

CONFINED PLACENTAL MOSAICISM IN SHORT TERM CULTURE

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Finding of fetal chromosomal mosaicism complicates genetic counseling, as well as pregnancy management. The aim of this study was to determine the risk of confined placental mosaicism in short term culture of chorionic villous samples. We conducted a retrospective review of karyotype analysis results obtained after chorionic villous sampling (CVS) in two years period. A 420 samples of chorionic villi were taken transabdominally and obtained by a semidirect method (overnight incubating culture). All fetuses with CVS mosaicism were under the intensive perinatal care. In all cases of chromosome mosaicism the additional karyotyping was performed from fetal blood samples after 22nd gestational week in order to exclude true fetal mosaicism. After delivery newborns were examined by experienced pediatrician. From 420 analyzed samples in 11 (2,6%) cases we found placental mosaicism. No anomalies were seen in genetic sonogram of this fetuses and mosaicism was confirmed only in one case. Confined placental mosaicism (CPM) was found in 2,1% (9/420) of all analyzed cases, and it made 90% of all placental mosaicism. In 60% (6/10) of placental mosaicism cases we found mosaicism with single aberrant cell. Trisomy 21 mosaicism was the most frequent aberration found in 30% of cases. Finding of mosaicism in chorionic villi sample is at special importance for genetic counseling, because every case has to be revealed individually regarding the type and level of mosaicism. Anyway, in every case of placental mosaicism intensive antenatal monitoring is necessary, with additional chromosome analysis from different tissue in consideration of previous findings.

Keywords: confined placental mosaicism, short term culture.

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INTRODUCTION

Chorionic villous sampling (CVS) is method for invasive prenatal diagnosis in early pregnancy.

This procedure is usually done at 10-13 weeks of gestation, with estimated adverse pregnancy outcome of 0,22% (AKOLEKAR *et al.*, 2015).

One way of processing obtained villi is semidirect preparation of rapidly dividing cytotrophoblast cells of the placenta (incubation; short term culture-STC), when cytogenetic analysis and results may be available within 24-48 hours.

Chorionic villi tissue and fetus both rise from trophoblast, so chromosome constitution of placenta should reflect fetal karyotype. But, in 1-2% of CVS, chromosomal mosaicism, defined as the presence of two or more cell lines with different chromosomal complement in an individual or tissue sample, is present (HAHNEMANN and VEJERSLEV, 1997, STRACHAN and READ, 2011).

Finding of CVS mosaicism complicates genetic counseling, as well as pregnancy management.

From the laboratory point of view, there are two types of mosaicism. One is pseudomosaicism which is an artifact occurring in tissue culture. Second is true mosaicism that can be present both in fetal and placental tissue, or just in placental tissue with no abnormality of the fetus, phenomenon known as confined placental mosaicism (CPM) (KALOUSEK and DILL, 1983).

The aim of this study was to determine the risk of confined placental mosaicism in short term culture.

MATERIALS AND METHODS

We conducted a retrospective review of karyotype analysis results obtained after chorionic villous sampling (CVS) in four years period. Study included 420 fetuses examined for chromosomal abnormalities in Clinic for gynecology and obstetrics, Clinical center of Serbia. Samples of chorionic villi were taken transabdominally, with ultrasonographic guidance by one of experienced maternal-fetal medicine physicians. A CVS karyotype was obtained by a semidirect method (overnight incubating culture). In all cases of chromosome mosaicism the additional karyotyping was performed from fetal blood samples after 22nd gestational week in order to exclude true fetal mosaicism. All fetuses with CVS mosaicism were under the intensive perinatal care through ultrasonographic monitoring in 14th, 18th, 20th and 22nd week of gestation. In 20th week of gestation "genetic sonogram" was performed in order to exclude fetal anomalies. After delivery newborns were examined by experienced pediatrician.

In cases of confirmed mosaicism (true fetal mosaicism), on parents demand, after genetic counseling and ethics committee approval, pregnancies were terminated.

RESULTS

From 420 analyzed samples in 11 (2,6%) cases we found placental mosaicism. Findings of mosaicism, together with cell proportion, indications and fetal blood karyotype analysis results are given in table 1.

Table 1. Indications and mosaicism findings in CVS, and fetal blood karyotype findings

	Indication	CVS mosaic karyotype	Cell proportion	Fetal blood karyotype
1.	AMSST	47,XX+7/46,XX	1:29	46,XX
2.	St post IVF	47,XX+6/46,XX	1:20	46,XX
3.	AMA	47,XXY/46,XX	18:2	/
4.	St post IVF	47,XY+18/46,XY	1:17	46,XY
5.	AMSST	47,XY+21/46,XY	1:21	46,XY
6.	BOH	69,XXX/46,XX	2:8	46,XX
7.	St post IVF	47,XY+21/46,XY	6:4	46,XY
8.	AMA	45,X/46,XX	10:10	45,X/46,XX (50%:50%)
9.	St post IVF	47,XY+5/46,XY	1:14	46,XY
10.	AMSST	45,X/46,XX	1:13	46,XX
11.	BOH	47,XX+21/46,XX	2:16	46,XX

AMSST-abnormal maternal serum screening test, St post IVF-pregnancy after in vitro fertilization, AMA-advanced maternal age, BOH-bed obstetric history

One pregnancy with placental mosaicism was ended immediately, and was not informative. In the rest ten cases pregnancies were continued and monitored ultrasonographically. No anomalies were seen in genetic sonogram in period at 18 to 22 weeks of gestation.

After cytogenetic analysis of fetal blood, mosaicism was confirmed only in one case (10%). This was the case of mosaicism with 50% of cells with monosomy X, and after genetic counseling, on parents demand and ethical commission approval, pregnancy was terminated.

Confined placental mosaicism (CPM) was found in 2,1% (9/420) of all analyzed cases, and it made 90% of all placental mosaicism.

In 60% (6/10) of CPM cases we found mosaicism with single aberrant cell. Trisomy 21 mosaicism was the most frequent aberration found in 30% of cases.

All nine newborns with confined placental mosaicism were born in term and they were healthy, with all clinical and laboratory parameters in physiological boundaries.

DISCUSSION

Cytogenetic analysis remains the "gold standard" for obtaining the fetal karyotype. Chorionic villous sampling, as procedure for obtaining fetal cells for karyotype analysis, has its own risks, benefits and limitations. One of the limitations is confined placental mosaicism (CPM). CPM represents a chromosomal abnormality in the extraembryonic tissue which is absent from fetal tissue (KALOUSEK and DILL, 1983).

In our study CPM was detected in 2,1% of CVS. This percentage correlates with those previously published in literature ranging CPM from 1% to 2% (HAHNEMANN and VEJERSLEV, 1997, KARAMPETSOU *et al.*, 2014).

Regarding the abnormal cell line location it is possible to distinguish three types of placental mosaicism: type I in the cytotrophoblast, type II in the villous' stroma and type III in both locations (OLIVAL *et al.*, 2011).

Type I CPM may be a meiotic origin, when non mosaic chromosomal aberration is found, or the result of mitotic accident, when chromosomal abnormality is mosaic (WOLSTENHOLME, 1996). Type I CPM is observed only after short term culture.

Type II CPM is usually result of an error in meiosis and it can be found only after long term culture (LTC). Type III also has meiotic origin and chromosomal abnormality is present both after STC and LTC (TOUTAIN *et al.*, 2010).

Regarding our method it is obvious that our findings of CPM represent the first type. From the laboratory point of view, mosaicism in CVS cultures is classified on three levels (WORTON and STERN, 1984). Level I represents a single cell abnormality that is not confirmed in the same or other cultures, so it is considered to be a cultural artifact with no clinical significance. In level II mosaicism, multiple cells with the same abnormality in one culture, are not found in any other culture. It is predominately pseudomosaicism that results in normal baby, but in 1% of cases it can reflect a true fetal chromosomal abnormality (WORTON and STERN, 1984, GRATI, 2014, FRYBURG *et al.*, 1993, LEDBETTER *et al.*, 1992). Level III mosaicism occurs when two or more cells with the same chromosome aberration are found in multiple cultures, and it is indicative of true fetal mosaicism (MCKINLAY-GARDNER *et al.*, 2012).

In our study in 54,5% (6/11) of mosaic samples level I mosaicism was found. In consideration we done semi direct method, we were not able to find mosaicism level III. But in three cases we found mosaicism with two cells that had different karyotype. In the first case, in the majority of cells we found 47,XXY and only in two normal female karyotype. On parents demand, and after ethical commission approval, this pregnancy was terminated, so we can only assumed that these two cells with normal chromosomal complement were present due to maternal cell contamination, occurring in about 15% of CVS (SCHRECK *et al.*, 1990).

In second case triploidy was found in two cells and in eight cells normal female karyotype. Triploidy in placenta is often sign of partial hydatidiform mole, but also there are reports of completely trisomic placenta in normal fetuses (ZHANG *et al.*, 2000, DANIEL *et al.*, 2003).

In the third case we found two cells with trisomy 21 in two and normal female karyotype in sixteen cells. This finding may be due to a vanishing twin phenomenon, reflecting the non viability of twin with abnormal karyotype (SCHRECK *et al.*, 1990, HALDER, 2005).

Interesting finding in our study is mosaic placental karyotype with majority of aneuploid cells 47,XX+21/46,XX (6:4). This was finding in second twin, where first twin had diagnosed full trisomy 21. Twin with trisomy 21 had sonographical sings, increased nuchal translucency and absent nasal bone, but second twin, with mosaic trisomy 21 had all parameters in physiology boundaries. This mosaic finding can be explained as contamination during CVS procedure, or confined placental chimerism (HALDER, 2005).

In summary, in our investigation CPM found in CVS in 60% of cases was type I, level I, and in 20% type I, level II. Our study confirms previously published data, that type I, level I or II CPM represents an artifact, e.g. pseudomosaicism, that has no impact on the fetus.

As in study of Phillips *et al.*, we find true fetal mosaicism only in 10% of cases when placental mosaicism was found, in case where normal and aberrant cell lines were found in the same percentage (PHILLIPS *et al.* 1996). Some other studies found true fetal mosaicism after placental mosaicism in 20%-30% (GOLDBERG and WOHLFERD, 1997, BATTAGLIA *et al.*, 2014).

In our investigation in 36,4% CPM was found in pregnancies after assisted reproductive techniques (ART), but previous investigations demonstrated that in this pregnancies incidence

of CPM is not significantly different comparing with spontaneous conceptions (JACOD *et al.*, 2008).

Some studies find CPM in direct correlation with intrauterine growth retardation, oligohydramnios, spontaneous abortions and fetal loss (KALOUSEK *et al.*, 1991, CORBACIOGLU, 2012). Our investigation showed no adverse pregnancy outcome in correlation with CPM.

Interpretation of mosaicism is among the most difficult challenges in genetic counseling for prenatal diagnosis because accurate prediction of clinical outcome based on information describing mosaicism may be impossible (HADAD *et al.*, 2013). Even when further analyses from different tissue at different gestational stages show that fetus have normal karyotype, good laboratory practice would be to include in reports that CPM cannot be excluded (KARAMPETSOU *et al.*, 2014).

CONCLUSIONS

Finding of mosaicism in chorionic villi sample is at special importance for genetic counseling, because every case has to be revealed individually regarding the type and level of mosaicism. Anyway, in every case of placental mosaicism intensive antenatal monitoring is necessary, with additional chromosome analysis from different tissue in consideration of previous findings.

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OGRANIČEN POSTELJIČNI MOZAICIZAM U KRATKOTRAJNOJ KULTURI

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Izvod

Nalaz hromozomskog mozaicizma kod fetusa ne komplikuje samo genetičko savetovanje, nego i dalje perinatološke procedure. Cilj ove studije je da utvrdi rizik za pojavu ograničenog placentnog mozaicizma u uzorcima horionskih resica nakon kratkotrajne kultivacije. Ovom retrospektivnom studijom evaluirani su nalazi analiza kariotipa iz 420 uzoraka horionskih resica obrađenih u periodu od četiri godine. Uzorci su uzeti transabdominalno i kultivisani 24h (kratkotrajne/inkubaciona kultura), pre praparacije hromozoma. Svi fetusi kod kojih je detektovan mozaicizam u uzorku horionskih resica bili su pod intenzivnim perinatološkim nadzorom. U svim slučajevima placentnog mozaicizma, a u cilju isključivanja pravog fetalnog mozaicizma, dodatno je urađena analiza kariotipa iz uzorka fetalne krvi nakon 22. nedelje gestacije. Po rođenju novorođenčad sa placentnim mozaicizmom pregledana su od strane iskusnog pedijatra. Od 420 analiziranih uzoraka, placentni mozaicizam je nađen u 11 (2,6%) slučajeva. Genetički sonogram nije otkrio strukturne anomalije kod ovih fetusa, a mozaicizam je potvrđen samo u jednom slučaju. Ograničeni placentni mozaicizam je nađen u 2,1% (9/420) svih analiziranih uzoraka i činio je 90% placentnog mozaicizma. U 60% (6/10) slučajeva placentnog mozaicizma nađen je mozaicizam sa samo jednom aberantnom ćelijom. Mozaicizam koji je uključivao trizomiju hromozoma br. 21 je nađen sa najvećom učestalošću, u 30% slučajeva. Nalaz mozaičnog kariotipa u uzorcima horionskih resica ima veliku važnost za genetičko savetovanje jer svaki slučaj treba evaluirati zasebno, u odnosu na tip i nivo mozaicizma. Svakako, u svim slučajevima placentnog mozaicizma neophodan je intenzivan antenatalni nadzor i dodatna analiza hromozoma iz uzorka nekog drugog tkiva u cilju isključivanja pravog fetalnog mozaicizma.

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