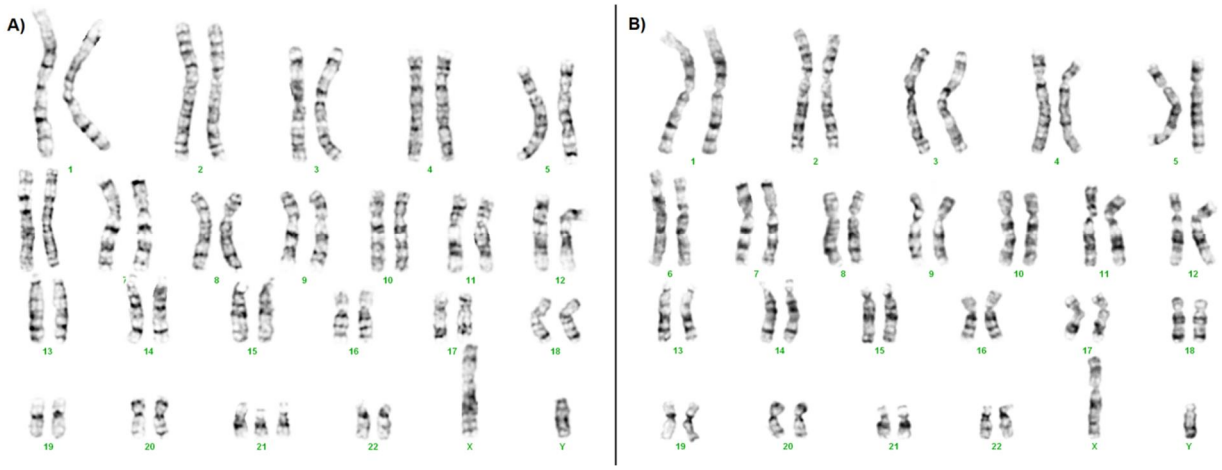

Figure 1. A case of 47,XY,+21/46,XY mosaicism revealed after amniocentesis. Karyogram showing trisomy 21 (A), and 46,XY cell line (B)

RESULTS

During a 13-year period (2003-2015), a total of 5837 prenatal cytogenetic investigations were performed, while mosaicism involving two cell lines, one with a normal karyotype, and another with trisomy 21, was observed in seven cases (0.1%). One case was detected from chorionic villus culture and six from amniotic fluid culture. Indications for prenatal diagnosis are presented in Table 1. The mean maternal age at the time of diagnosis was 33 years, ranging from 27 to 41 years.

Three fetuses were males and four were females. The percentage of trisomic cell line ranged from 4% to 42%. Four cases were re-evaluated through repeated amniocentesis in combination with cordocentesis, one case solely by amniocentesis, one only using cordocentesis, while culture of fetal skin was performed in one case. Confirmation studies were positive in five cases (5/7, 71%).Abnormal ultrasound findings were diagnosed in two cases confirmed to be mosaic for trisomy 21: bilateral pyelectasis measuring 6 mm, and cystic hygroma measuring 6.1 mm associated with anasarca. In two cases evaluated from amniotic fluid culture and fetal blood, the percentages of trisomic cells were 23% and 31%, and 6% and 13%, respectively (Figure 1). In the case where mosaicism was detected on CVS, a 47,XX,+21/46,XX karyotype was detected in both, short- and long-term cultures (Figure 2). A 12-week old fetus with cystic hygroma and anasarca ended in spontaneous abortion after invasive procedure. A confirmation study was performed on fetal skin fibroblasts culture, showing 20% of trisomic cells. In one case, the presence of mosaic trisomy level of 38% was confirmed from fetal blood culture. In a case of amniocentesis performed due to advanced maternal age and risk for trisomy 21 of 1:85, obtained by double test, cytogenetic analysis revealed the proportion of trisomic cells of 4%. Subsequent amniocentesis was performed, and trisomy 21 was confirmed in 9% of metaphases from two flasks. However, FISH analysis on uncultured amniotic fluid cells revealed a trisomy 21 in 16.8% of analyzed cells (Figure 3).

After extensive genetic counseling, the parents decided to terminate the pregnancies in all cases confirmed as true fetal mosaicisms. Pregnancy outcome in two negative cases was uneventful, resulting in delivery of phenotypically normal infants. A seven-year postnatal course was normal.

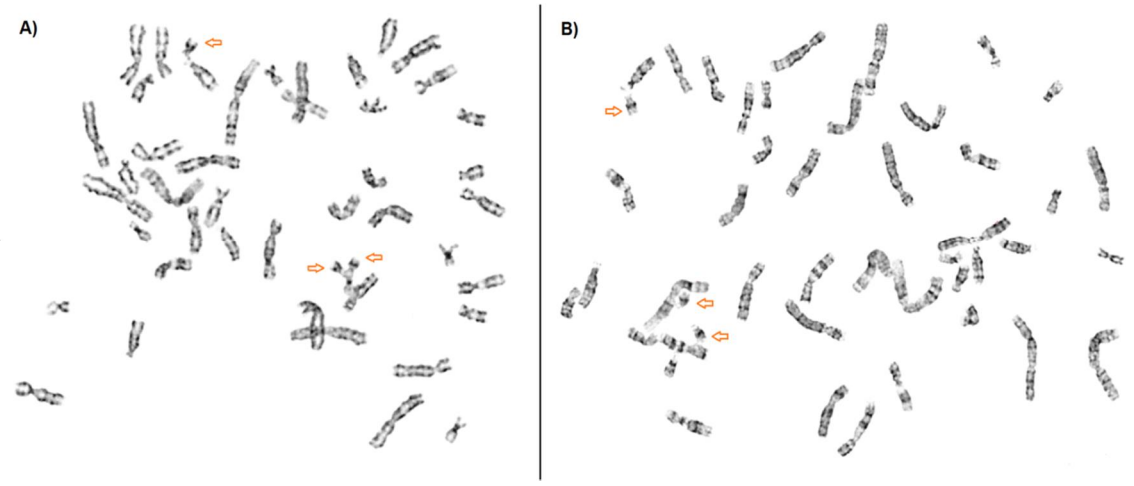

Figure 2. Chromosome preparations obtained after CVS. Metaphase chromosomes with trisomy 21 from short-term culture (A), and long-term culture (B)

DISCUSSION

Although Down syndrome is the most common chromosomal abnormality disclosed among live births, its etiology and mechanisms underlying the appearance of trisomy 21 remain unclear. It has been estimated that in 95% of non-mosaic trisomy 21 cases, the extra chromosome 21 originates from non-disjunction during first or second meiotic divisions, while postzygotic mitotic errors occur in 5% of cases. Furthermore, approximately 90-95% of free trisomy 21 are maternal in origin, and, to date, advanced maternal age has been established as the only certain risk factor for conceiving a pregnancy with Down syndrome.[9,10] For mosaic trisomy 21, two possible mechanisms by which mosaicism could arise have been revealed (Figure 4). In a case when meiotic non-disjunction results in a trisomic zygote, the loss of the extra chromosome, i.e. trisomic rescue, occurs in some mitotic divisions during fetal development. In the other scenario, the zygote is initially chromosomally normal, while trisomic cell line arose from mitotic nondisjunctional events during somatic divisions.[11] It is assumed that meiotically derived mosaicism could be in correlation with maternal age in the same way as free trisomy 21. However, the study of Pangalos et al. on mechanisms underlying mosaic trisomy 21 showed that meiotic errors occurred in only 58.8% of cases, while the rest were due to a postzygotic event.[12] Investigating maternal age dependence for trisomy 21 mosaics, Morris reported the mean maternal age of 33.1 years within the group with mosaicism, in comparison with 35.0 years among free trisomy 21 cases.[13] Furthermore, 33% of mothers who had a pregnancy with the mosaic form were younger than 35 years. Our results correlate with the report of Morris, since the mean maternal age was 33 years in the present study, while 5 out of 7 mothers (28.6%) were younger than 35. These results indicate a significantly weaker association of maternal age and mosaic trisomy 21 in comparison with non-mosaic Down syndrome, and most likely the presence of other possible risk factors, causing difficulties in genetic counseling on the risk-estimation of the mosaicism recurrence in subsequent pregnancies. Furthermore, the differences in fetal sex ratio have been observed between mosaic Down syndrome and full trisomy 21, with the female proportion of approximately 60% and 45%, respectively.[13, 14] In the present study, the male to female ratio of 0.75 was also observed.

Though chromosomal mosaicism is not an uncommon finding during prenatal diagnosis, it still presents a challenge for interpretation. Depending on the abnormal cell line distribution detected during cytogenetic analysis, three levels of mosaicism are distinguished. Level I mosaicism represents the finding of a single abnormal cell, which is almost certainly a cultural artefact and is referred to as pseudomosaicism. When two or more cells with the same aberration are found in a single flask or colony, it usually represents a pseudomosaicism, with an *in vitro* origin of the abnormality (level II mosaicism). Finally, level III mosaicism or true mosaicism denotes the presence of two or more cells with the same aberration found in multiple flasks/colonies.[5] Although guidelines for the management of the detected mosaicism have been proposed, the distinction between pseudomosaicism and true mosaicism could still cause difficulties in praxis. Furthermore, a true fetal chromosomal abnormality may exist in approximately 1% of cases with level II mosaicism. With the proportion higher than 5%, Hsu et al. found mosaic trisomy 21 as one of

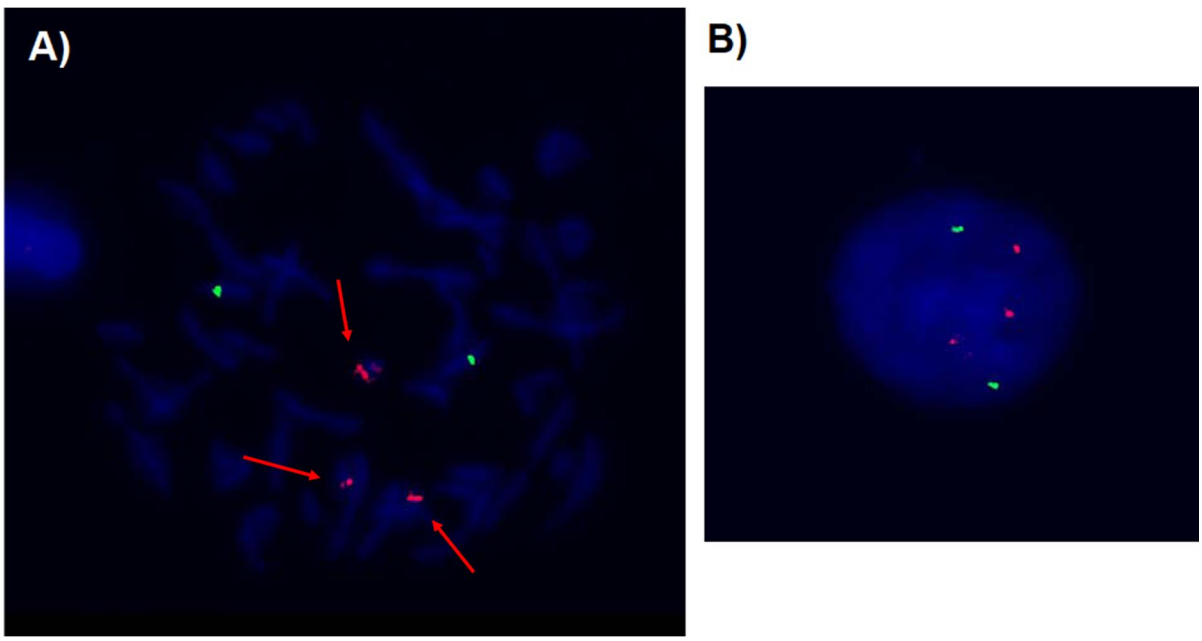

Figure 3. FISH analysis using 21q22.1 specific region probe performed after amniocentesis. A) Three red signals indicate the presence of trisomy 21 on metaphase chromosomes from cultured amniocytes. B) An interphase nucleus with trisomy 21 (three red signals) from native amniotic fluid sample

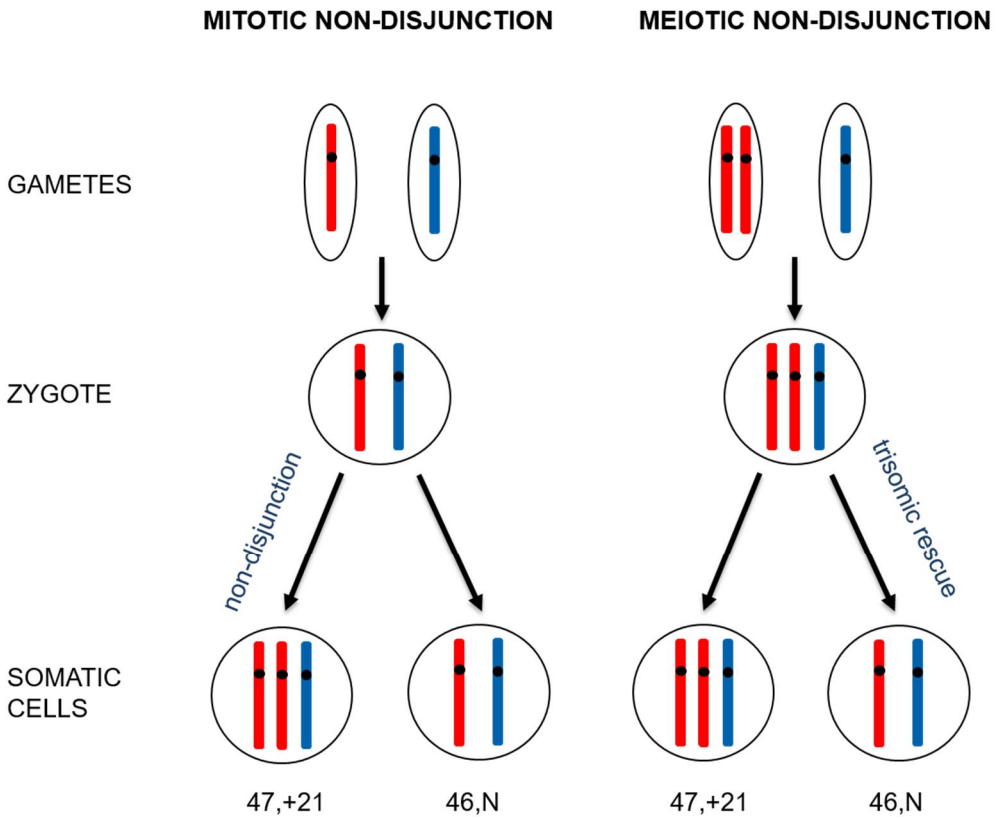

Figure 4. Shematic representation of mechanisms leading to mosaic trisomy 21. In the case of mitotic non-disjunction, the zygote is chromosomally normal and non-disjunction occurs postzygotically. In the case of meiotic non-disjunction, the zygote is initially trisomic, while trisomic rescue occurs during somatic mitotic divisions.

the most common aneuploidies occurring among level I and II mosaicisms.[15] However, based on karyotype-phenotype correlation data, the authors proposed that extensive workup, and probably confirmation studies, should be performed when mosaic trisomy 21 is suspected. All cases of mosaicism presented in our study were level III mosaicism. Mosaic trisomy 21 was found in 0.1% of all prenatal investigations performed. Still, confirmation studies and postnatal follow-up revealed false positive results in two out of seven cases, which could be the reason for the slightly higher proportion observed in the present study in comparison with frequencies of 0.02% and 0.07% found by Hahnemann and Vejerslev and Hsu, respectively.[2, 6]

Cases of cryptic mosaicism present another challenge in prenatal diagnostics. One such case was disclosed in our study. A mosaicism with 4% and 9% trisomic cells was revealed by cytogenetic analysis of the first and subsequent amniotic fluid samples, respectively. However, FISH analysis on uncultured amniocytes showed a trisomy 21 with a proportion of 16.8%. It has been estimated that for exclusion of mosaicisms lower than 10% with a 95% or 99% confidence, at least 35 or more than 50 metaphases should be examined, respectively.[16] For this purpose, FISH analysis has been proven as the method of choice, since it enables the examination of a substantial number of metaphase as well as interphase cells. Furthermore, the possibility of the overgrowth of karyotypically normal cell line due to long-term culturing, and the descending proportion of trisomic cells during subculturing, could not be excluded. In such a manner, interphase FISH on uncultured amniocytes provides more accurate information on the trisomy 21 mosaic percentage, more precisely reflecting the degree of aneuploid cells present *in vivo*.[17]

When mosaicism is found during prenatal diagnosis, the prediction of fetal outcome always requires special attention in genetic counseling. In the present study, abnormal outcome was revealed in 71% of cases, while ultrasound anomalies were recorded in two cases. Wallerstein et al. reported an abnormal fetal outcome in 51% of trisomy 21 mosaicisms disclosed at amniocentesis.[18] However, out of 48 normal cases there were only seven phenotypically normal infants, while 41 cases were apparently normal abortuses. Since confirmatory studies revealed a normal karyotype in only 12 abortuses, it is likely that at least a certain proportion of terminated pregnancies would have had an abnormal outcome. Although authors suggested that the risk of abnormal outcome is significantly higher in cases with the proportion of trisomic cells greater than 50%, the percentage range of 1% to 95% was observed among abnormal cases.[19] Furthermore, the proportion of trisomic cells in a range of 4% to 38% observed among abnormal cases in our study suggests that the fetal outcome is not dependent on the mosaicism level, causing difficulties in genetic counseling, especially in

those cases with the absence of ultrasound anomalies.

The majority of mosaic cases detected on CVS present a confined placental mosaicism (CPM), which means that abnormal cell line is restricted only to placenta, while true fetal mosaicism (TFM) is confirmed in approximately 10-15% of cases. During cytogenetic analysis, abnormal cells could be found solely in short- or long-term culture, or in both, and all three possibilities could be associated with CPM or TFM, implying six possible combinations, i.e. six types of mosaicism.[2] Depending on the cell line distribution and type of chromosomal abnormality, the risk of fetal involvement is settled. Confirmatory analysis on the amniotic fluid sample should be performed to discriminate between CPM and true fetal involvement. In the case of mosaicism revealed in our study, trisomic cell line was detected in both, short- and long-term culture. Although a finding of mosaicism for other chromosomopathies in both cytotrophoblast and mesenchymal stroma (CPM type III or TFM type VI) carries the risk of TFM of approximately 24%, the presence of placental generalized mosaicism for trisomy 21 is associated with a risk of 72.7%.[20] Furthermore, it is important to take into consideration that the presence of abnormal cell line in placental tissue carries an additional risk of fetal loss and intrauterine growth restriction.[21]

In conclusion, when mosaic trisomy 21 is discovered during prenatal diagnosis, confirmatory studies using amniocentesis and/or cordocentesis should be performed. FISH analysis on native amniocytes is the method of choice in resolving low-level or cryptic mosaicisms. During genetic counseling, it should be considered that fetal outcome is not dependent on the level of abnormal cell line. However, increasing proportion of trisomic cells, and especially the presence of ultrasound anomalies, correlates with high risk for fetal abnormality.

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