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CHIMERISM AND MULTIPLE NUMERICAL CHROMOSOME IMBALANCES IN A SPONTANEOUSLY ABORTED FETUS

We report on a case of chimerism and multiple abnormalities of chromosomes 21, X and Y in spontaneous abortion specimen. To the best our knowledge the present case is the first documented chimera in a spontaneously aborted fetus. The application of interphase fluorescence in situ hybridization (FISH) using chromosome enumeration and site-specific DNA probes showed trisomy X in 92 nuclei (23 %), tetrasomy X in 100 nuclei (25 %), pentasomy of chromosome X in 40 nuclei (10 %), XXY in 36 nuclei (9 %), XXXXXYY in 12 nuclei (3 %), XXXXXYYYYY in 8 nuclei (2 %), trisomy 21 and female chromosome complement in 40 nuclei (10 %), normal female chromosome complement in 72 nuclei (18 %) out of 400 nuclei scored. Our experience indicates that the frequency of chimerism coupled with multiple chromosome abnormalities should be no less than 1 : 400 among spontaneous abortions. The difficulties of chimerism identification in fetal tissues are discussed.

Introduction. Chimerism — the presence in an organism of several cell populations derived from different zygotes — is an extremely rare event in human conceptuses. Despite of the achievements in biomedical science made throughout last years this phenomenon is poorly documented [1]. Although no fewer than two cases of chimerism diagnosed prenatally were described [2, 3], to the best our knowledge there was no any communication concerning the incidence or consequences of chimerism coupled with aneuploidy in human pregnancies. Here, we report a first case of chimerism and multiple chromosome abnormalities in a spontaneously aborted fetus.

Materials and methods. A 24-year-old primigravida has experienced spontaneous abortion at 48th day of gestation with a diagnosis of intrauterine growth retardation. Ultrasonography was unable to establish real gestational age suggesting severe intrauterine growth retardation of 1—2 weeks. Twinning pregnancy was excluded. Additionally, gestational and yolk sac but not fetal heart beat were observed. Conventional cytogenetic analysis revealed normal karyotype in lymphocytes of both parents.

Chorionic villus sampling was performed according to previously described protocol [4]. A set of chromosome enumeration DNA probes for chromosomes 1, 9, 13/21, 14/22, 15, 16, 18, X and Y was applied for interphase fluorescence in situ hybridization (FISH). Site-specific DNA probe for chromosomes 21 was applied. DNA probes and multicolor FISH performance were described previously in details [4—6]. In order to confirm chromosome abnormalities detected multicolor FISH was applied using the combination of site-specific probe for chromosome 21 and centromeric probes for chromosomes X and Y. Analysis of FISH results was performed through scoring 400 interphase nuclei per each FISH assay.

Results and discussion. Chromosome abnormalities detected were referred to trisomy 21 and mosaic polysomy of sex chromosomes. The analysis of remaining autosomes did not demonstrate abnormality. The results of molecular cytogenetic studies carried out are summarized in table. The specimen possessed two distinct cell populations characterized by female/male chromosome complement. Twinning pregnancy was excluded by ultrasonography. Therefore, the occurrence of chimerism in the index case was concluded.

Human chimerism is assumed to be essentially manifested as 46,XX/46,XY karyotype. Additio-

Summary of FISH studies performed for the analysis of the index cases

Combination of DNA probes (chromosomes)	Results
1—X—Y	nuc ish 1qh(D1Z1x2),Xcen(DXZ1x3)[91]/1qh(D1Z1x2),Xcen(DXZ1x4)[101]/1qh(D1Z1x2),Xcen (DXZ1x5)[39]/1qh(D1Z1x2),Xcen(DXZ1x2),Ycen(DYZ3x1)[34]/1qh(D1Z1x2),Xcen(DXZ1x6), Ycen(DYZ3x2)[15]/1qh(D1Z1x2),Xcen(DXZ1x5),Ycen(DYZ3x5)[8]/1qh(D1Z1x2), Xcen(DXZ1x2)[112]
16—18	nuc ish 16qh(D16Z1x2),18cen(D18Z1x2)[403]
13/21—14/22	nuc ish 13cen/21cen(D13Z1/D21Z1x4),14cen/22cen(D14Z1/D22Z1x4)[354]/13cen/21cen(D13Z1/ D21Z1x5),14cen/22cen(D14Z1/D22Z1x4)[46]
9—15	nuc ish 9qh(D9Z1x2),15cen(D15Z1x2)[398]
X—Y—21*	nuc ish 21q22(cCMP21.ax2),Xcen(DXZ1x3)[89]/21q22(cCMP21.ax2),Xcen(DXZ1x4)[98]/ 21q22(cCMP21.ax2),Xcen(DXZ1x5)[40]/21q22(cCMP21.ax2),Xcen(DXZ1x2),Ycen(DYZ3x1)[38]/ 21q22(cCMP21.ax2),Xcen(DXZ1x6),Ycen(DYZ3x2)[10]/21q22(cCMP21.ax2),Xcen(DXZ1x5), Ycen(DYZ3x5)[9]/21q22(cCMP21.ax3),Xcen(DXZ1x2)[40]/21q22(cCMP21.ax2),Xcen(DXZ1x2)[70]

* Site-specific DNA probe for chromosome 21.

nally, it was occasionally described in dizygotic twins (prenatal diagnosis), newborns, and allogeneic organ transplantants [1—3]. Since the presence of maternal cell lines in chorionic villus or another fetal tissue processed for genetic analysis can not be excluded, the 46,XX/46,XY karyotype detected in spontaneous abortion specimens is usually explained by maternal cell contamination. The latter is probably the cause of chimerism underestimation in fetal tissues. Therefore, multiple chromosome imbalances associated with different sex chromosome complement appear to be the unique indication for chimerism. Numerical chromosome abnormalities account for over the half of spontaneous abortions. Although conventional cytogenetic analysis requiring fetal tissue culture remains the golden standard for cytogenetics of spontaneous abortions, the diminished success rate implies for the application of more reliable techniques allowing the investigation of uncultured somatic tissues. The most applicable among these ones is FISH [4, 7]. The presence of normal female cells detected may be explained by maternal contamination. However, the occurring of male and female cell populations with abnormal chromosome complement evidences for the index case to be a spontaneously aborted chimera.

Chromosome abnormality presently described is unlikely to be accurately assessed through routine cytogenetic technique because cells with abnormal chromosome complement are poorly cultivable [7]. This implicates FISH as the appropriate technique for aneuploid chimerism detection. Chromosomal imbalances identified are likely to be sporadic and could be attributed to meiotic errors occurred in maternal and paternal gametes followed by mitotic errors in fetal tissues which are probably occurred through clonal evolution.

The index case represents the first evident example of chimerism in a spontaneous abortion revealed by FISH. It gives evidence that complex numerical chromosome abnormalities in chimeric fetuses can be associated with variable level of mosaicism and result in inviability. Hence, in spite of the rarity and difficulties of the identification, chimerism coupled with numerical chromosome abnormalities should be considered as an additional source for early pregnancy losses.

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РЕЗЮМЕ. Представлен анализ случая химеризма и сочетанной хромосомной аномалии с участием хромосом 21, X и Y в клетках спонтанного абортуса. Исходя из данных литературы, можно сделать вывод о том, что случаи химеризма у спонтанных абортусов ранее не описывались. Применение метода интерфазной флюоресцентной гибридизации in situ (FISH) с использованием центромерных и сайтспецифических ДНК проб позволило идентифицировать трисомию хромосомы X в 92 клетках (23 %), тетрасомию хромо-

сомы X в 100 клетках (25 %), пентасомию хромосомы X в 40 клетках (10 %), XXY — в 36 клетках (9 %), XXXXXXYY — в 12 клетках (3 %), XXXXXYYYYY — в 8 клетках (2 %), трисомию хромосомы 21 при нормальном женском кариотипе в 40 клетках (10 %), нормальный женский кариотип в 72 клетках (18 %) из 400 проанализированных клеток. Молекулярно-цитогенетические исследования хромосомного набора показывают, что частота химеризма среди спонтанных абортусов составляет, скорее всего, не менее 1:400. Обсуждаются также сложности определения химеризма в клетках спонтанных абортусов.

РЕЗЮМЕ. Представлено аналіз випадку химеризму та сполучної хромосомної аномалії за участю хромосом 21, X та Y у клітинах спонтанного абортуса. Виходячи з даних літератури, можна зробити висновок про те, що випадки химеризму у спонтанних абортусів раніше не описувались. Використання методу інтерфазної флуоресцентної гібридизації in situ (FISH) центромірних та сайтспецифічних ДНК проб дозволило ідентифікувати трисомію хромосоми X у 92 клітинах (23 %), тетрасомію хромосоми X у 100 клітинах (25 %), пентасомію хромосоми X у 40 клітинах (10 %), XXY — у 36 клітинах (9 %), XXXXXXYY — у 12 клітинах (3 %), XXXXXYYYYY — у 8 клітинах (2 %), трисомію хромосоми 21 при нормальному жіночому каріотипі у 40 клітинах (10 %), нормальний жіночий каріотип у 72 клітинах (18 %) із 400 проаналізованих клітин. Молекулярно-цитогенетичні дослідження показують, що частота химеризму серед спонтанних абортусів становить, скоріш за все, не менше 1 : 400. Обговорюються також складності визначення химеризму в клітинах спонтанних абортусів.

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